Proceedings of the Florida State Horticultural Society 2019

Contents

VOLUME CXXXII PRINTED JUNE 2019

Published Papers

Krome Memorial Institute (Tropicals)

Citrus Section

Handling & Processing Section

Ornamental, Garden & Landscape Section

Agroecology and Natural Resources Section

Poster Section

FLORIDA STATE HORTICULTURAL SOCIETY
Board of Directors 2019

Eric Simonne
Chair of the Board

Gene McAvoy
President

Chair of the Board	**Eric Simonne** Gainesville
President	**Gene McAvoy** *LaBelle*
President-elect	**Jeffrey Williamson**, *Gainesville*
Secretary–Treasurer	**Jamie Burrow** *Lake Alfred*
Program Coordinator	**Cami Esmel McAvoy** *LaBelle*
Proceedings Editor	**Mary Lamberts** *Miami Springs*
Marketing Coordinator/Webmaster	**Steve Rogers** *Highland City*
Board Member-at-Large	**Adrian Hunsberger**, *Homestead*
Board Member-at-Large	**Eric Waldo** *Tampa*
Newsletter Editors	**Tatiana Sanchez** *Gainesville* **Alicia Lamborn**, *Macclenny*
Student Best Paper Awards	**Michelle Danyluk**, *Lake Alfred*

Sectional Vice Presidents

Agroecology and Natural Resources	**Zachary Brym**, *Homestead*
Citrus	**Jawwad Qureshi**, *Immokalee*
Handling and Processing	**Yu Wang**, *Lake Alfred*
Krome Memorial Institute	**Alan Chambers**, *Homestead*
Ornamental, Garden and Landscape	**Wayne Hobbs**, *Green Cove Springs*
Vegetable	**Shinsuke Agehara**, *Balm*

2019 MERITORIOUS SERVICE AWARDS

For significant contributions to Florida horticulture

Presidential Gold Medal Award
(not awarded)

President's Industry Award
(not awarded)

Outstanding Commercial Horticulturist Award
(not awarded)

2019 Patron Members

Members who annually pledge additional support for the student programs of the Society

Anne Plotto

Eric Simonne

Friends of the Florida State Horticultural Society Award

To recognize individuals for their longtime commitment and support of the Florida State Horticulture Society

Dr. Gene Albrigo

Mr. Theodore "Ted" Winsburg

Florida Tomato Research Award

Rafia A. Khan, Dakshina R. Seal, and Shouan Zhang

UF IFAS Tropical Research and Education Center, Homestead, FL 33031

Vegetable Crops as Hosts of Thrips (Thysanoptera: Thripidae) Vectoring
Tomato Chlorotic Spot Virus In Tomatoes

Proc. Fla. State Hort. Soc. 131:165–168. 2018

2019 FSHS Awards

BEST PAPER AWARDS

From the Proceedings of the Florida State Horticultural Society Volume 131

Awarded to the best and most meritorious paper as printed in the previous year's Proceedings for its respective section

Agroecology and Natural Resources Section

Lyn A. Gettys and Kimberly A. Moore

University of Florida, IFAS, Fort Lauderdale Research and Education Center, Fort Lauderdale, FL 33314

Title: Greenhouse Production of Native Aquatic Plants. Proc. Fla. State Hort. Soc. 131: 265–269. 2018.

Citrus Section

Ed Stover[1], Robert G. Shatters, Jr.[1], David G. Hall[1], Jude Grosser[2], Barrett Gruber[3], and Gloria A. Moore[4]

[1]U.S. Horticultural Research Laboratory, USDA, ARS, Fort Pierce, FL 34945, [2]UF IFAS Citrus Research and Education Center, Lake Alfred, FL 33850, [3]UF IFAS Indian River Research and Education Center, Fort Pierce, FL 34945, [4]UF IFAS Horticultural Sciences Dept, University of Florida, Gainesville, FL 32611

Title: Huanglongbing-related Responses of 'Valencia' Sweet Orange on Eight Citrus Rootstocks during Greenhouse Trials. Proc. Fla. State Hort. Soc.131: 88-94. 2018.

Handling & Processing Section

Anna Marín[1], Anne Plotto[2], Lorena Atares[1], and Amparo Chiralt[1]

[1]Instituto de Ingeniería de Alimentos para el Desarrollo, Departamento de Tecnología de Alimentos, Universitat Politècnica de València, España, [2]USDA-ARS U.S. Horticultural Research Laboratory, USDA, ARS, Fort Pierce, FL 34945

Title: Lactic Acid Bacteria Incorporated into Edible Coatings to Control Fungal Growth and Maintain Postharvest Quality of Grapes Proc. Fla. State Hort. Soc. 131: 200–206. 2018.

Krome Memorial Institute

Jonathan H. Crane[1], Daniel Carrillo[1], Romina Gazis[1], Edward Evans[1], Jude Grosser[2], Kim D. Bowman[3], James Colee[4], and David Herbella[1]

[1]UF IFAS Tropical Research and Education Center, Homestead, FL 33031, [2]UF IFAS Citrus Research and Education Center, Lake Alfred, FL 33850, 3USDA-ARS, Ft. Pierce, FL 34945, 4 UF IFAS Department of Statistics, Gainesville, FL 32611

Title: Preliminary results of a trial of 'Tahiti' lime and three lime-hybrid scions grafted to five citrus rootstocks established at TREC. Proc. Fla. State Hort. Soc. 131:36–44. 2018.

Ornamental, Garden & Landscape Section

Jianjian Xu and Zhanao Deng

[1]University of Florida, IFAS, Environmental Horticulture Department, Gainesville, FL 32611

Title: Progress in Genetic Sterilization of *Lantana camara* through Ploidy Manipulation. Proc. Fla. State Hort. Soc. 131:220–223. 2018.

Vegetable Section

Stephen Deschamps and Shinsuke Agehara

UF IFAS Gulf Coast Research and Education Center, University of Florida IFAS, Wimauma, FL 33598

Title: Optimization of Microenvironments by Metalized-Striped Plastic Mulch Improves Earliness and Total Yield of Strawberry. Proc. Fla. State Hort. Soc. 131:164–170. 2018.

BEST STUDENT ORAL PRESENTATION AWARDS

Pictured (left to right): Gene McAvoy, *FSHS President,* Vincent Njung'e Michael, Mary Dixon, Gustavo F. Kreutz, and Michelle Danyluk, *FSHS Student Awards*.

BEST ORAL PRESENTATION

First Vincent Njung'e Michael, Yuqing Fu, and Geoffrey Meru

UF/IFAS Tropical Research and Education Center, Homestead, FL 33031

Title: Inheritance of Resistance to *Phytophthora* Crown Rot in *Cucurbita pepo*

Second Mary Dixon[1], Harry Klee[1], Eric Simonne[1], Thomas Obreza[2], and Guodong Liu[1]

[1]UF/IFAS Horticultural Sciences Department, Gainesville, FL 32611, [2]UF/IFAS Soil and Water Sciences Department, Gainesville, FL 32611

Title: Genotypic Variability in Phosphorus Use Efficiency by Hydroponically-grown Tomato

Third Gustavo F. Kreutz, Germán V. Sandoya, and Jesse Murray

UF/IFAS Everglades Research and Education Center, Belle Glade, FL 33430

Title: Lettuce Performance in Two Types of Soil in Florida

STUDENT BEST WRITTEN PAPER AWARDS

These awards are given to encourage student participation in the published FSHS Proceedings, and are given to the students with the best papers submitted for publication in the Proceedings.

BEST WRITTEN PAPER (prepared for the 2019 Proceedings)

First Syuan-You Lin and Shinsuke Agehara
UF/IFAS Gulf Coast Research and Education Center, Wimauma, FL 33598

Title: Cane Management of Floricane-fruiting Blackberries Grown in Central Florida

Second Kenneth J. Sweeney[1], Morgan Jones[2], Adam Cruz[2], Carolyn Cureton[2], Malcolm M. Manners[3], and John L Griffis, Jr.[4]
[1]Biology Department, Florida Gulf Coast University, Fort Myers, FL, [2]Biology Department, Florida Southern College, Lakeland, FL, [3]Horticultural Science Department, Florida Southern College, Lakeland, FL, [4]Marine & Ecological Sciences Dept., Florida Gulf Coast University, Fort Myers, FL

Title: Relationships between Leaf Chlorophyll Content, Fertilizer Application Rates and Visual Grades of Greenhouse-grown Homalomena 'Emerald Gem'

2019 STUDENT SCHOLARSHIP AWARDS

Catherine Belisle • Shahrzad Bodaghi • Daniel Bodakye • Sarah Brewer • Lan-Yen Chang
Mary Dixon • Marie Dorval • Xiangiu Fu • Susmita Gaire • Gustavo Kreutz • Syuan-You Lin
Jean-Maude Louizias • Natalia Macan • Trequan McGee • Riphine Mainviele • Vincent Michael
James Orrock • Carina Theodore • Xhou Zou

Also pictured: Gene McAvoy (far left) and Michelle Danyluk, (far right, back row).

2019 PRESIDENTIAL ADDRESS

Gene McAvoy

President of the Florida State Horticultural Society

Greetings, members of the Florida State Horticultural Society (FSHS). I would like to thank you for choosing me as your president and for your confidence in me. For over 130 years, FSHS has united horticulturists across the state of Florida. In the process, the society has emerged as one of the most prestigious and respected horticultural societies in both the United States and the world. For over a century, the *Proceedings of the Florida State Horticultural Society* (*Proceedings*) have served as a repository of the collective knowledge of our members, professional and amateur horticulturists. The *Proceedings* document advances in the horticultural sciences, new crops and varieties, new and emerging technologies, and many more topics, all of which make them an invaluable reference and resource for horticulturists around the world.

Like all societies our strength is in our members. One of these strengths is the diversity of the FSHS community. This diversity takes many forms: scientific interests (production, postharvest, breeding); commodity interests (citrus, ornamentals, tropical and non-citrus fruit, vegetables, other crops, postharvest products, and natural resources); professional status of Society members (students, post-docs, early career professionals, and accomplished professionals); and the ecotypes in which our members work.

Despite our strengths, the Society has faced challenges in recent years. Unfortunately, these could eventually undermine the status and perhaps even the existence of FSHS if not addressed in a timely, thoughtful manner. Some of the challenges have been structural, some fiscal while others are more nebulous, but will be yet no less serious if ignored.

Over the past few years, FSHS's financial status has declined due to declining membership, fewer sponsors, failure to collect page charges and quite simply spending more than the society has taken in, something which happens more in some years and less in others.

The Board has taken several actions to try to ensure that the society remains strong for the next century. One was to revise the By-Laws to re-establish the position of Treasurer. The Board hopes this will increase scrutiny of budgetary matters and tighten financial controls. You—the members—will be asked to approve this and other changes to the By-Laws at the Annual Business Meeting.

Another action is to reduce expenditures by scaling back on reception functions at the annual meeting, including the presidential reception.

Next year, as Chairman of the Board, I plan to continue to work with incoming President Williamson and the new Board to continue to evaluate our finances and look for additional ways to reduce expenditures and increase revenue.

Like all societies, FSHS is only as strong as its members and their commitment to the Society. This year I was disappointed that I was unable to fill all the committees described in the By-Laws due to members being reluctant or unwilling to step up and serve on a committee.

I appeal to you that, when you are asked to serve in the future, you consider this as an opportunity rather than as a burden and work to help maintain the pre-eminent status of the Florida State Horticultural Society.

FSHS also has a continuing problem of authors not turning in papers (delinquent authors) or turning them in late. Please be responsible and professional and work with the editor and your sectional vice president(s) to submit your paper(s) in a timely fashion.

This coming year, I look forward to working with the Board and individual members to continue to strengthen the society.

Before I yield the floor, I would like to recognize two individuals for their longtime commitment to and support of the Florida State Horticulture Society by giving them the Friend of the Florida State Horticultural Society Award. This award goes Dr Gene Albrigo and Mr. Theodore "Ted" Winsberg. Please join me in giving them a big round of applause for their long service to and support of the Society.

We have a great meeting planned this year and a wide variety of interesting presentations scheduled, so I hope you enjoy the next few days. I look forward to catching up with old friends and making new acquaintances.

All the best for a great meeting.

Presidents of the Florida State Horticultural Society, 1888–Present

1888–96	Dudley W. Adams
1897–1904	George L. Tabor
1905–06	C.T. McCarty
1907–08	P.H. Rolfs
1909	William C. Richardson
1910–22	H.H. Hume
1923–29	L.B. Skinner
1930–36	John S. Taylor
1937	C.W. Lyons
1938–40	Charles I. Brooks
1941	T. Ralph Robinson
1942	Henry C. Henricksen
1943–47	Frank M. O'Byrne
1948	William F. Ward
1949	Frank Stirling
1950	Leo H. Wilson
1951	G. Dexter Sloan
1952	Frank L. Holland
1953	R.S. Edsall
1954	M.U. Mounts
1955	H.A. Thullbery
1956	R.A. Carlton
1957	R.E. Norris
1958	A.F. Camp
1959	S. John Lynch
1960	W.L. Thompson
1961	Ruth S. Wedgworth
1962	John H. Logan
1963	Herman J. Reitz
1964	Willard M. Fifield
1965	Ernest L. Spencer
1966	Arthur F. Mathias
1967	Ed H. Price, Jr.
1968	J.R. Beckenbach
1969	G.M. Talbott
1970	F.E. Gardner
1971	O.R. Minton
1972	R.A. Dennison
1973	B.E. Colburn
1974	G.G. Norman
1975	Leon Miller
1976	John W. Sites
1977	J.B. Pratt
1978	R.R. Reed
1979	J.F. Morton
1980	C. Wayne Hawkins
1981	W. Grierson
1982	Roger Young
1983	Charles A. Conover
1984	Carl W. Campbell
1985	Fred Bistline
1986	Al H. Krezdorn
1987	Richard F. Matthews
1988	T.T. Hatton
1989	W.H. Krome
1990	Tom J. Sheehan
1991	Larry K. Jackson
1992	Daniel J. Cantliffe
1993	Michael O. Taylor
1994	Salvadore J. Locascio
1995	Mohamed A. Ismail
1996	Walter J. Kender
1997	Fred Saunders
1998	Larry E. Beasley
1999	David W. Buchanan
2000	Will E. Waters
2001	Frederick S. Davies
2002	William S. Castle
2003	Jonathan H. Crane
2004	Craig Campbell
2005	George J. Hochmuth III
2006	Jacqueline K. Burns
2007	Peter McClure
2008	James P. Syvertsen
2009	Mary Lamberts
2010	Jeffrey K. Brecht
2011	Richard Tyson
2012	Juanita Popenoe
2013	Nancy Roe
2014	Ed Etxeberria
2015	Steve Sargent
2016	Chris Oswalt
2017	Mark Ritenour
2018	Eric Simonne
2019	Gene McAvoy

Honorary Members (1988 to Present)*

Albrigo, L. Gene	2012
Anderson, J.B.	1922
Anderson, Shirley F.	2002
Beckenbach, J.R.	1967
Berckmens, P.J.	1893
Berger, E.W.	1940
Berry, Robert E.	1987
Blackmon, G.H.	1964
Bosanquet, L.P.	1924
Brecht, Jeffrey K.	2014
Brown, Arthur C.	1952
Bryan, Herbert H.	2003
Bullock, Robert C.	2008
Burgis, Donald S.	1980
Calvert, David V.	1997
Camp, A.F.	1956
Campbell, Beverly	2010
Campbell, C.W.	1988
Cantliffe, Daniel J.	2006
Carlton, R.A.	1962
Castle, William S.	2011
Chase, J.C.	1939
Chase, S.O.	1939
Childers, N.F.	1993
Clayton, H.G.	1956
Colburn, Burt	1970
Commander, C.C.	1952
Cooper, W.C.	1981
Dickey, R.D.	1968
Edsall, R.S.	1967
Etxeberria, Ed	2016
Everett, Paul H.	1986
Fairchild, David	1922
Fifield, Willard M.	1955
Flagler, H.M.	1903
Floyd, Bayard F.	1944
Floyd, W.L.	1939
Ford, Harry	1985
Forsee, W.T., Jr.	1973
Gaitskill, S.H.	1909
Gardner, Frank E.	1967
Garrett, Charles A.	1951
Goldweber, Seymour	1984
Grierson, William	1979
Guzman, Victor L.	1987
Haden, Mrs. Florence P.	1934
Hall, David J.	2011
Harding, Paul L.	1968
Hart, W.S.	1909
Hastings, H.G.	1939
Hatton, Thurman T.	1987
Hayslip, Norman C.	1981
Henricksen, H.C.	1939
Holland, Frank L.	1962
Holland, Spessard L.	1945
Hoyt, Avery S.	1950
Hoyt, R.D.	1914
Hubbard, E.S.	1922
Hume, H. Harold	1927
Ismail, Mohamed A.	1996
Jackson, Larry K.	2000
Jamison, F.S.	1962
Johnson, Warren O.	1965
Jones, John Paul	1997
Kender, Walter J.	2000
Knight, Robert J., Jr.	2006
Koo, R.C.J.	1978
Krezdorn, A.H.	1979
Krome, William H.	1973
Krome, William J.	1927
Krome, Mrs. Isabelle B.	1960
Lawrence, Fred P.	1973
Lipsey, L.W.	1924
Locascio, Salvadore J.	1996
Logan, J.H.	1965
Lynch, S. John	1975
MacDowell, Louis G.	1968
Magie, Robert O.	1977
Mathias, A.F.	1972
Matthews, Richard F.	1992
Mayo, Nathan	1940
McCornack, A.A.	1986
Menninger, Edwin A.	1964
Miller, Leon W.	1972
Miller, Ralph L.	1972
Montelaro, James	1985
Morton, Julia F.	1989
Mounts, M.U.	1958
Mowry, Harold	1950
Murdock, Del I.	1984
Newell, Wilmon	1940
Norman, Gerald G.	1967
Norris, Robert, E.	1962
O'Byrne, Frank M.	1962
Overman, A.J.	1988
Painter, E.O.	1909
Peterson, J. Hardin	1950
Pratt, J.B.	1980
Redmond, D.	1893
Reed, R.R.	1970
Reitz, Herman J.	1970
Reitz, J. Wayne	1955
Robinson, T. Ralph	1942
Rolfs, P.H.	1921
Rolfs, Mrs. P.H.	1921
Ruehle, George D.	1958
Sargent, Steven A.	2018
Saunders, Fred	1999
Sharpe, Ralph H.	1974
Shaw, Miss Eleanor G.	1927
Sherman, Wayne B.	2003
Showalter, Robert K.	1984
Singleton, Gray	1962
Skinner, L.B.	1931
Sloan, G. Dexter	1964
Smith, Paul F.	1972
Smoot, John J.	1986
Spalding, Donald H.	1987
Spencer, E.L.	1962
Stamps, Robert	2010
Steffani, C.H.	1958
Stephens, James M.	1995
Stevens, H.B.	1934
Swingle, W.T.	1941
Syvertsen, James P	2013
Taber, George L.	1914
Tait, W.L.	1941
Talbott, George M.	1980
Tenny, Lloyd S.	1956
Thompson, Ralph P.	1962
Thompson, W.L.	1962
Thullbery, Howard A.	1962
Todd, Norman	1991
Tucker, David P.	1999
Veldhuis, M.K.	1972
Ward, W.F.	1962
Waters, Will E.	1997
Webber, H.J.	1941
Wedgworth, Ruth S.	1965
Wenzel, F.W.	1973
Wheaton, T. Adair	1994
Wilfret, Gary J.	1998
Wilson, Lorenzo A.	1934
Wiltbank, William J.	1987
Winsberg, Theodore W.	2007
Winston, J.R.	1960
Wolfe, H.S.	1964
Young, T.W.	1978
Yothers, W.W.	1976
Ziegler, L.W.	1976

**Date listed is the year in which the award was received.*

Outstanding Growers or Commercial Horticulturists

1997	Norman Todd	Grove Crafters, Labelle, FL
1998	Ted Winsberg	Green Cay Farms, Boynton Beach, FL
1999	Larry K. Jackson	Horticultural Consultant, Auburndale, FL
2000	Gary E. Zill	Zill High Performance Plants, Boynton Beach, FL
2001	Murray J. Corman	Garden of Delights, Davie, FL
2002	Craig Campbell	Valent Biosciences, Orlando, FL
2003	Derek Burch	Masterworks, Plantation, FL
2004	Scott Emerson	Citrus & Vegetable Magazine, Tampa, FL
2005	James "Buster" Pratt	Hanes City, FL
2008	David J. Hall	HDH Agri Products, Tavares, FL
2009	Kenneth Shuler	Stephen's Produce, Jupiter, FL
2010	Peter McClure	Evans Properties, Okeechobee, FL
2011	Michael Edenfield	Bayer CropScience, Windemere, FL
2012	Robert T. McMillan, Jr.	Kerry's Bromeliads Nursery Inc., Homestead, FL
2013	Alvin Cheng	JBT FoodTech, Lakeland, FL
2014	*not awarded*	
2015	*not awarded*	
2016	Steve Rogers	Ecostat Inc., Highland City, FL
2017	Noris Ledsesma	Fairchild Tropical Bodanic Garden, Homestead, FL
2018	Erin Harlow	Dubal County Extension, Jacksonville, FL
2019	*not awarded*	

FSHS Annual Meeting Host Cities

1888	Ocala
1889	Orlando
1890	De Land
1891	Interlachen
1892	Ormond Beach
1893	Pensacola
1894	Jacksonville
1895	Jacksonville
1896	Jacksonville
1897	Orlando
1898	Orlando
1899	Jacksonville
1900	Jacksonville
1901	St. Augustine
1902	Tampa
1903	Miami
1904	Jacksonville
1905	Jacksonville
1906	Jacksonville
1907	St. Petersburg
1908	Gainesville
1909	Daytona
1910	Orlando
1911	Jacksonville
1912	Miami and Havana, Cuba
1913	DeLand
1914	Palatka
1915	Tampa
1916	Arcadia
1917	Arcadia
1918	Ft. Myers
1919	Orlando
1920	Ocala
1921	Miami
1922	Lakeland
1923	Orlando
1924	Tampa
1925	Eustis
1926	Cocoa Beach
1927	Bradenton
1928	Winter Haven
1929	Clearwater
1930	Sebring
1931	Miami and Homestead (spring meeting) Mariana (fall meeting)
1932	Gainesville
1933	Lake Wales
1924	Orlando
1935	Vero Beach
1936	DeLand
1937	Ocala
1939	Winter Haven
1939	Hollywood
1940	Tampa
1941	Orlando
1942	West Palm Beach
1943	Winter Haven
1944	Winter Haven
1945	Orlando
1946	Miami
1947	St. Petersburg
1948	West Palm Beach
1949	Tampa
1950	Winter Haven
1951	West Palm Beach
1952	St. Petersburg
1953	Daytona Beach
1954	Miami Beach
1955	Clearwater
1956	Orlando
1957	Miami Beach
1958	Clearwater
1959	Miami
1960	Tampa
1961	Miami Beach
1962	Miami Beach
1963	Miami Beach
1964	Miami Beach
1965	Miami Beach
1966	Miami Beach
1967	Miami Beach
1968	Miami Beach
1969	Miami Beach
1970	Miami Beach
1971	Miami Beach
1972	Miami Beach
1973	Miami Beach
1974	Miami Beach
1975	Lake Buena Vista
1976	Miami Beach
1977	Lake Buena Vista
1978	Miami Beach
1979	Lake Buena Vista
1980	Miami Beach
1981	Lake Buena Vista
1982	Miami Beach
1983	Daytona Beach
1984	Miami Beach
1985	Tampa
1986	Miami Beach
1987	Orlando
1988	Miami Beach
1989	Tampa
1990	Lake Buena Vista
1991	Miami Beach
1992	Tampa
1993	Miami Beach
1994	Orlando
1995	Orlando
1996	Orlando
1997	Orlando
1998	St. Petersburg
1999	Stuart
2000	Lake Buena Vista
2001	Stuart
2002	Marco Island
2003	Orlando
2004	Orlando
2005	Tampa
2006	Tampa
2007	Palm Beach Gardens
2008	Ft. Lauderdale
2009	Jacksonville
2010	Crystal River
2011	St. Petersburg
2012	Delray Beach
2013	Sarasota
2014	Clearwater
2015	St. Augustine
2016	Stuart
2017	Tampa
2018	Fort Lauderdale
2019	Maitland

Proc. Fla. State Hort. Soc. 132:1–3. 2019.

Sensory Evaluation of 15 Mango Cultivars in South Florida

Noris Ledesma*[1], Luzmila Andrea Nieto[2], and Luisa Pacheco[2]

[1]*Fairchild Tropical Botanic Garden, 10901 Old Cutler Rd, Coral Gables, FL 33156*

[2]*University of Magdalena, Santa Marta, Colombia*

Additional index words. 'Ah Ping', 'Angie', 'Rosigold', 'Rosa', 'Cosghall', 'Duncan', 'Kensington Pride', 'Mallika', 'Nam Doc Mai', 'Espada', 'Rapoza', 'Valencia Pride', 'Young', 'Osteen', 'Omer'

The mango industry in Colombia is primarily for domestic consumption. In addition to local cutlivars, Colombia produces mango cutlivars such as 'Tommy Atkins' and 'Keitt'. In this work, 15 mango cultivars were selected for characteristics and attributes that make them desirable for the mango industry (Ledesma, 2018). 'Ah Ping', 'Angie', 'Rosigold', 'Rosa', 'Cosghall', 'Duncan', 'Kensington Pride', 'Mallika', 'Nam Doc Mai', 'Espada', 'Rapoza', 'Valencia Pride', 'Young', 'Osteen', and 'Omer' were evaluated and compared with 'Tommy Atkins' through sensory analyses in different locations in South Florida. The survey was conducted from May to July 2018, during main mango season in South Florida. For the study, mature fruit from the genetics collection of Fairchild Tropical Botanic Garden in Coral Gables, FL, were used. The sensory evaluations were performed in public places following established protocols. Sensory attributes include appearance, aroma, taste, and texture. Ethnicity, age, and gender of the panelists was recorded. Results using 392 panelists per variety show that 'Rapoza', 'Mallika', and 'Rosa' are the favorite mangos in terms of general appearance, aroma, flavor, and texture. The study also shows differences in preference according ethnicity, but no significant differences based on age or gender.

Mango is one of the most important tropical fruits produced worldwide. Mango production in Colombia is mainly for fresh fruit consumption, which accounts for more than 95% of national production. In Colombia , the main variety is Hilacha or mango crioyo (native mango). Colombia introduced mango cultivars from South Florida in the 1980s: including 'Haden', 'Kent', 'Keitt', 'Ruby', 'Tommy Atkins', 'Van Dyke', 'Palmer', and 'Irwin'. New mango plantations were established in 2011 and 2012, but the fresh mango industry is still in its infancy. The mango industry in Colombia is interested in introducing new cultivars. (Ledesma et al, 2018).

The increase in mango production and consumption has generated a search for new varieties in different countries. Although Colombia currently has varieties with potential for the international market, they are not yet known outside of the country. For the industry to grow, it is necessary to introduce new cultivars with superior characteristics and high quality (Campbell and Ledesma, 2006, 2015). A sensory analysis is a first step in obtaining valuable information about consumer preferences and is a tool to determine the potential of new cultivars for commercialization.

Materials and Methods

This study was done using mature fruit from the Fairchild Tropical Botanic Garden mango collection from May to July 2018. The Fairchild mango collection is located near Homestead, FL, geographic coordinates: 25°32'12.91" N lat. and 80°25'55.17" W long.

The collection is composed of a single tree per cultivar. Each tree is averagre of 12 years-old.. Trees were been grafted onto 'Turpentine' rootstock and have been hand-pruned every year following harvest. Fertilization and disease control involve very few chemical inputs. No irrigation is applied.

The varieties selected for the study are: 'Ah Ping', 'Angie', 'Rosigold', 'Rosa', 'Cosghall', 'Duncan', 'Kensington Pride', 'Mallika', 'Nam Doc Mai', 'Espada', 'Rapoza', 'Valencia Pride', 'Young', 'Osteen', and 'Omer'.

Fruit preparation. The fruit was harvest at a physiologically mature state, stored at temperatures of 70 °F to 75 °F until reaching the ideal ripeness for tasting. For harvesting and postharvest processes, maturity was judged by the color of the flesh, firmness, and the shape of the shoulders of the fruit. The fruit was evaluated at physiological maturity before the survey. Brix degrees and a cut test were performed.

Survey. Color, flavor, and texture of fresh mango are critical factors to consumer acceptance and the success of fresh mango cultivars in the United States. In this research we examined desirable and undesirable quality attributes of the 15 different mangos cultivars compared with 'Tommy Atkins.' The survey included a chart designed to evaluate the preferences in overall appearance of the 15 mango varieties. Color, flavor, texture, quality of fresh-cut mango, and flavor preference were evaluated in different locations in South Florida.

The ethnicity and age range were included in the study to evaluate the preference among U.S. consumers. Age range was divided in 4 groups: (10- to 15-year-old); (15 to 25-year-old),

*Corresponding author. Email: norisledesma.mango@gmail.com

(25- to 45-year-old), and older than 45-years-old. Ethnicity was grouped as: Asian; Hispanic/Latino; White/Caucasian; Black/African American; American Indian/Alaska Native; Native Hawaiian/Pacific islander; and other. At least two hundred (200) people sampled each cultivar.

The form was designed to consider consumer characteristics including gender, ethnicity and age. The test has two interval scales: The first scale is graphical (facial hedonic) and evaluates the appearance and aroma of three varieties of mango compared with 'Tommy Atkins'. The faces correspond to a numerical scale: 1 = "I like it very much", 2 = "I like it", 3 = "I do not dislike it", 4 = "I don't like it", and 5 = "I do not like it at all." The second scale was linear, to determine the intensity of aroma (low, moderate, and very aromatic) and fiber content (a of lot fiber, moderate fiber, and no fiber).

Following the methodology used by Reis et al., 2006 for the preparation of samples, each group of panelists only evaluated four samples (1, 2, 3, and T), with T being the 'Tommy Atkins' cultivar used as a control since it is widely known. The survey was measured using the central tendency to define differences.

Results

Results using 392 panelists per cultivar show that the highest score and acceptability per characteristic evaluated were:

Appearance = 'Ah Ping', 'Rapoza', 'Osteen' and 'Omer'

Flavor = 'Rapoza', 'Rosa', 'Angie', 'Young', 'Mallika'

Aroma = 'Mallika', 'Rosa', 'Rapoza'

Texture = 'Rosa' and 'Espada', (cultivars with more fiber).

Latin ethnic group chose fiber as a positive quality in a mango.

Among the 15 mango cultivars selected for the study, the highest score based on flavor and appearance among all cultivars evaluated taken across all ethnicities, genders, and ages were: 'Rapoza' (3.57); 'Mallika' (3.54); 'Rosa' (3.46); 'Osteen' (3.23); and 'Espada' (3.21). The margin of error was 0.102, with a 95% confidence interval. (Table 1, Fig. 1).

Preferences by Ethnicity, Age, and Gender

The study also showed differences in preference based on ethnicity (Table 2), age (Table 3), and gender (Table 3).

There were significant differences for acceptability based on age or gender. For a small percentage of the evaluations, participants did not mark their gender and it was difficult to identify them based on their name so they were not included.

In general all participants, based on gender, considered the cultivar selected as a control, 'Tommy Atkins' as having a pleasant appearance, but it was not the favorite based on taste; it was also the cultivar with the highest amount of fiber.

Discussion and Conclusions

Sensory analyses showed that several new mango cultivars have potential for commercialization in South Florida: 'Rapoza', 'Mallika', 'Osteen', 'Rosa', and 'Espada'. These cultivars were characterized by consumers as having an attractive color and shape, a pleasant aroma and texture, which means little fiber. Other cultivars, such as Omer, Ah Ping, Angie, Rosigold, and

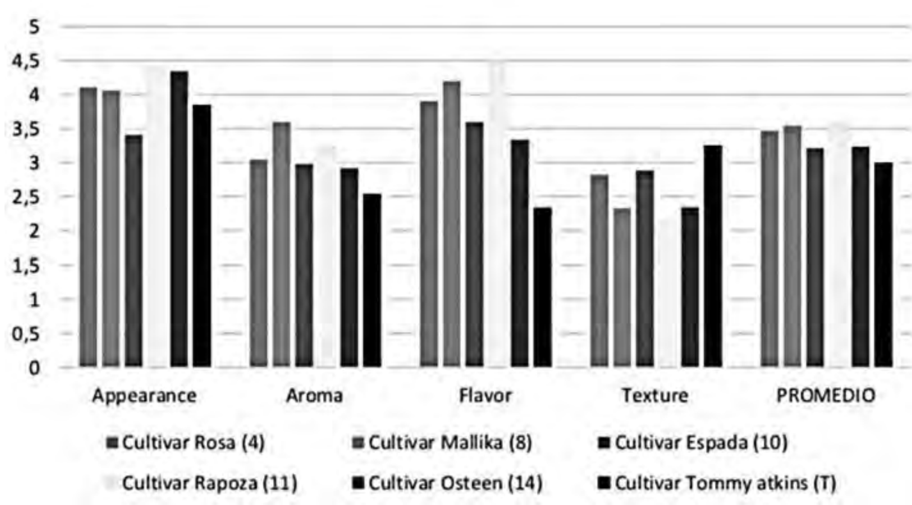

Fig 1. The top cultivars in the survey

Table 1. Average acceptability[z] of the top five cultivars across ethnicity, gender and age.

	Cultivar					
Characteristics	Rosa	Mallika	Espada	Rapoza	Osteen	Tommy Atkins (T)
Appearance	4.11	4.06	3.41	4.41	4.34	3.85
Aroma	3.05	3.60	2.98	3.25	2.92	2.54
Flavor	3.91	4.2	3.59	4.48	3.34	2.34
Texture	2.82	2.33	2.88	2.15	2.34	3.25
Average	3.47	3.55	3.22	3.58	3.23	2.99

[z]Ratings based on a 1–5 scale with 1 = not acceptable and 5 = highly desirable.

Table 2. Average mango cultivar ratings[z] for flavor and appearance by different ethnic groups.

Ethnicity	Flavor	Appearance
Asian		
	Mallika (4.6)	Osteen (4.75)
	Nam Doc Mai (4.3)	Mallika (4.375)
	Osteen (4.25)	Omer, Rapoza, Rosa (4)
	Tommy Atkins (2.48)	Tommy Atkins (3.55)
White/Caucasian		
	Rapoza (4.46)	Omer (4.56)
	Angie (4.27)	Rapoza (4.38)
	Nam Doc Mai (4.09)	Ah Ping (4.35)
	Ah Ping, Cosghall (4)	Osteen (4.31)
	Tommy Atkins (2.39)	Rosa (4.26)
		Tommy Atkins (3.9)
Hispanic/Latino		
	Rapoza (4.68)	Ah ping (4.65)
	Mallika (4.48)	Rapoza (4.57
	Angie (4.28)	Osteen (4.2)
	Nam Doc Mai (4.23)	Mallika (4.16)
	Cosghall (4.15	Valencia Pride (4.13)
	Tommy Atkins (2.21)	Tommy Atkins (3.78)
Black/Afrian American		
	Rapoza (5)	Mallika (4.66)
	Mallika (4.66)	Rapoza, Valencia Pride, Osteen (4.5)
	Angie (4.66)	Rosa (4.33)
	Nam Doc Mai, Rosa (4)	Tommy Atkins (3.64)
	Tommy Atkins (1.82)	
Native Hawaiian/Pacific Islands		
	Ah Ping, Rapoza (5)	Ah ping, Rapoza (5)
	Young (4.5)	Duncan, Valencia Pride (4)
	Tommy Atkins (2.66)	Tommy Atkins (4)

[z]Ratings based on a 1–5 scale with 1 = not acceptable and 5= highly desirable.

Table 3. Preference ratings[z] for appearance, flavor and most aromatic by age group and gender.

	Appearance	Flavor	Aroma
Age			
10–15	Omer (4.8)	Mallika (4.8)	Rosa (4.1)
16–25	Cosghall (4.6)	Rapoza (4.6)	Mallika (4.5)
26–45	Rapoza (4.5)	Mallika (4.5)	Mallika (3.7) Angie (2.2)
45+	Ah Ping (4.5)	Rapoza (4.2)	Mallika (3.4)
Gender			
Women	Ah Ping (4.4)	Rapoza (4.4)	Mallika (3.5)
Men	Rapoza (4.4)	Mallika (4.4)	Mallika (4.4)

[z]Ratings based on a 1–5 scale with 1 = not acceptable and 5 = highly desirable.

Cosghall, also stood out for certain qualities. 'Rapoza' and 'Mallika' stood out for their organoleptic qualities.

The control 'Tommy Atkins' was not a favorite. It was characterized as having a higher fiber content than the other cultivars.

The different ethnic groups present in South Florida described (Gómez, 1994), that the consumption of mango in the United States has been increasing due to ethnic populations, which have contributed to the expansion of the market. Likewise, a study carried out by the National Mango Board (2008–14) where they learned that the demand for mango in the United States depends on the consumer, with ethnicity being the main factor driving the market. New mango varieties can open new possibilities for the mango market in the United States.

Literature Cited

Campbell, Richard J., and N. Ledesma. 2006. A Plan for the Future of the Export Mango Industry of the Western Hemisphere. Proc. Interamer. Trop. Hort.

Campbell, R.J. and N. Ledesma. 2015. Fairchild's quick guide to mangos of the world. Fairchild Tropical Botanic Garden, Miami, FL.

Gómez, Rosario. 1994. La comercialización de mango fresco en el mercado Norteamericano. Centro de investigación (CIUP), Universidad del Pacifico, Lima, Perú. 20 May 2019. <https://core.ac.uk/download/pdf/51208779.pdf>.

Kramer, Amihud. 1965. Quality Control for the Food Industry: Fundamentals v. 1. AVI Publishing Co., Westport, CT.

National Mango Board. (2008–14) Conduciendo la demanda para mangos en los EEUU: El impacto de los programas de la national mango board. 20 May 2019. <http://www.mango.org/Mangos/media/Media/Documents/Research%20And%20Resources/Research/Industry/Consumer/Mango-Demand_Impact-of-NMB-Programs_Executive-Summary_Final_SPN.pdf >.

Ledesma, N., F. Algarin, F. Varela, and M.E. Morales. 2018. Fruit morphology characterization of 10 commercial Mango cultivars (*Mangifera indica* L.) with potential for pulp agro-industry in Colombia, Proc. Fla. State Hort. Soc. 131:45-48.

Reis, R.C., A.M. Ramos, A.J. Regazzi, V.P.R. Minim, and P.C. Stringueta. 2006. Almacenamiento de mango secado análisis fisicoquímico, microbiológico, color y sensorial. Ciencia y Tecnologia Alimentaria 5(3):214-225.

Proc. Fla. State Hort. Soc. 132:4–8. 2019.

Morphological Characterization of Anthers from 17 Accessions of *Mangifera* L. species

Pedro del Llano Rivas[1], Noris Ledesma*[3], Iris Grisel Galván Escobedo[2], and Rodrigo Vásquez López[1]

[1]*Universidad Autónoma Chapingo, Texcoco, Estado de Mexico, Mexico*

[2]*Colegio de Postgraduados, Montecillo, Estado de Mexico, Mexico*

[3]*Fairchild Tropical Botanic Garden, 10901 Old Cutler Rd., Coral Gables, FL 33156*

Additional index words. *Mangifera laliwija, M. rubrapatela, M. odorata, M. laurina, M. zeylanica,* mango breeding

Several qualitative and quantitative morphological variables were extracted from the anthers of 17 accessions of *Mangifera* L. species from the Fairchild Tropical Botanic Garden (Fairchild Farm). Characters were described and multivariate statistical analyzes were applied to the information obtained in order to find significative differences between the morphology of the anthers that allow us to distinguish between the different accessions. It was possible to find a statistically significant difference between all the accessions analyzed with the exception of *M. zeylanica* vs. *M.* sp. 'Poh gedon'. The study suggests that it is possible to use qualitative and quantitative morphological characters of mango anthers for the separation of materials. Superior depth, superior shape and superior angle were the best ones for this purpose.

The genus *Mangifera* L. contains 69 taxonomically recognized species and is one of 73 genera in the family Anacardiaceae (Kostermans and Bompard, 1993). The majority of commercially cultivated mangoes are cultivars derived from *Mangifera indica* L. so most of the research has been focused on that species. The other *Mangifera* species have seen little or no research or production emphasis, and have experienced significant degradation of genetic resources (Bompard, 1993).

There are several morphological studies based on general characteristics of the tree, which may or may not include international standards used by the International Plant Genetic Resources Institute (IPGRI). Characterization studies of this collection have already been carried out including specifics of flower morphology (Ledesma et al., 2018) (Ledesma et al., 2014), but there are none specifically of the anthers.

This morphological study of *Mangifera* species contributes to descriptions of the morphology of the unstudied species. It also provides information on specific anther characteristics which, together with morphological and molecular characteristics of a species, could be used to resolve controversial taxonomic problems.

Materials and Methods

Experimental location and plant material conditions. The experiment was conducted at the Fairchild Tropical Botanic Garden experimental farm "The Fairchild Farm" located at 25 °32'12.91"N and 80°25'55.17"W. It is within the subtropical humid forest according to the Holdridge life zones (Ledesma et al., 2018). The trees have an average of 12 years of age, and have mostly been grafted onto *Mangifera indica* 'Turpentine'.

Sampling. Flowers were collected from a single tree per accession during Jan. to Mar. 2019, between 9:00 am and 11:00 am. The collections were made daily during the blooming timeframe. The examinated species were identified with a code for the results: *Mangifera laliwija* (Lal), *M. rubrapatela* (Rub), *M. odorata* (Odo), *M. laurina* (Lau), *M. zeylanica* (Zey) and other unidentified accessions 'Tenom' (Ten), 'Poh Gedon' (Poh), 'Rampagni' (Ram), 'Butterfly' (But), 'Gedon Ginco' (Ged), 'Juana Diaz 1' (JD1), 'Juana Diaz 5' (JD5), 'Juana Diaz 7 (JD7)', 'Mempelam' (Mem), 'Depih Biasa' (Dep), 'Bua Pelam' (Bua), and 'BRFC' (BRF).

Separately, five anthers from accessions JD1, Rub, Odo, JD5, Zey, Dep, Lau, Lal, Ten, Mem, Ram, and But, were collected at the same time for pollen quantification and anther accession.

Anther evaluation. Healthy anthers were randomly selected and photographed with an AmScope SM-3TZZ-54S stereoscopic microscope with a scale to analyze the images with ImageJ software. Based on these images, anther color and morphological variables were evaluated.

Anther color. Anther color was determined using the Royal Horticultural Society (RHS) Colour Chart (1986). Additional flowers were collected to take color pictures using a confocal microscope.

Anther morphological variables: Nine quantitative morphological variables and four qualitative variables were defined for the anthers (Table 1). All variables were observed and measured for at least 20 anthers by accession.

Statistical analysis. Anther morphological variable observations by accession were organized in a data matrix. For each

*Corresponding author. Email: norisledesma.mango@gmail.com

Table 1. Qualitative and quantitative characters of anthers.

Variable	Character	Abbreviation	Value
Sex	Qualitative	Sex	1 = male 0 = hermaphrodite
Color	Qualitative	C	1 = homogeneous 2 = heterogeneous 3 = No data
Inferior shape	Qualitative	IS	1 = curved 2 = squared 3 = angulated
Superior shape	Qualitative	SS	1 = merged 2 = pointed 3 = crested 4 = curved
Length	Quantitative	L	μm
Width	Quantitative	W	μm
Inferior depth	Quantitative	IDe	μm
Superior depth	Quantitative	SDe	μm
Inferior distance	Quantitative	IDi	μm
Superior distance	Quantitative	SDi	μm
Inferior angle	Quantitative	IA	angle
Superior angle	Quantitative	SA	angle
Area	Quantitative	A	mm^2

variable, basic statistical parameters were estimated: mean, minimum value, maximum value and standard deviation.

Data normality was examined in the matrix before multivariate statistical analysis with a normality test of Shaphiro-Wilk (1965). Variables that did not have a normal distribution were transformed to $\log_{10}$ (Hammer, 2017).

Multivariate statistical methods were used to find differences between the anther morphology among accessions: multivariate analysis of variance (MANOVA) and analysis of principal components (PCA). The test statistic used in MANOVA was *Wilks' lambda of possibilities*. To determine specifically the accessions with significant differences, a comparison was made using pairs of accessions. The test statistic was the *P* value with *Bonferroni correction;* the comparisons with $P < 0.05$ were considered statistically different. All statistical analyzes were carried out with the software PAST version 3.24.

Pollen quantification. Anthers were placed on a slide. At the opening of the anther, a drop of distilled water was added and, with the help of a needle, the pollen was released and dispersed on the slide. Later a drop of potassium iodide was added to dye the pollen grains which were covered with a coverslip with drawn lines and the pollen count was carried out with an Leica dm1000 led microscope with 10× lenses. Pollen counts were recorded as pollen grains/anther/accession, and their average value, minimum value, maximum value, and standard deviation were obtained.

Results

Anther evaluation.

Anther color: In general, anthers are purple (RHS color code between 59A-B and 71A-B) (Table 2) with a rough surface and the exact shade varies in hue by accession and is not always

Table 2. Main anther colors on the Royal Horticultural Society Colour Chart (1986).

Accession	DJ1	Rub	Odo	JD5	Zey	Dep	Lau	Ged	Lal	Ten	Poh	Mem	JD7	Ram	But	Bua	BRF
Color code	60[a]	59B	59A	60A	59B	60A	59B	71A	61A	59C	64A	60D	71A	59A	71A	60C	60B

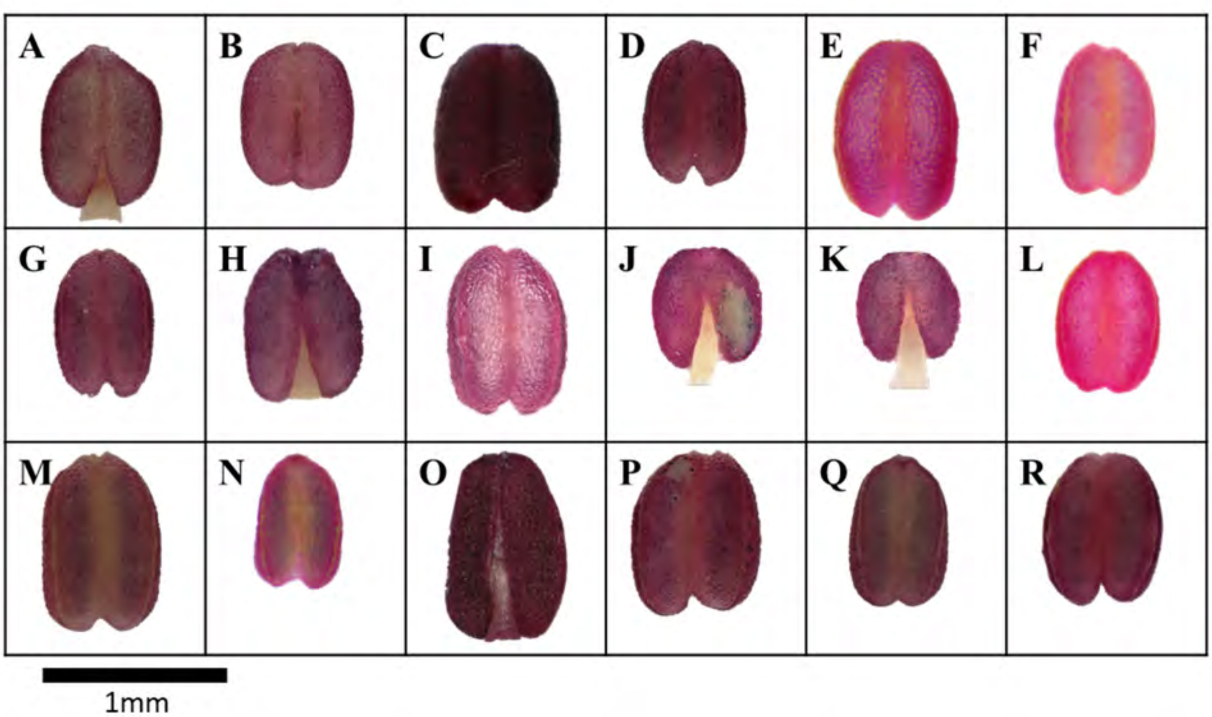

Fig. 1. Pictures of the anthers: **A**) JD1, **B**) Rub, **C**) Odo, **D**) JD5, **E**) Zey, **F**) Dep, **G**) Lau, **H**) Ged, **I**) BRF, **J**) Lal, **K**) *Mangifera casturi*, **L**) Ten, **M**) Poh, **N**) Mem, **O**) Ram, **P**) But, **Q**) Bua, and **R**) JD7. *Manguifera casturi* was included for photographic use only because there was limited material.

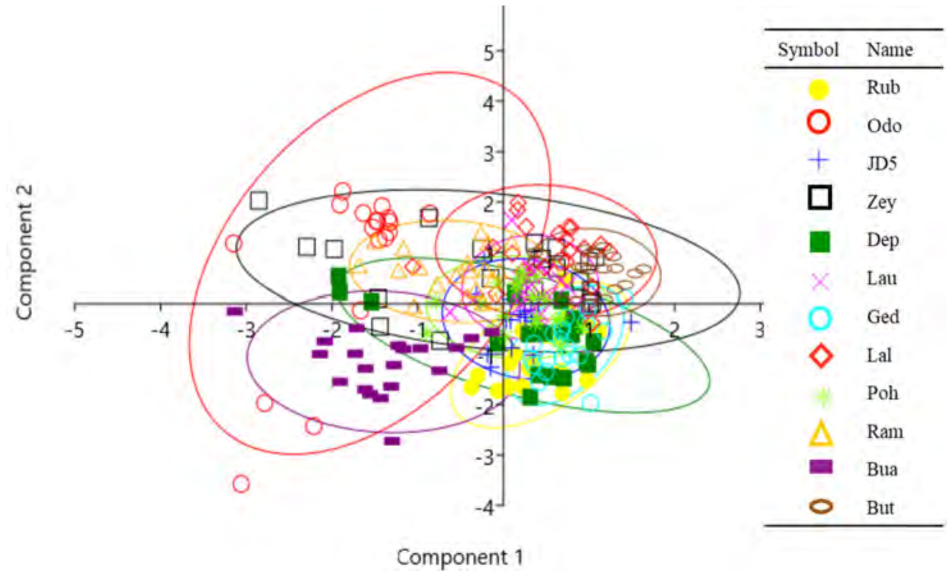

Fig. 2. PCA scatter plot with 90% ellipses with eigenvalues scale.

Table 3. Anther morphological variables.

Acc[z]	Sex	C	IS	SS	L	W	IDe	SDe	IDi	SDi	IA	SA	A
Rub	1.5 ± .5	1.7 ± 0.5	1 ± 0	2.9 ± 1	764 ± 51	588 ± 43	50 ± 11	26 ± 7	210 ± 34	80 ± 36	102 ± 15	89 ± 23	0.38 ± 0.5
	1–2	1–2	1–1	2–4	637–843	495–650	33–75	14–42	147–270	34–187	79–130	39–121	0.29–0.45
Odo	1.5 ± .5	1 ± 0	1.1 ± 0.5	1.3 ± 0.8	1017 ± 33	710 ± 20	80 ± 12	6 ± 6	248 ± 27	116 ± 62	66 ± 20	167 ± 14	0.60 ± 0.03
	1–2	1–1	1–3	1–4	972–1076	684–762	66–108	1–28	213–306	2–195	26–113	129–180	0.57–0.67
JD5	1 ± 0.2	1.9 ± 0.4	2.9 ± 0.4	1.95 ± 0.4	803 ± 76	589 ± 35	93 ± 17	25 ± 12	218 ± 27	94 ± 33	89 ± 29	97 ± 29	0.37 ± 0.05
	1–2	1–3	1–3	1–3	621–891	505–658	46–118	12–66	170–273	42–177	45–148	63–163	0.24–0.44
Zey	1.4 ± 0.5	1.9 ± 0.6	1.9 ± 1	2.75 ± 1.4	935 ± 62	660 ± 33	88 ± 11	19 ± 12	243 ± 29	113 ± 32	73 ± 16	119 ± 31	0.51 ± 0.04
	1–2	1–3	1–3	1–4	806–1030	587–713	57–107	1–38	186–295	46–170	51–106	62–176	0.45–0.60
Dep	1.6 ± 0.5	2 ± 0	1.3 ± 0.7	2.85 ± 1.2	819 ± 46	572 ± 28	41 ± 11	23 ± 10	182 ± 21	113 ± 29	110 ± 16	106 ± 35	0.39 ± 0.03
	1–2	2–2	1–3	1–4	718–900	538–627	22–61	3–40	152–231	55–178	72–135	43–178	0.34–0.45
Lau	1.5 ± 0.5	2 ± 0	1 ± 0	3.1 ± 0.4	832 ± 59	681 ± 33	64 ± 17	21 ± 6	236 ± 34	111 ± 21	65 ± 15	123 ± 17	0.46 ± 0.04
	1–2	2–2	1–1	2–4	729–947	627–744	40–105	8–37	181–299	70–164	37–95	91–157	0.40–0.54
Ged	1 ± 0	1 ± 0	1.2 ± 0.6	2.4 ± 0.8	937 ± 41	621 ± 29	58 ± 12	35 ± 10	192 ± 36	85 ± 26	77 ± 13	54 ± 14	0.48 ± 0.04
	1–1	1–1	1–3	2–4	842–1011	564–668	38–83	24–66	133–239	56–172	56–105	34–90	0.41–0.55
Lal	1.5 ± 0.5	1.8 ± 0.4	1 ± 0	3.4 ± 1.1	858. ± 27	694 ± 30	109 ± 14	27 ± 10	285 ± 34	23 ± 38	54 ± 10	123 ± 25	0.48 ± 0.03
	1–2	1–2	1–1	1–4	801–919	637–746	83–146	9–44	236–353	162–231	34–71	63–157	0.43–0.54
Poh	1 ± 0	2 ± 0	1.1 ± 0.4	2.7 ± 1.1	897 ± 54	653 ± 38	66 ± 12	20 ± 5	214 ± 32	97 ± 21	71 ± 15	102 ± 32	0.49 ± 0.06
	1–1	2–2	1–3	1–4	748–980	588–744	45–84	9–35	154–282	70–152	43–102	42–173	0.34–0.59
Ram	1.55 ± 0.5	1 ± 0	1 ± 0	1.8 ± 0.4	1187 ± 77	705 ± 33	93 ± 16	13 ± 5	312 ± 28	71 ± 19	66 ± 16	120 ± 24	0.69 ± 0.07
	1–2	1–1	1–1	1–2	989–1296	644–768	61–115	3–22	240–369	46–129	34–99	67–178	0.54–0.78
Bua	1 ± 0	2 ± 0	1 ± 0	1.55 ± 1	735 ± 41	525 ± 49	47 ± 13	7 ± 3	178 ± 40	56 ± 14	97 ± 19	157 ± 14	0.31 ± 0.04
	1–1	2–2	1–3	1–4	667–808	430–614	26–74	1–15	85–230	26–75	73–146	119–179	0.24–0.38
But	1.6 ± 0.5	1.6 ± 0.5	1 ± 0	3.9 ± 0.4	879 ± 53	714 ± 55	76 ± 17	36 ± 10	284 ± 35	149 ± 33	84 ± 7	88 ± 20	0.52 ± 0.05
	1–2	1–2	1–1	2–4	741–966	631–838	53–113	17–60	226–360	91–228	73–103	55–135	0.40–0.65
JD1	1.1 ± 0.4	1.9 ± 0.4	1 ± 0.0	3.1 ± 0.5	844 ± 26	625 ± 19	56 ± 9	28 ± 11	210 ± 19	95 ± 27	78 ± 10	97 ± 18	0.43 ± 0.05
	1–2	1–2	1–1	2–4	798–889	593–650	35–68	13–45	186–241	48–135	59–94	71–133	0.38–0.46
Ten	1.1 ± 0.3	1 ± 0.0	1.3 ± 0.7	2.8 ± 1.4	764 ± 23	582 ± 23	50 ± 13	13 ± 4	198 ± 25	119 ± 31	79 ± 18	136 ± 21	0.37 ± 0.02
	1–2	1–1	1–3	1–4	735–816	543–627	33–75	6–20	155–242	70–135	59–113	95–160	0.34–0.41
BRF	1.3 ± 0.5	3 ± 0.0	1 ± 0.0	3.8 ± 0.6	796 ± 28	632 ± 24	81 ± 15	30 ± 8	211 ± 31	129 ± 29	75 ± 13	83 ± 10	0.41 ± 0.02
	1–2	3–3	1–1	2–4	733–830	596–673	60–110	18–43	159–269	73–182	57–117	68–101	0.35–0.43
Mem	1.2 ± 0.4	2.1 ± 0.6	1 ± 0.0	1.2 ± 0.4	713 ± 59	477 ± 47	54 ± 14	5 ± 4	186 ± 58	42 ± 18	85 ± 34	148 ± 46	0.28 ± 0.05
	1–2	1–3	1–1	1–2	589–811	381–538	33–81	0–14	80–255	14–72	55–117	54–179	0.16–0.35
JD7	1.5 ± 0.5	1 ± 0.0	1 ± 0.0	1 ± 0.0	738 ± 54	616 ± 51	71 ± 20	11 ± 7	250 ± 28	98 ± 28	74 ± 13	157 ± 26	0.38 ± 0.06
	1–2	1–1	1–1	1–1	616–799	540–704	37–102	4–20	206–309	50–146	54–96	92–178	0.27–0.47

[z]Acc = accession, C = color, IS = inferior shape, SS = superior shape, L = length, W = width, iIDe = inferior depth, SDe = superior depth, IDi = inferior distance, SDi = superior distance, IA = inferior angle, SA = superior angle, A = area.

Mean ± standard derivation, minimum value – maximum value.

Table 4. Pairwise comparisons between accessions.

	Rub	Odo	JD5	Zey	Dep	Lau	Ged	Lal	Poh	Ram	Bua	But
Odo	6.45×10^{-10}											
JD5	2.44×10^{-07}	4.70×10^{-11}										
Zey	2.04×10^{-06}	8.18×10^{-06}	1.78×10^{-05}									
Dep	0.02235	1.61×10^{-09}	1.50×10^{-07}	1.58×10^{-05}								
Lau	9.87×10^{-05}	2.06×10^{-08}	2.60×10^{-08}	0.00021434	4.75×10^{-06}							
Ged	2.61×10^{-07}	1.15×10^{-08}	8.23×10^{-10}	3.00×10^{-08}	2.49×10^{-07}	7.82×10^{-10}						
Lal	7.62×10^{-07}	2.55×10^{-09}	2.99×10^{-08}	3.24×10^{-05}	5.39×10^{-09}	0.034713	7.99×10^{-11}					
Poh	0.00034062	4.38×10^{-07}	4.16×10^{-07}	0.075	0.00052056	0.018548	1.64×10^{-06}	3.50×10^{-05}				
Ram	5.15×10^{-11}	0.00037063	4.33×10^{-12}	2.10×10^{-07}	2.74×10^{-10}	1.58×10^{-10}	1.69×10^{-08}	1.36×10^{-10}	1.70×10^{-08}			
Bua	9.22×10^{-05}	2.03×10^{-10}	1.37×10^{-07}	9.80×10^{-08}	0.00038296	1.22×10^{-07}	4.87×10^{-10}	1.32×10^{-09}	1.11×10^{-06}	6.47×10^{-12}		
But	0.00013403	1.63×10^{-08}	5.14×10^{-09}	7.89×10^{-05}	5.54×10^{-07}	0.012053	1.20×10^{-08}	0.0048645	0.0010386	1.39×10^{-09}	7.05×10^{-10}	

$P < 0.05$ = statistically significant difference.

homogeneous (Fig. 1). The most intense colors were found in *M. odorata* and 'Rampagni', while less intense colors were found in 'Mempelam', 'Depih Biasa', and 'Bua Pelam'.

Anther morphological variables: 'Juana Diaz 1', 'Tenom', 'BRFC', 'Mempelam', and 'Juana Diaz 7' were not included in the statistical analysis because there were too few.

Statistical analysis. The mean, standard deviation, minimum value and maximum value per accession are shown in Table 3. On average, anthers were 889 microns long and 643 microns wide. The longest anthers were from 'Rampagni' and *M. odorata*, while the shortest ones were 'Bua Pelam' and 'Juana Diaz 5'. As expected, the same accessions are near the highest and lowest values for anther area.

The MANOVA analysis gave *Wilks´ lambda* = 0.0006 and $P = 5.39 \times 10^{-259}$. This means that there are statistically significant differences among the accessions, $P < 0.05$ in most cases, which means every accession is different from every other accession other with the exception of *M. Zeylanica* vs. 'Poh Gedon' where $P = 0.07$ (Table 4).

The PCA among accessions showed that the first three principal components accounted for 69% of total variance. The first component accounted for 43% of the variance, followed by the second (14%) and the third (12%) (Table 5). For cumulative variance, the most important load factors were: 1) superior depth, 2) superior shape, 3) superior angle, 4) superior distance, and 5) inferior depth.

The scatter plot of the PCA showed a conglomerate for most of the accessions, which indicates a high correlation in the morphological variables of the anthers. However, the accessions *M. odorata*, *M. Zeylanica,* 'Depi Biasa', 'Bua Pelam' and 'Rampagni' showed a dispersed distribution relative to the other accessions, which indicates that there is more variation in anther morphology of these accessions (Fig. 2).

Pollen quantification. As shown in Fig. 3, there was in the pollen content between the different accessions and for different anthers within the same tree.

The average value per accession ranged from 639 to 902. The accessions with the lowest amount of pollen were 'Juana Diaz 1', 'Mempalam', and *M. laurina* with 639, 659, and 660 respectively. The accessions with the highest average amount of pollen were 'Butterfly', *M. zeylanica,* and 'Rampagni' with 902, 792, and 783, respectively.

Table 5. Principal components analysis. Eigen values and accumulated variance of three main components and load factors of component per variable.

Component	1	2	3
Eigen value	0.167	0.054	0.048
Variance (%)	42.688	13.825	12.233
Accumulated variance (%)	42.688	56.513	68.746
	Load factors		
Variable	PC 1	PC 2	PC 3
Color	0.046	–0.097	–0.398
Superior Shape	0.447	0.095	–0.107
Inferior Depth	0.001	0.420	0.412
Superior Depth	0.815	–0.175	0.197
Inferior Distance	0.020	0.251	0.215
Superior Distance	0.244	0.686	–0.560
Inferior Angle	0.022	–0.275	–0.301
Superior Angle	–0.259	0.250	–0.162
Area	–0.003	0.243	0.292

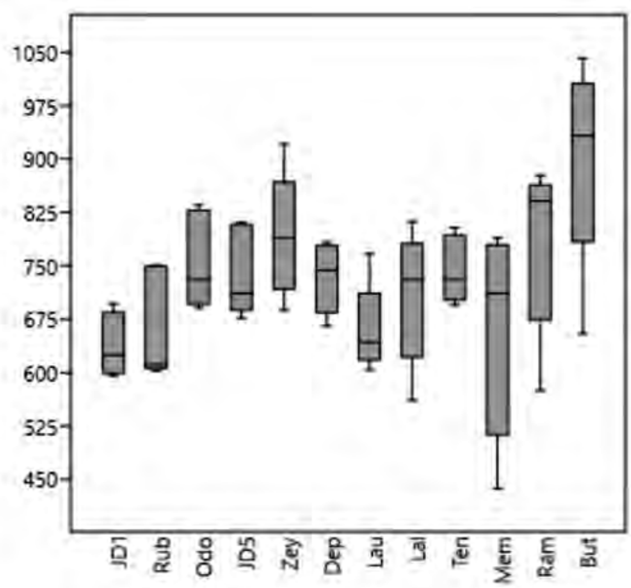

Fig. 3. Box plot pollen quantification/anther/accession.

Discussion

There were subtle variations in the color of the anthers. Therefore, it was necessary to use a color chart. It was also important to take the measurements as quickly because the color began to change after a few hours.

The most stable qualitative characters within the accessions were color homogeneity and the superior shape of the anther. The inferior shape was less stable as seen in the standard deviations in Table 3.

The statistical analyzes were necessary to separate the different materials. The MANOVA showed that all the evaluated accessions were statistically different from each other with the exception of *M. zeylanica* vs. M. *sp*. 'Poh gedon' with a P value > 0.05.

The PCA showed that the most important characters to separate the groups were: 1) superior depth, 2) superior shape, 3) superior angle, 4) superior distance and, 5) inferior depth, all of which are found in the upper part of the anther. Unfortunately, values such as length and with, which are easier to measure, were less important.

Conclusions

This study suggests that it is possible to use both the qualitative and quantitative morphological characters of mango anthers to separate materials. The most accurate qualities are: superior depth, a superior shape, and a superior angle.

The amount of pollen per anther is quite variable and there are differences by accession, which also seems to be influenced by the size of the anthers. This is a preliminary study, so further work is necessary.

Literature Cited

Bompard, J.M. 1993. The genus *Mangifera* rediscovered: the potential contribution of wild species to mango cultivation. Acta Hortic. 341 (341), 69–77.

Hammer, Ø. 2017. PAST. Paleontological Statistics Version 3.15. Reference Manual. 10 Apr. 2017. <https://folk.uio.no/ohammer/past/past3manual.pdf_>

Kostermans, A.J.G.H. and J.M. Bompard. 1993. The Mangoes: Their botany, nomenclature, horticulture and utilization. Academic Press. 233.

Ledesma, N., R.J. Campbell, H.W. Poor, J.J.Figueroa, S. and Zona, 2017. Floral morphology of seven *Mangifera* species. Acta Hortic. 1183:1–10. https://doi.org/10.17660/ActaHortic.2017.1183.1

Ledesma, N., V.F. Segundo and A. F. David. 2018. Morphological characterization of inflorescences and flowers of 200 mango varieties (*Mangifera indica* L.) Proc. Fla. State Hort, Soc. 131:49-51

Royal Horticultural Society, 1986. The RHS Colour Chart. The Royal Horticultural Society, in association with the Flower Council of Holland.

Sankaran, M., M.R. Dinesh, N. Chaitra, and K. Ravishankar. 2018. Morphological, cytological, palynological and molecular characterization of certain *Mangifera* species. Current Science. 15(7):1379-1386.

Shapiro, S., and M. Wilk. 1965. An Analysis of Variance test for normality (complete samples). Biometrika, 52: 591-611.

Proc. Fla. State Hort. Soc. 132:9–11. 2019.

‘Angie’ Mango for South Florida

Noris Ledesma*

Fairchild Tropical Botanic Garden, 10901 Old Cutler Rd., Coral Gables, FL 33156

Additional index words. mango cultivars, home garden, state agriculture, dwarf tree

‘Angie’ was selected for Fairchild Tropical Botanic Garden as a home garden and estate agriculture cultivar in South Florida due to its compact growth habit, disease tolerance, and overall fruit quality. The fruit are 400 g, oblong, and saffron yellow with Indian orange blush on the sun-exposed shoulders. The skin is smooth and without visible lenticels. The flesh is tangerine orange and without fiber. The flavor is classified in the ‘Alphonso’ class of mangos with a deep sweetness and sophisticated profile rich in apricot. The disease tolerance is excellent and given its early season, it often can be harvested before the rainy season in South Florida. The tree is dwarf and highly manageable with annual pruning. Size can be maintained at or below 3 m with consistent production. In South Florida, ‘Angie’ keeps increasing in popularity because of its small size and potential for high production per unit land area. In places like Florida, “estate” type agriculture involves relatively small plantations, with production destined for specialized ethnic markets.

‘Angie’ is originally from Florida. It is from a seedling of unknown origin, planted in 1988 at Four Fillies Farm at Fairchild Tropical Botanic Garden, Coral Gables, FL. In 2006, Fairchild Tropical Botanic Garden named the cultivar after Angie Whitman, wife of Mr. Bill Whitman who was a Garden trustee.

Description

The tree (Fig. 1) is small in size, forming a round canopy. Trees in South Florida reach a height of 7–12 ft with a spread of 7 ft, but with annual pruning they can be kept smaller. The tree flushes once a year, and has small internodes and stems compared with most cultivars. The leaves are often twisted in a characteristic manner. Trees are easy to handle and highly productive, they can be kept at 8 ft, while maintaing fruiting and tree health.

Trees begin to bloom and produce fruit 3 years after planting, either in the field or in a container. In South Florida, trees often have yellowish leaves due to iron deficiency, which is easy to control with applications of iron chelate. Flowering occurs over a fairly long period in the winter, which can lead to multiple crops.

Disease tolerance is excellent. Given its early season, ‘Angie’ can often be harvested before the rainy season. The tree is easy to grow if nitrogen is kept low and the tree is not over-watered or grown in soils prone to flooding or with a high water table.

The fruit (Fig. 2) have a 400 g average weight, with a rounded base, a stool stem inserted obliquely in a level manner, a rounded apex with a small lateral beak, and a slightly undulating surface. The fruit is firm, with a soft texture and few lenticels. The flesh is orange-yellow, firm and melting, with very little fiber and a lot of juice.

The flavor of ‘Angie’ resembles that of ‘Alphonso’ mango and it has been gaining popularity because of its good quality. This fruit is characterized by subtle aromas of spices with a slight smell of peach, cucumber, and wood, and fine aroma of honeysuckle and an interesting aroma of pineapple. The flavor is excellent—rich and aromatic—with a strong component of peach and some cantaloupe and Cucurbitaceous relatives, plus strong accents of pineapple and papaya. The after taste is very low in acidity, astringency, and bitterness. It has a creamy smooth texture with a Brix of 22% and a long shelf-life in storage. The best flavor is obtained when the fruit is tree ripened, but it maintains a good flavor after proper storage. It is a fruit with high overall quality.

It is a polyembrionic cultivar. The seed is 3.8 in long, 2 inwide, and 1.4 in thick.

The selection of ‘Angie’ was based on trying to find a small sized mango with disease tolerance, productivity, and good fruit quality. Today trees have been propagated by grafting from the mother tree at the Fairchild Farm in Homestead, FL.

Fig 1. ‘Angie’ tree.

*Corresponding author. Email: norisledesma.mango@gmail.com

Fig 2. 'Angie' fruit.

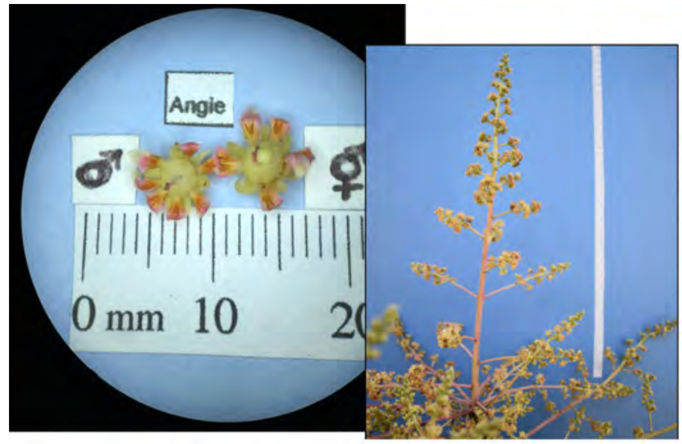

Fig 3. 'Angie' flowers and inflorescence.

Maturity date for harvesting. Fruit production is between May and July in South Florida.

Detailed Description

Tree

Size: 7–12 ft in height
Vigor: low.
Figure: compact, rounded habit
Productivity: very good, 310 kilo per mature tree
Regularity of bearing: regular bearing

Leaves (IPGRI, 2006)

Size: very large as compared with other mango varieties
Average length—17 cm
Average width—8 cm
Shape: Lanceolate
Tip—acute, with some twisting
Base—broad angular
Crossection—incurved
Pubescence—none
Color:
Upwardly disposed surface—pea green (144A) [Royal Horticultural Society (RHS), 2015]
Downwardly disposed surface—Typically same as upper or with a slight more yellow tint
Marginal form: generally smooth to slightly wavy
Petiole:
Length—4–5 cm
Diameter—0.3–0.4 cm
Color—gray
Young leaves:
Color—yellow-green (154A) (RHS, 2015)

Flowers. Produced on terminal inflorescences with thousands of individual flowers that typically set less than 1.5% in natural pollination. Flowers are shown in Fig. 3. 'Angie' has high percentage of hermaphrodite flowers (50% to 70%) compared with other cultivars.
Petals and sepals—both male and hermaphrodite flowers have 5 petals and sepals
Color—white to cream to red with maturity (Ledesma et al., 2018).
Flower buds:
Size—2.5 mm average
Surface texture—Firm
Flowers: Multiple flower inflorescences with thousands of flowers each.
Date of bloom—mid-December to early February
Size—generally 2.2 mm, when full open—4.3 mm
Petals and sepals: 5 petals and sepals
Color—white to cream to red with maturity
Petiole and peduncle: small and many-branched
Color—pink, red or green
Anther: five; color: color
Ovary: single color, white/red (stigma)

Fruit. 'Angie' has a fruit size of 4.8 in length, 3.9 in width, and 3 in thickness with an oblong shape. The fruit is firm and colorful. The predominant color is saffron yellow with Indian orange blush (30A). (RHS, 2015). The fruit are ripe for harvesting and shipment approximately May through June in South Florida.
Size (cm):
Typical average length—4.8 in
Typical average diameter—3.9 in
Typical average: thickness—3 in
Typical average weight—400 g
Shape: Oblong to oval with a slightly undulating skin surface, a rounded base, slender stem with a squared insertion, no cavity and a bluntly pointed apex with a small lateral beak.
Shape—oblong oval
Surface—slightly wavy
Appendix—lightly dotted

Peak—small; side
Shape—bluntly pointed

*Stem***:**
Peduncle—hard; cylindrical
Length—10 cm–16 cm
Diameter—0.3 cm
Color—brown to gray

Skin:
Thickness—thin, tender and adhesive
Smoothness—moderately undulating
Lenticels—few, small, yellow lenticels
Skin color—predominant color is saffron yellow with Indian orange blush (30A). (RHS, 2015)

Flesh: firm, melting and juicy with very little fiber
Flesh color—deep yellow to orange
Fiber—fiber
Flavor—excellent
Brix—22 °Brix
Texture—firm and slightly presence of fiber

Resistance to disease: good tolerance to anthracnose (*Colletotrichum gloeosporioides*); moderate susceptibility to powdery mildew (*Oidium mangiferae*)

Harvesting: by hand

Sap burn: sap burn not a problem

Literature Cited

Campbell, R.J. and N. Ledesma. 2004. A new generation of mangos for Florida. Proc. Fla. State Hort. Soc. 117:204–205.

Ledesma, N., F.S. Varela, F.D. Algarin, and R.J. Campbell. 2018. Morphological characterization of influences and flowers of 200 mango varieties. Proc. Fla. State Hort. Soc. 131:49–51.

IPGRI. 2006. Descriptors for Mango (*Mangifera indica* L.). International Plant Genetic Resources Institute, Rome, Italy.

Royal Horticultural Society, 2015. The RHS Colour Chart. The Royal Horticultural Society, London, in association with the Flower Council of Holland.

Proc. Fla. State Hort. Soc. 132:12–14. 2019.

Mango Pollen Collection and Preservation Methods for *Mangifera odorata*, *M. lalijiwa,* and *M. laurina*

Noris Ledesma*[1], Rodrigo Lopez Vazquez[2], Pedro del Llano Rivas[2], and Sergio Roberto Marquez[2]

[1]*Fairchild Tropical Botanic Garden, 10901 Old Cutler Rd., Coral Gables, FL 33156*

[2]*Autonomous University of Chapingo, Chapingo, Mexico*

Additional index words. cryopreservation, genetic resources, mango breeders

Pollen collection and storage tests of *Mangifera odorata, M. lalijiwa,* and *M. laurina* were evaluated to generate a protocol for these species. Genetic improvement, using interspecific crosses between different species related to *M. indica* are ongoing at the Fairchild Farm Genetic Resources Center, in Homestead, FL, which is part of Fairchild Tropical Botanic Garden. Different species flower at different times, which hinders manual pollination. The preservation of mango pollen could help conserve genetic resources and aid in pollination, both for the production of commercial fruit and for reproduction. This study revised and proposes a practical technique for the breeder that includes pollen collection methods, storage periods of up to 2 months, and germination and viability tests. *M. lauraina* and *M. lalijiwa* have a germination rate of over 73% using for Silica 2 hours and storage at –32.8 °F. The results also show the germination was stable during the period of evaluations compared with other species. The proposed methodology includes guidance to determine pollen viability of the *Mangifera* species studied, in vitro germination, and two months after being collected.

The mango is produced in more than 90 countries. Asia produces 77%, the Americas 13% and Africa 9% of world production. India is the largest producer of mango, with 38.6% of world production from 2003 to 2005. During this period, the mango harvest in India averaged 10.79 million metric tons, followed by China and Thailand with 3.61 million metric tons (12.9%) and 1.73 million metric tons (6.2%), respectively (FAOSTAT, 2007). Mango is the primary tropical fruit produced worldwide, followed by pineapple, papaya, and avocado. These four are considered major tropical fruits, according to world market studies (FAO, 2004).

Genetic improvement in mangoes is limited by several factors, such as the identification of new species, and the spatial and temporal isolation of the parents. There are very few studies of mango pollen storage and morphology, as well as viability, collection and transport.

It is necessary to breed and select new mangos with desirable characteristics (Campbell and Zill, 2006). Fairchild Tropical Botanic Garden includes collections of tropical fruits, orchids and native plants. Currently, the Fairchild Farm, a tropical fruit experimental farm is dedicated to research on and preservation of different species of *Mangifera*. This collection is the source of basic germplasm for breeding programs. It includes more than 650 varieties of *Mangifera indica* and more than 23 species of *Mangifera*. Interspecific crosses have been made over the last five years with the aim of creating ultra-tropical mangos that do not need artificial floral induction in the tropics (Ledesma, et al., 2016).

Researchers from Fairchild Tropical Botanic Garden are conducting a mango breeding program to create interspecific hybrids. To use conventional breeding programs, synchrony in flowering and/or matching gametes is required. Many of these species have different flowering times, which often occur before most cultivars of *M. indica* (Ledesma and Campbell, 2014). Pollen storage becomes an alternative for these breeders. Gene banked pollen can be made available to breeders, to facilitate research programs, and may include shipping pollen overseas.

Pollen storage studies indicate that it is possible to maintain viability at low temperatures. Other studies show that when pollen is collected and stored at a range of temperatures, low temperatures are more suitable for long-term pollen (Dutta et al., 2013). On the other hand, pollen can be stored efficiently for a few weeks at temperatures of –20 and –4 °C (Dutta et al., 2013). It is necessary to generate pollen storage protocols or methods for use in manual pollination when other species are used as parents. For storage and transport, different pollen collection techniques are necessary (Dutta et al., 2013), and pollen viability studies, among other things, are necessary.

The longevity of pollen depends on many factors specific to individual cultivars or species (Ganeshan and Alexander, 1991 --is year correct?). In the present work, in vitro germination is considered indicative of the effectiveness of mango pollen storage tests. The study was conducted with *Mangifera odorata*, *M. lalijiwa,* and *M. laurina* with *M. indica* 'Tommy Atkins' used as a control. All the specimens mentioned are living accessions established at the Fairchild Farm Genetic Resources Center, located in Homestead, FL.

Materials and Methods

Project location. The present work was conducted at the Fairchild Farm Genetic Resources Center, located in Homestead,

*Corresponding author. Email: norisledesma.mango@gmail.com

FL. The three species include *M. odorata*, *M. laurina*, and *M. lalijiwa*. The control was *Mangifera indica* 'Tommy Atkins'.

Collecting samples. Anther collection was conducted from 8:00 am to 10:00 am Mango pollen is sticky and difficult to separate from the anthers. The collected anthers were purple or red (Dutta et al., 2013). Anthers were stored at 75 °F daylight to induce dehiscence. For storage, a plastic container with humid tissue paper was used.

Drying. Four treatments were used.

1) Silica: Drying hours: 2 and 3 h.
2) Freeze dryer: Drying hours: 1 and 2 h. (Dutta et al., 2013).
3) Drying, benzene and filtered: (Khan and Perveen, 2009).
4) Drying with liquid benzene: (Khan and Perveen, 2009).

Storage. Each of the different drying treatments is subjected to different storage temperatures: ambient temperature (control), 39.2 °F, 24.8 °F, and –32.8 °F. Evaluations were made every 15 days for a two-month period.

In vitro germination test. The eppendorf tubes were removed from the freezer, opened and the pollen was collected with a clip by placing on a slide. The pollen had to be rehydrated to 90% to 100% for 1 h.

Counting of germinated pollen grains. When pollen began immerging in to the culture medium (at 6–8 h) it was counted under a microscope. Pictures were taken to archive information for future reference.

The statistical analysis was conducted using a completely randomized factorial design with five replications. All data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test.

Results and Discussion

Pollen germination of three different *Mangifera* species has been compared, stored under four different conditions and with four drying methods. The in vitro germination test demonstrated the ability of the pollen to remain viable after storage for a period of two months.

The three species had different percentages of germination. Among the three species, *M. lalijiwa* and *M. laurina* had germination rates of 74.33% and 75% at 45 days, using the drying treatment with silica for 2 h and storage at –32.8 °F (Fig. 4). *Mangifera lauraina*, show than pollen can be stored 24.8 °F in vitro germination with good results (Fig 3). We did not have results using other treatments with these species.

M. odorata had a germination rate of 40% with silica gel drying for a period of two hours and storage at –32.8 °F for 45 days (Fig. 4). A pollen germination rate of 30.67% was obtained after 45 days of storage at 39.2 °F using drying on silica gel for 2 hours (Fig. 2). Lower germination rates were obtained with 16.33% of germination with samples using 39.2 °F, drying in a freeze dryer for one hour and 45 days of storage.

M. laurina showed the most resistance for all storage treatments including the control, storage room temperature. The control treatment had a germination of 12.33% (Fig. 1). The best germination results were for storage at 24.8 °F with freeze dryer for 1 h, which had a germination of 57.33% after 45 days of storage. Germination of 50% was obtained with a storage period of 45 days for *M. laurina* after freeze-drying for two hours. The pollen at 39.2 °F, with drying in a freeze dryer had a germination rate of 31.67% at 45 days (Fig. 2). Using silica gel

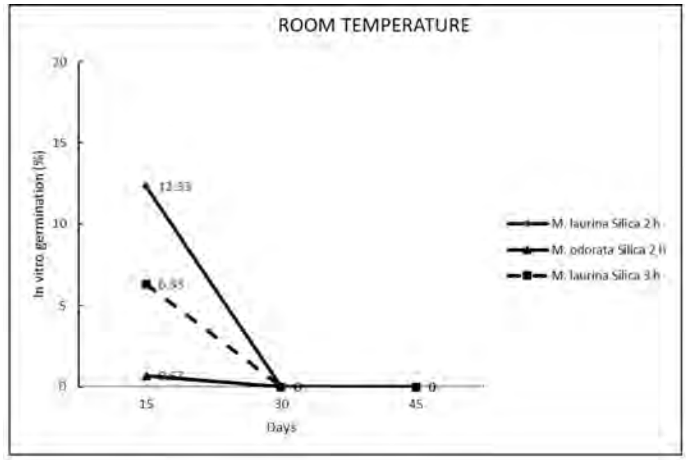

Fig 1. Percentage of pollen germination at room temperature.

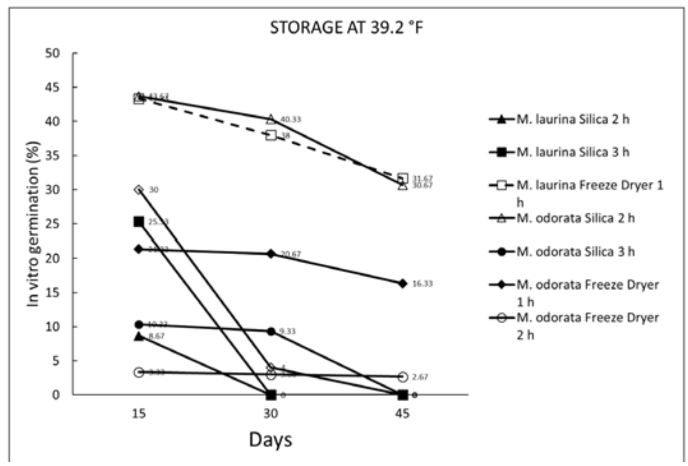

Fig 2. Percentage of pollen germination after storage at 39.2 °F.

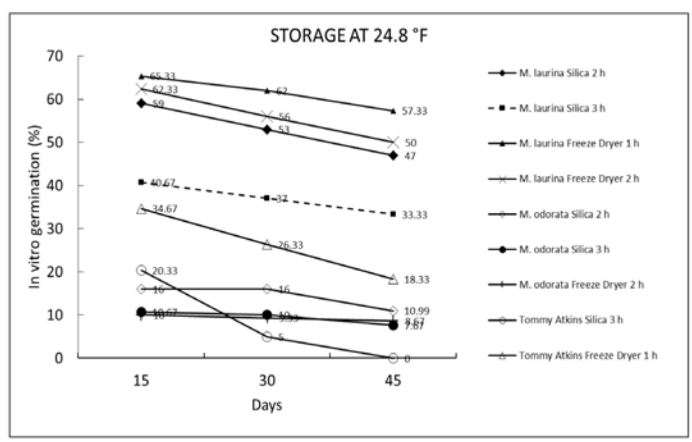

Fig 3. Percentage of pollen germination after storage at 24.8 °F.

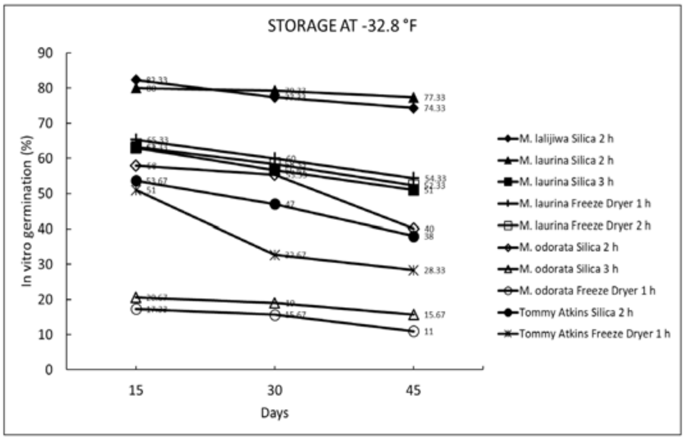

Fig 4. Percentage of pollen germination after storage at –32.8 °F.

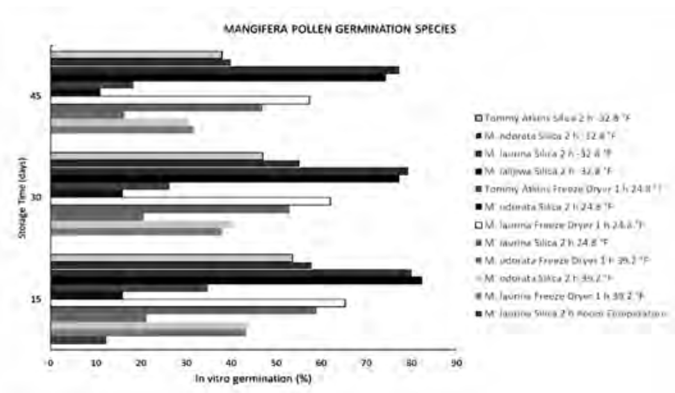

Fig. 5. Storage time vs. species and treatments.

for three hours, pollen can be stored up to 15 days, but dies after 30 days of storage under room temperature. Other treatments had no effect at this storage temperature.

'Tommy Atkins' had lower germination compared to the three species of *Mangifera* (Fig. 4). Using silica gel drying for two hours and a temperature of –32.8 °F, germination was 47% at 30 days of storage. The treatment with –32.8 °F dried in a freeze dryer in 1 hour had a germination of 32.67% at 30 days and 28.33% at 45 days.

The best treatments for all species tested was silica gel for two hours, storage at –32.9 ° F. For long period storage, preliminary data suggests lyophilizing for 1 h.

M. lauraina had good germination with the different treatments and was the most stable during the evaluation period compared with other species (Fig. 5).

M. lauraina can be stored in silica per 2 h., at –32.8 °F. The same treatment also worked well for *M. lalajiwa* (75% germination).

The results also show than *M. lauraina* can be stored in a freeze dryer for 1 h at 24.8 °F with 68% germination; and good results were found storing in silica 2 h at 24.8 °F (66% germination). Storage Freeze dryer storage for 1 h at 39.2 °F showed 43% germination of *M. lauraina*.

M. odorata storage in silica for 2 h at 39.2 °F showed 44% germination, and freeze dryer storage 2 h at –32.8 °F showed 59% germination.

Future Research

These results are preliminary and future evaluations are suggested including further trials of pollen storage for long periods. Cryopreservation in liquid nitrogen for long-term storage is recommended. Suitable methods for transport of pollen over long distances also need to be standardized. Interspecific hybrids made using stored pollen should be evaluated. Field pollination after storage should be studied to maximize pollen survival and success in setting fruit.

Literature Cited

Campbell, R.J. 2007. The potential of new *Mangifera* species in Florida. Proc. Fla. State Hort. Soc. 120:11–12.

Dutta, S.K., M. Srivastav, R. Chaudhary, K. Lal, P. Patil, S.K. Singh, and A. K. Singh. 2013. Low temperature storage of mango (*Mangifera indica* L.) pollen. Scientia Horticulturea, 161: 93–197.

FAO. 2004. Frutas tropicales. Perspectivas a plazo medio de los productos básicos agrícolas. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAOSTAT. 2007. FAO Statistics, Food and Agriculture Organization of the United Nations, Rome, Italy.

Ganeshan A. and M.P. Alexander. 1986. Effect of freeze-drying of pollen germination in vitro in papaya (*Carica papaya*) and tomato (*Lycopersion esculentum*), Gartenbauwissenschaft. 51:17–20.

Khan, S.A and A. Perveen. 2009. Pollen germination capacity of three mango cultivars (*Mangifera indica* L., Anacardiaceae) from Pakistan. Pak. J. Bot., 41:1009–1012.

Ledesma, N. and R.J. Campbell. 2014. Conservation and Commercial Development of *Mangifera* Species (Wild Mangos) in Florida. Proc. Fla. State Hort. Soc. 127:10–13.

Ledesma, N., R.J. Campbell, M. Hass, and T.B. Campbell. 2017. Interspecific hybrids between *Mangifera indica* and related species. Acta Hortic. 1183:83–88. https://doi.org/10.17660/ActaHortic.2017.1183.12

Proc. Fla. State Hort. Soc. 132:15–16. 2019.

Salinity Tolerance of Five Mango Ecotypes (*Manginfera indica* L.) in Santa Marta, Colombia

Noris Ledesma*[1], Felipe Andrés Arias Acevedo[2], and
Yeiser Javier Estrada Púa[2]

[1]*Fairchild Tropical Botanic Garden, 10901 Old Cutler Rd, Coral Gables, FL 33156*

[2]*University of Magdalena, Santa Marta, Colombia*

Additional index words*:* salinity, mango, growth tolerance

The Department of Magdalena, Colombia, is an important area for the mango industry. Mango production has been increasing for commercial purposes, and new challenges have appeared including nutritional problems and saline soils. Some species can tolerate high concentrations of salt in the environment before being affected in a negative way, but for mango, this not the case as it is very sensitive to salinity. This work evaluates the effect of salinity on the growth and development of mango trees selected for salt tolerance. Five selections of 'Hilacha' were evaluated every two days for 3 months under nursery conditions using water treatments with different salt concentrations. The study was conducted at the Center of Universidad del Magdalena.

The mango (*Mangifera indica* L.) is one of the fruit trees widely found in the tropics, as it is beneficial for human health and desired around the world for fresh consumption or for its byproducts. Colombia ranks 45th in mango production worldwide with production of more than 200,000 t.

The Department of Magdalena, ranking third in production, occupies an important place in the production of mango in Colombia. 'Hilacha' is one of the most widely cultivated mangos in the north coast of Colombia. It is a polyembryonic cultivar, which is found alone or in commercial plantations throughout Colombia. There are various 'Hilacha' types because they are propagated from seeds. The trees are highly productive and have good disease tolerance. The fruit has a rich flavor with sweet notes of melon and citrus. On the Atlantic coast, 'Hilacha' grows in flooded conditions and the fruit fall to the ground when the water level drops. Trees in this region have been able to adapt to saline conditions and, when started from seed, begin to produce fruit in 6–8 years (Ledesma, 2016).

Saline soils are those that contain such excessive concentrations of soluble salts that plant productivity is drastically reduced (Vieccelli et al., 2017). Some plant species tolerate a range of salt concentrations in the environment before being adversely affected (Chaitanya et al., 2014). Mango trees, by contrast, are very sensitive to salinity (Zuazo et al., 2004). Studies show that mango seedlings, grafted died after they been exposed to salinity levels as low as 1.2 μS/m as they are very sensitive to soil salinity at younger stages (Dubai et al., 2007).

Many seedling mango cultivars are sensitive to saline soils. '13-1', 'Gomera 1', and *M. zeylanica* are some of the mango cultivars and species related of mango with resistance to salinity. Salt ions accumulate in toxic amounts in mango leaves. Symptoms of sodium injury include chlorotic areas in leaves, tips, and even stems. (Dubai et al., 2007)

*Corresponding author. Email: norisledesma.mango@gmail.com

The objective of this experiment was to establish the effect of salt on the development of young plants under nursery conditions and to determine their maximum tolerance to levels of salinity.

Materials and Methods

The project was conducted at the agricultural and forestry development center of the University of Magdalena, Colombia, located at coordinates of 11°13'18¨ N, 74°11'10¨ W. This geographical position has a warm-dry climate, with an annual average rainfall of 573 mm, average annual temperatures of 25.3 to 32 ° C and a relative humidity of 81.5% .

Adaptation of the nursery. A mixture of 50% river sand, 25% sand, and 25% organic matter (2:1:1) was used. Trees were transplanted in 5-gallon plastic pots (buckets). The containers had holes in the bottom for drainage. The containers where place above the soil to avoid direct contact with the ground. The ecotypes collected were healthy, vigorous plants of the same age (approximately eight months).

Selected trees. The plants for the study came from seeds of 5 adult trees locally called 'Hilacha' that were selected for their ability to grow and produce fruit under extreme saline conditions. The ecotypes EC1, EC2, EC3, EC4, and EC5 were evaluated using five (5) replicates per treatment.

The mango 'Hilacha'. 'Hilacha' is a local naturalized mango selection in Colombia. It is found in all mango areas in Colombia between level sea and 1,667 m.a.s.), including regions of Tolima, Cundinamarca, Magdalena, Cesar, Córdoba, Bolívar, Huila, and Antioquia. It is a polyembryonic cultivar, is found as single trees or in commercial plantations. .

The selected trees came from the area of Sitionuevo, Magdalena, Colombia. The original trees were selected by the corporation (CEA, SA) for exclusive propagation in their operations. The original trees were found scattered in the Magdalena Farm and are ~70 years old. The trees have survived for years despite regular

Table 1. Salt concentrations and treatments.

Treatments	Concentration (NaCl)	Water
T1	10 mmol (CE 1084 $\mu S \cdot cm^{-1}$)	300 mL
T2	20 mmol (CE 2143 $\mu S \cdot cm^{-1}$)	300 mL
T3	40 mmol (CE 4031 $\mu S \cdot cm^{-1}$)	300 mL
T4	80 mmol (CE 7631 $\mu S \cdot cm^{-1}$)	300 mL
Control T5	No salt application	300 mL

floods, salty soils, and an electrical conductivity up to 1.8 μS/m. Trees received no irrigation and were randomly distributed. Five trees were selected from improved seedling selection of 'Hilacha'. Propagation was done by grafting, using cuttings grown from the mother tree. The propagation for this study was conducted at the Magdalena Farm using same mother tree as a rootstock.

The five selections of 'Hilacha', with an average age of 8 months, were evaluated under nursery conditions. They were transplanted into 5-gallon plastic containers for the experiment. The trees were place one meter above the ground before making the salt applications.

Experimental design. A completely randomized design was used. The treatments used distilled water and salt rates shown in Table 1.

The control (T5) received no salt. One replication per treatment was used due to limited plant material. 300 mL of water per tree and the prepared solution was applied every 3 days for a period of 4 months.

Evaluations. Before treatment applications, pH and electrical conductivity (CE) were measured. Additional measurements to determine plant vigor included: height, number of leaves, thickness of the stem and number of burned leaves.

Variables evaluated

Electric conductivity (EC). EC was measured using a conductivity meter. Distilled water was added to the soil sample using a 2:1 ratio, then stirred for 30 minutes, and allowed to rest for 10 minutes prior to measuring EC.

pH. The pH was determined using the same procedure as outlined above. This process was performed every 30 days for four (4) months.

Tree vigor. Every three days for a period of 16 weeks, plant height (HP) and stem diameter (DT) were measured. Manual counts of the total numbers of leaves (NH), and burned leaves were made.

Salinity tolerance. The tolerance to salinity was determined in terms of relative biomass, that is, the relationship between the biomass generated at a certain salt concentration and the biomass generated without salinity. Salt stress in mango produces symptoms as scorched leaf tips and margins, which can develop into necrotic areas (Litz, 2009).

In addition, the stress tolerance index was determined from the relationship between salt concentrations and biomass.

The statistical analysis was conducted using a completely randomized design with five replications. All data were analyzed using the analysis of variance procedure, with mean separation using Duncan's Multiple Range test.

Results and Discussion

The data show differences among treated and non-treated plants. Leaf tissue damage increased with the rate of salt application. EC increased up to 97%, indicating the high concentration of salts in the pots (Table 2).

Fully grown trees can tolerate higher levels of salt than young trees (Dubai and Srivastav, 2007). New trials are suggested to evaluate salinity resistance over time. Taste tests of these selections are also recommended.

Literature Cited

Chaitanya, K., C. Krishna, G. Ramana, and S. Beebi. 2014. Salinity stress and sustainable agriculture a review. Agricultural Reviews. 35:34.

Dubai, A. K. and Manisg Srivastav. 2007. Salt strees studies in mango —A review, Agric.Rev. 28(1):75–78.

Ledesma, Noris. 2016. Chapter 18. Mango of Colombia, The mango tree encyclopedia. Royal Courts Affairs. Sultanate of Oman. Vol. 2.2:798–831.

Litz, R.E. 2009. The mango botany, production and uses. p. 68–89. In: Iyer and Schnell (eds.). Breeding and genetics. USDA–ARS, National Germplasm Repository. Miami, FL.

Mendoza, F., E. Hernández, and L. Ruiz. 2015. Efecto del escaldado sobre el color y cinética de degradación térmica de la vitamina C de la pulpa de mango de Hilacha (*Mangífera indica* var Magdalena river).

Shannon, M. 1984. Breeding, selection and genetics of salt tolerance. p. 231–254. In: Salinity tolerance in Plants. Staples R.C. Toenniessen (eds.) John Wiley & Sons, New York.

Vieccelli, J., L. Araujo, U. Lopes, D. Siqueira, F. Rodrigues, J. Viecelli, and F. Rodrigues. 2017. Development of mango wilt in mango cultivars submitted to salt stress. Bragantia, 76(3):372–377.

Zuazo, V., A. Raya, and J. Ruiz. 2004. Impact of salinity on the fruit yield of mango (*Mangifera indica* L. cv. 'Osteen'). European J. Agronomy. 21(3):323–334.

Table 2. Averages of the effects of salinity on plant development, pH, and electrical conductivity.

NaCl conc.	Stem diam.	# Leaves	Plant hgt.	# Burned leaves	pH	EC
10 mM	1.4 (± 0.17)	60.14 (± 6.7)	111.34 (± 4.77)	16.52 (± 7.11)	8.34 (± 0.21)	0.99 (± 0.42)
20 mM	1.58 (± 0.21)	76.08 (± 11.7)	119.11 (± 6.19)	16.57 (± 7.6)	8.34 (± 0.29)	1.14 (± 0.49)
40 mM	1.44 (± 0.2)	64.62 (± 6.43)	106.23 (± 5.73)	17.79 (± 7.55)	8.08 (± 0.14)	1.50 (± 0.8)
80 mM	1.38 (± 0.19)	67.89 (± 11.1)	111.57 (± 6.5)	12.74 (± 5.86)	7.99 (± 0.14)	1.52 (± 0.88)
Control	1.51 (± 0.18)	68.77(± 10.27)	16.21 (± 7.99)	15.08 (± 6.65)	8.00 (± 0.27)	1.08 (± 0.44)

Proc. Fla. State Hort. Soc. 132:17–18. 2019.

Genomics-based Diversity of *Vanilla planifolia* and Native Florida Vanilla Species

Alan Chambers*[1], Ying Hu[2], Marcio F.R. Resende Jr.[2], Aureliano Bombarely[3], Maria Brym[1], and Elias Bassil[1]

[1]*University of Florida/IFAS, Tropical REC, 18905 SW 280th Street, Homestead, FL 33031*

[2]*Horticultural Sciences, University of Florida/IFAS, P.O. Box 110690, Gainesville, FL 32611-0690*

[3]*School of Plant and Environmental Sciences, Virginia Tech, Smyth Hall MC0404, 185 Ag-Quad Ln, Blacksburg, VA 24061*

Additional index words. *Vanilla planifolia*, native species, diversity, GBS

Vanilla extract has near universal appeal around the world. Understanding the diversity of vanilla can help identify superior accessions for trailing in the United States. Additionally, our native, endangered vanilla species are aided by diversity analysis and conservation research. We have developed a *V. planifolia* draft genome and conducted genotyping-by-sequencing to improve genetic resolution among a collection of 112 accessions. The results show separation among species and identify previously unknown hybrids. Future work will investigate extract quality from many of these accessions.

Global demand for all-natural vanilla flavor outpaces supply, but its botanical source, *Vanilla planifolia*, faces critical challenges arising from a narrow germplasm base. Current morphological and molecular methods to identify diversity in *Vanilla* are severely limited. Genomic tools are the key to uncover diversity and enable advanced genetics and plant breeding for new cultivars with improved yield and quality. The objective of this work was to establish the genomic resources needed to facilitate analysis of diversity among *Vanilla* accessions and to provide a resource to analyze other *Vanilla* collections. A *V. planifolia* draft genome was assembled and used to identify over 500,000 single nucleotide polymorphism (SNP) markers using Genotyping-By-Sequencing (GBS). The draft genome had an estimated size of 2.40 Gb. A filtered set of 5,082 SNPs was used to genotype a living collection of 112 *Vanilla* accessions from 23 species including native Florida species. The native Florida species included *V. barbellata*, *V. dilloniana*, *V. phaeantha*, and *V. mexicana*. All native *Vanilla* species are endangered, and this research can support conservation in the future. Principal component analysis of all accessions revealed putative hybrids, misidentified accessions, significant diversity within *V. planifolia*, and evidence for 12 clusters that separate accessions by species. These results validate the efficiency of genomics-based tools to characterize and identify genetic diversity in *Vanilla* and provide a significant tool for genomics-assisted plant breeding supporting the establishment of a domestic *Vanilla* industry.

Materials and Methods

DNA extraction, genome assembly, SNP calling, and analysis. All methods and analyses are described in detail as previously reported (Hu et al., 2019).

*Corresponding author. Email: ac@ufl.edu

Results

The 112 accessions were successfully genotyped and used to accurately classify the native Florida species (Fig. 1). The leafless species including *V. barbellata* and *V. dilloniana* were separated from the commercial species on the PCA plot. The native *V. phaeantha* were similar to, but distinct from, the other *V. phaeantha* accessions in the study. One *V. phaeantha* collected from preservation lands was identified as a hybrid with *V. pompona*. *V. mexicana* was the most distantly related species among all tested, and is consistent with the results from other studies. (Fig. 2).

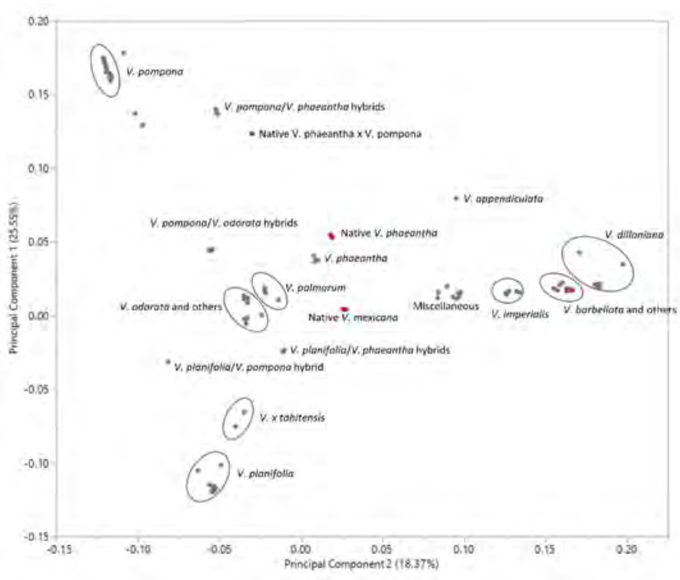

Fig. 1. PCA plot of the 112 accessions in this study with each represented by a single point. Putative species groupings are show with gray ovals. The native Florida species are shown as red dots.

Fig. 2. Pictures of *Vanilla* flowers in southern Florida. *V. planifolia* (top left), *V. pompona* (top middle), *V. barbellata* (top right), *V. dilloniana* (bottom left), *V. phaeantha* (bottom middle), and *V. mexicana* (bottom right) are shown.

Conclusions

New tools for *Vanilla* are making genomics based diversity analysis and species identification more robust. The identification of hybrids (~19% of accessions tested) suggests that gene flow among species may be common, or that remnants from historical *Vanilla* breeding programs are being maintained in collections. The identification of a *V. phaeantha* × *V. pompona* hybrid in preserve land suggests that this accession is either an escape from an intentional hybrid, or a chance seedling that arose from a natural, perhaps orchid bee facilitated, pollination between the commonly grown *V. pompona* and the native species. Future work includes the analysis of the expanded *Vanilla* collection and analysis of vanilla extract quality from diverse accessions.

Literature Cited

Hu, Y., M. Resende, A. Bombarely, M. Brym, E. Bassil, and A. Chambers. 2019. Genomics-based diversity analysis of *Vanilla* species using a *Vanilla planifolia* draft genome and Genotyping-By-Sequencing. Sci. Rpts. 9:3416.

Proc. Fla. State Hort. Soc. 132:19–23. 2019.

Efficacy and Phytotoxicity of Glufosinate and Glyphosate on Weeds Under Banana, Guava, and Lychee Grove Conditions at the Tropical Research and Education Center

Jonathan H. Crane*[1], Rebecca Tanenbaum[1], Adrian Casanola[1], Cliff Martin[1], James Colee[2], and Zachary Brym[1]

[1]*University of Florida/IFAS, Tropical Research and Education Center, 18905 SW 280 St., Homestead, FL 33031*

[2]*IFAS Statistical Consulting Unit, University of Florida/IFAS, P.O. Box 110339, Gainesville, FL 32611*

Additional index words. weed control, herbicide efficacy, *Musa* spp., *Psidium guajava, Litchi chinensis*

Weed pressure under south Florida's warm, marine subtropical climate is intense, especially during the summer months when high temperatures and frequent rainfall occur. A 0.14-acre 'Grand Nain' banana, a 0.37-acre 'Barbie Pink' guava, and a 0.16-acre 'Sweet Heart' lychee grove were used to investigate the efficacy and fruit crop phytotoxicity of herbicides from 14 May through 7 Oct. 2018. Treatments included non-treated control, low-rate glufosinate plus CLING (pinene polymer, nonionic surfactant and polydimethylsioxane; Diamond R Fertilizer, Ft. Pierce, FL); high-rate glufosinate plus CLING; low-rate glufosinate + glyphosate; and high-rate glufosinate + glyphosate arranged in a completely randomized design. Twenty-five different weed species were identified in these groves before the initial herbicide application. Percentages of the ground surface area covered with weeds ranged from ~20 to 100% prior to the first application. In general, all herbicide treatments significantly reduced the percentage of the ground covered with weeds compared to the non-treated control. The low- and high-rates of glufosinate and CLING and high-rate glufosinate + glyphosate performed better than the low-rate glufosinate + glyphosate treatments. There were no significant differences in crop phytotoxicity among the treatments or crops tested. Minor leaf necrosis and cupping was occasionally noted on a few lower canopy banana leaves. The percent ground covered by weeds was significantly lower for all herbicide treatments up to 58 days after the third application. However, by 86 days after the last application, the percent weed cover was similar among all treatments in the banana, lychee, and guava plantings. Treatments of glufosinate and glufosinate + glyphosate controlled 25 weed species under south Florida conditions.

Weeds compete with desirable plants for water and nutrients, often harbor insect pests and diseases, and if not controlled, may interfere with irrigation, nutrient management, and harvest practices. In addition, weeds may significantly reduce fruit crop growth and production and may increase the incidence of insect and diseases within the planting. Conversely, weeds and native plants increase native pollinator populations and have been shown to be beneficial to mango and avocado fruit set and production (Carvalheiro et al., 2010; Garibaldi, 2011; Wysoki et al., 2002).

In any pest control program, the repeated use of an herbicide with the same or similar modes of action may result in selection for resistance (Diepenbrock et al., 2019; Heap and Duke, 2017). This occurs in many tropical fruit groves in south Florida, where, for example, the noxious and invasive weed parthenium (*Parthenium hysterophorus* L.) has become glyphosate resistant (Barbier et al, 2012; Crane et al., 2006; Stamps, 2016). In addition, many minor tropical fruit crops in the United States have few herbicides registered for weed control. This potentially results in an economic disadvantage for growers.

Materials and Methods

We investigated the herbicidal efficacy and fruit crop phytotoxicity resulting from glufosinate treatments from 14 May to 7 Oct. 2018. A 0.14-acre 'Grand Nain' banana, a 0.37-acre 'Barbie Pink' guava grove, and a 0.16-acre section of a 2.5-acre lychee grove located at the Tropical Research and Education Center (TREC) in Homestead FL were used. The mean temperature at 2-ft height during this time was 79 °F (range: 66 to 92 °F). Soil temperatures averaged 83 °F, with 83% RH, 51 inches of rainfall, and mean evapotranspiration at 0.14 inches per month. Twenty-five weed species were identified in the plots before treatment applications (Table 1).

Banana plant mats were spaced 15-feet within rows by 20-ft between rows. Similarly, lychee trees were spaced 15-ft within and 25-ft between rows. Guava trees were spaced 20-ft within and between-rows. Treatments consisted of glufosinate (Rely®280, 24.5% ai, Group 10 herbicide) applied at two rates plus surfactant (CLING, pinene polymer, nonionic surfactant,

This work was partially funded by the USDA-NIFA Minor Crop Pest Management Program, Interregional Research Project #4 (IR-4), Rutgers University, New Brunswick, NJ, and the UF/IFAS Tropical Research and Education Center, Homestead, FL.

*Corresponding author. Email: jhcr@ufl.edu

Table 1. Twenty-five weed species identified in the banana, guava and lychee groves at the University of Florida/IFAS (UF/IFAS), Tropical Research and Education Center (TREC) prior to herbicide applications.

Common name	Scientific name
Green shrimp plant	*Ruellia blechum*
Spiny amaranth	*Amaranthus spinosus*
Beggarticks	*Bidens alba*
Straggler daisy	*Calyptocarpus vialis*
Parthenium	*Parthenium hysterophorus*
Spiny sowthistle	*Sonchus aper*
Asian false hawksbeard	*Youngia japonica*
Baldwin's flatsedge	*Cyperus croceus*
Cuban copperleaf	*Acalypha setosa*
Florida hammock sandmat	*Chamaesyce ophthalmica*
Fiddler's spurge	*Euphorbia heterophylla*
Trailing indigo	*Indigofera spicata*
Wild bushbean	*Macroptilium lathyroides*
Common fanpetals	*Sida ulmifolia*
Erect spiderling	*Boerhavia erecta*
Leafflower	*Phyllanthus amarus*
Southern sandbur	*Cenchrus echinatus*
Bermudagrass	*Cynodon dactylon*
Southern crabrass	*Digitaria ciliaris*
Indian goosegrass	*Elusine indica*
Feather lovegrass	*Eragrostis amabilis*
Tropical signalgrass	*Urochloa distachya*
Artillery plant	*Pilea microphylla*
Chancleta	*Hybanthus linearifolius*
Possum grape	*Cissus verticillata*

Table 3. Timing of herbicide applications with efficacy and crop damage ratings for 'Grande Nain' banana, 'Sweet Heart' lychee, and 'Barbie Pink' guava groves at TREC.

Application #	Treatment date	
1	18 May 2018	
2	15 June 2018	
3	13 July 2018	
Efficacy rating	Date data taken	Days after application
1	14 May 2018	–4[z]
2	1 June 2018	14
3	14 June 2018	27
4	30 June 2018	15
5	15 July 2018	2
6	28 July 2018	15
7	9 Aug. 2018	27
8	7 Oct. 2018	86
Crop damage	Date data taken	Days after application
1	1 June 2018	15
2	14 June 2018	28
3	30 June 2018	16
4	15 July 2018	2
5	28 July 2018	15
6	9 Aug. 2018	27
7	7 Oct. 2018	86

[z]Before the first treatment application.

and polydimethylsioxane; Diamond R Fertilizers, Ft. Pierce, FL); glufosinate plus glyphosate (Roundup Power Max, 48.8% ai, Group 9 herbicide) at two rates; and a non-treated control (Table 2). There were three tree plots per treatment; treatments were arranged in a completely randomized design in all three trials. Three herbicide applications were made (18 May, 15 June, and 13 July 2018) at 28-d intervals (Table 3). So as not to confound existing with potential new crop damage, a pre-herbicide application plant damage assessment was conducted on all fruit plants before applying any herbicides to detect existing nutrient deficiencies, plant deformities and plant damage. This helped to avoid the confusion of preexisting with potentially new crop damage from the tests

Herbicide applications were made with a backpack electric sprayer (Strom 18-volt Electric Backpack Sprayer, Green Touch Industries, Lake Park, FL) with a covered wand. Sprays were timed (3 seconds each per east and west side of each plant); they consisted of three passes using a 12-inch-wide spray swath to cover a 3-ft-wide × 7-ft-long (21 ft^2) spray area. Weeds ranged in size based on species from < 6" height to ~ 2-ft height. Herbicide applications were made on the east and west side of each banana mat. For the woody tree crops, herbicide efficacy and crop phytotoxicity (damage) evaluations were made over a 142-d period.

The percentage of weed ground cover in each plot was determined by visual estimation in each 21 ft^2 area on the east and west side of each tree four days before the first herbicide application (14 May 2018). Subsequently, herbicide efficacy was determined by estimating the percentage of live weeds (0 to 100%) found within a 21-ft^2 area on the east and west sides of each tree. Crop injury was rated as: 1) no injury; 2) minor burn, chlorosis, or cupping of leaves; 3) moderate, marginal leaf burn or necrosis; 4) severe leaf burn, chlorosis, or cupping; and 5) severe distortion or necrosis in the leaves or pseudostems. If present, any fruit damage (e.g., chlorosis, spotting, or necrosis) was also noted. Data were analyzed using ANOVA and means separated by Tukey-Kramer. Herbicide efficacy and crop phytotoxicity were determined seven times for each plant plot (Table 3).

Table 2. Herbicide treatments applied to banana, lychee, and guava plots at TREC.

Active ingredient	Brand name	Rate of products per acre	Spray volume per acre (gal)
Glufosinate plus CLING mix[z]	Rely®280 plus CLING	82 oz + 3 oz	37
Glufosinate plus CLING mix[z]	Rely®280 plus CLING	164 oz + 3 oz	37
Glufosinate plus glyphosate	Rely®280 plus Roundup Power Max®	82 oz + 74 oz	37
Glufosinate plus glyphosate	Rely®280 plus Roundup Power Max®	164 oz + 148 oz	37
Non-treated control	NA	NA	NA

[z]Includes CLING, pinene polymer, and a nonionic surfactant. Mixture formulations and polydimethylsioxane provided by Diamond R Fertilizer, Ft. Pierce, FL.

NA = not applicable

Results and Discussion

Banana. There were no significant differences in weed pressure before herbicide applications (Fig. 1); percentage ground cover by weeds ranged from 45% to 68%. There was a highly significant date-x-treatment effect with significantly less ground covered with weeds in all herbicide-treated plots compared with the non-treated control (Fig. 2). There were no significant differences in percent weed cover among herbicide treatments. Percentage of the ground covered with weeds in herbicide-treated plots ranged from < 1% to 21% depending on days after following the previous herbicide application (Fig. 3, Fig. 4). However, by 86 d after the last herbicide application, there were no significant differences among treatments, and all plots had > 73% weed ground coverage. There were no significant differences in crop damage ratings among any treatments on any date (data not shown). On four occasions within one replication of the glufosinate plus glyphosate treatments, there was some leaf cupping (rating [2]) on an emerging pseudostem ("suckers" or "pup") (Fig. 5). However, the suckers continued to develop, and the symptoms did not persist. This may have been due to slight

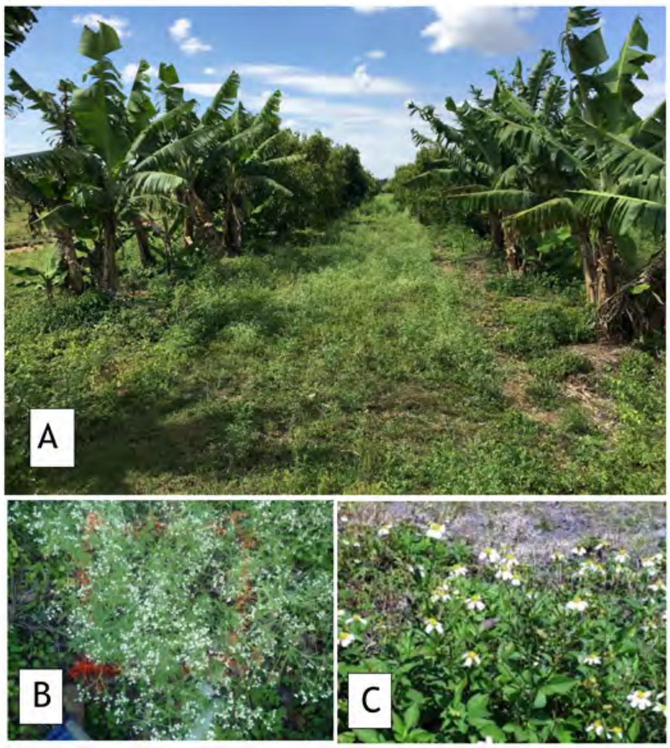

Fig. 1. (**A**) Example of weed pressure shortly before herbicide applications in the 'Grand Nain' banana planting. Two common weeds, (**B**) parthenium (*Parthenium hysterophorus*) , and (**C**) beggarticks (*Bidens alba*) [Photos: (**A** & **C**)—J. Crane, (**B**)—M. Barbier.].

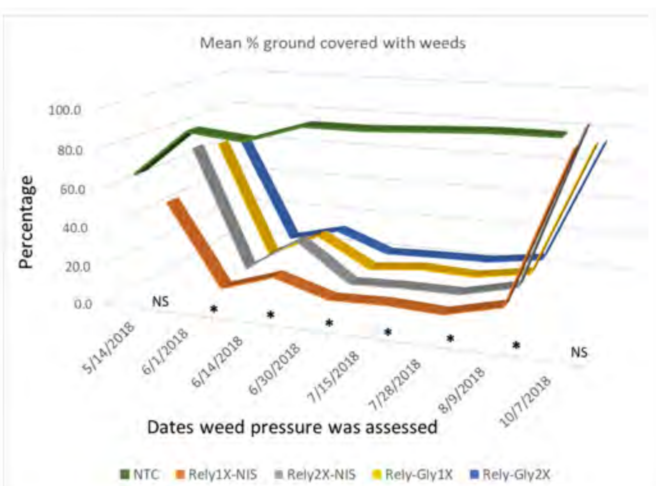

Fig. 2. The percentage of ground covered in weeds after three herbicide applications in a 'Grand Nain' banana plot at UF/IFAS TREC, Homestead, FL. Nontreated control (NTC); glufosinate at 1× rate and a non-ionic surfactant (Rely 1X-NIS)); glufosinate at 2× rate and a non-ionic surfactant (Rely 2X-NIS); glufosinate and glyphosate each at 1× rates (Rely-Gly1X); and glufosinate and glyphosate each at 2× rates (Rely-Gly2X).

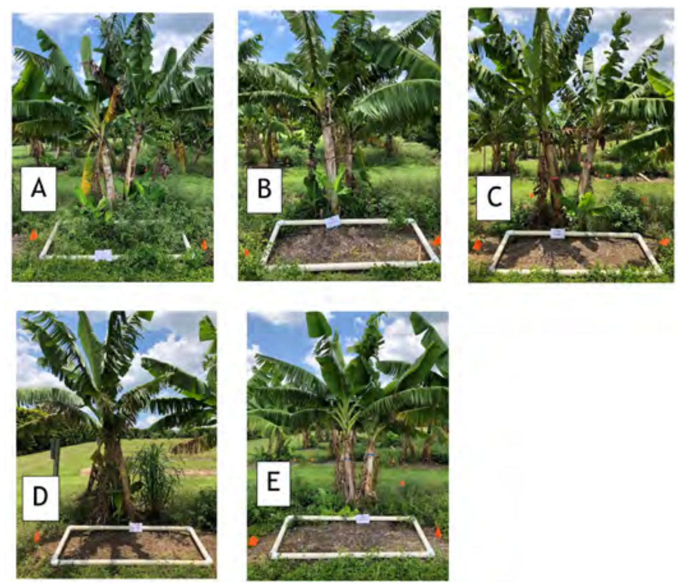

Fig. 3. 'Grand Nain' banana plots 2 d after the third herbicide application (13 July 2018). (**A**) Non-treated control; (**B**) Rely 1X; (**C**) Rely 2X; (**D**) Rely 1X + Roundup 1X; and (**E**) Rely 2X + Roundup 2X.

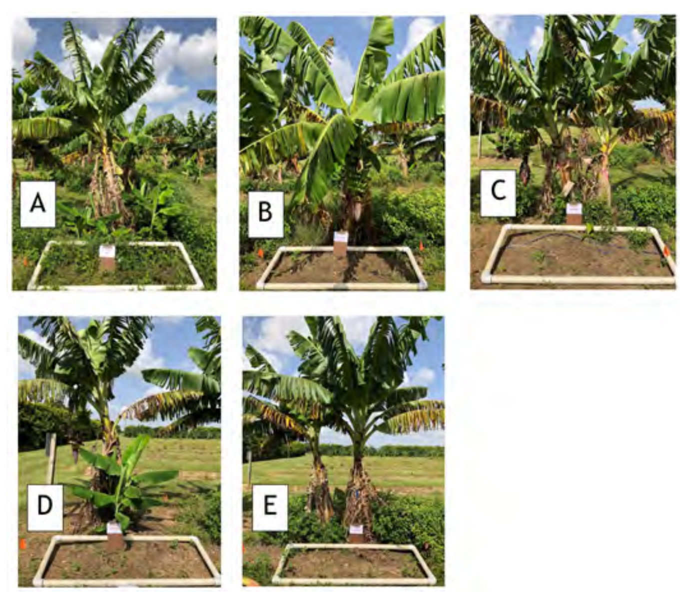

Fig. 4. 'Grand Nain' banana plots 27 d after the third herbicide application (13 July 2018). (**A**) Non-treated control; (**B**) Rely 1X; (**C**) Rely 2X; (**D**) Rely 1X + Roundup 1X; and (**E**) Rely 2X + Roundup 2X.

Fig. 5. Suckers showing mild leaf cupping and distortion on one replication of the 'Grand Nain' plots after three applications of Rely 1X + Roundup 1X (A) and Rely 2X + Roundup 2X (B). The damage was considered minor and acceptable.

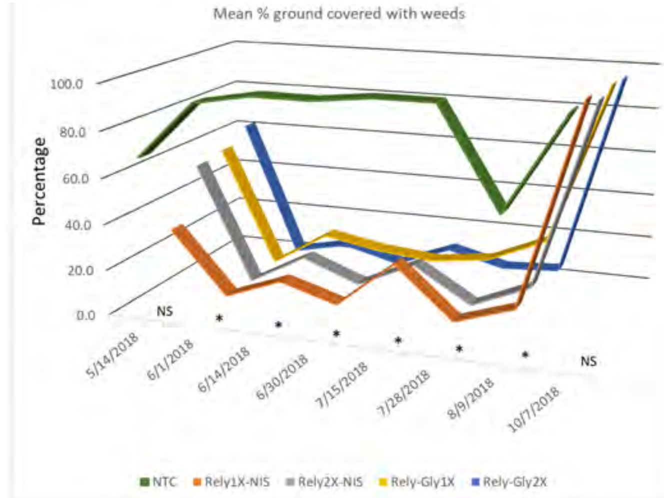

Fig. 7. The percentage of ground covered in weeds after three herbicide applications in a 'Barbie Pink' guava plot at UF/IFAS TREC, Homestead, FL. Nontreated control (NTC); glufosinate at 1× rate and a non-ionic surfactant (Rely 1X-NIS); glufosinate at 2× rate and a non-ionic surfactant (Rely 2X-NIS); glufosinate and glyphosate each at 1× rates (Rely-Gly1X); and glufosinate and glyphosate each at 2× rates (Rely-Gly2X).

exposure to the herbicides just prior to or when they first emerged from the ground as a covered boom was used to avoid spray drift.

Lychee. There was no significant difference in weed pressure before herbicide application; the weeds covered 73% to 90% of the ground. There was a highly significant date-x-treatment effect with significantly less ground coverage by weeds in all herbicided plots than in the non-treated control plots (Fig. 2). There were no significant differences in percent weed cover among herbicide treatments. However, by 86 days after the last herbicide application, there were no significant differences among treatments and all plots had complete weed ground coverage. A few leaves on some stems adjacent to the ground had some marginal leaf necrosis or leaf browning. However, this occurred in both herbicided and non-herbicided plots; thus, attributing it to herbicide exposure seems unmerited.

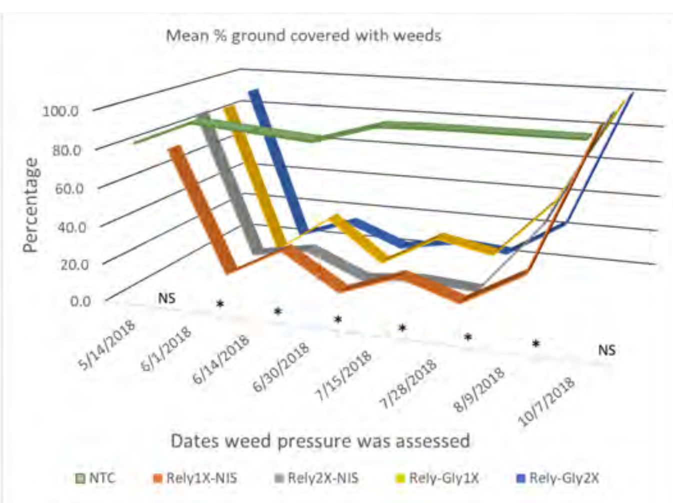

Fig. 6. The percentage of ground covered in weeds after three herbicide applications in a 'Sweet Heart' lychee plot at UF/IFAS TREC, Homestead, FL. Nontreated control (NTC); glufosinate at 1× rate and a non-ionic surfactant (Rely 1X-NIS); glufosinate at 2× rate and a non-ionic surfactant (Rely 2X-NIS); glufosinate and glyphosate each at 1× rates (Rely-Gly1X); and glufosinate and glyphosate each at 2× rates (Rely-Gly2X).

Guava. There was a significant difference in the percentage of ground covered with weeds before the first herbicide application (Fig. 7). Before applying herbicides, significantly more weed ground cover occurred in plots of the non-treated control (68%) and high-rate glufosinate plus glyphosate (62%) than in the low-rate glufosinate plots (30%). Plots that were later sprayed with high-rate glufosinate and low-rate glufosinate plus glyphosate had weed coverage intermediate to other plots (53 to 55%). However, all herbicide treated plots had significantly lower percentages of weed ground cover after the herbicide applications (Fig. 7). There were no significant differences in percent weed cover among herbicide treatments. However, 86 d after the last herbicide application, there were no significant differences among treatments and all plots had > 95% weed ground coverage. No herbicide damage occurred within the guava plots in any treatment or at any time.

Conclusions

At the low and high rates tested, glufosinate alone and glufosinate plus glyphosate successfully controlled numerous weed species adjacent to banana, lychee and guava plants under south Florida climatic conditions. To reduce the potential for the development of herbicide resistance in weeds, glufosinate alone or mixed with glyphosate should be incorporated into weed management programs for banana, lychee and guava crops in south Florida.

Literature Cited

Barbier, M., J. Crane, J. Castillo, and Y. Li. 2012. Effectiveness of flumioxazin alone and in combination with glufosinate-ammonium for control of parthenium (*Parthenium hysterophorus*) under grove conditions in Homestead, Florida. Proc. Fla. State Hort. Soc. 125:1–5.

Carvalheiro, L.G., S.L. Seymour, R. Veldtman, and S.W. Nicloson. 2010. Pollination services decline with distance from natural habitat even in biodiversity-rich areas. J. Appl. Ecol. 47:810–820.

Crane, J.H., R. Stubblefield, and C.W. Meister. 2006. Herbicide efficacy to control parthenium (*Parthenium hysterophorus*) under grove conditions in Homestead, Florida. Proc. Fla. State Hort. Soc. 119:9–12.

Diepenbrock, L.M., M.M. Dewdney, and R. Kanissery. 2019. 2019–2020 Florida production guide: Pesticide resistance and resistance management. Entomology and Nematology Dept., UF/IFAS Extension. 4 p. <https://edis.ifas.ufl.edu/cg026>.

Garibaldi, L.A., I. Steffan-Dewenter, C. Kremern, J.M. Morales, R. Bommarco, S.A. Cunningham, L.G. Carvlheiro, N.P. Chacoff, J.H. Dudenhöffer, S.S. Greenleaf, A. Hozschuh, R. Isaacs, K. Krewenka, Y. Mandelik, M.M. Mayfield, L.A. Morandin, S.G. Potts, T.H. Richetts, H. Szentgyörgyi, B.F. Viana, C. Westphal, R. Winfree, and A.M. Klein. 2011. Stability of pollination services decreases with isolation from natural areas despite honey bee visits. Ecology Letters 14:1062–1072.

Heap, I. and S.O. Duke. 2017. Overview of glyphosate-resistant weeds worldwide. Pest Mgt. Science 74:1040–1049.

Stamps, R.H. 2016. Identification, impacts and control of ragweed parthenium (*Parthenium hysterophorus* L.). Environmental Hort. Dept., UF/IFAS Extension. 10 p. <https://edis.ifas.ufl.edu/ep448>.

Wysoki, M., M.A. van de Berg, G. Ish-Am, S. Gazit, J.E. Peña, and G.K. Waite. 2002. Pests and pollinators of avocado. p. 232–293. In: (J.E. Peña, J.L. Shapr, and M. Wysoki, (eds.) Tropical Fruit Pests and Pollinators: Biology, Economic Importance, Natural Enemies and Control, CABI International, NY, NY.

Proc. Fla. State Hort. Soc. 132:24–28. 2019.

Cane Management of Floricane-fruiting Blackberries Grown in Central Florida

Shinsuke Agehara* and Syuan-You Lin

Gulf Coast Research and Education Center, University of Florida/IFAS, 14625 CR 672, Wimauma, FL 33598

Additional index words. caneberry, primocane, *Rubus* subgenus *Rubus*

Subtropical climates in Florida extend the vegetative growth period of blackberry (*Rubus* subgenus *Rubus*) by inducing early initiation of primocanes and delaying the onset of dormancy. Excessive primocane growth makes cane management laborious and time-consuming, while negatively affecting light interception, disease management, and nutrient partition. This study aimed to develop optimum cane management practices for blackberries grown under Florida's climatic conditions. We conducted a field trial in Balm, FL in the 2017–2018 season to evaluate three pruning methods using three floricane-fruiting erect-type cultivars, 'Natchez', 'Navaho', and 'Ouachita'. Tested cane management practices were: 1) summer pruning and tipping (Summer PT)—prune to five primocanes per plant in August and tip canes when they reach the top wire; 2) summer pruning without tipping (Summer P)—prune to five primocanes per plant in August, and train canes to grow downward when they reach the top wire; 3) winter pruning and tipping (Winter PT)—allow all primocanes to grow before dormancy, and prune to five primocanes per plant and tip them in late-February. Neither yield variables (fruit number, average size, and marketable yield) nor fruit quality (°Brix) were affected by cane management practices. Winter PT was not practical because of increased difficulties to manage vigorously-developed canes. Training canes to grow downward for Summer P was more labor-intensive than tipping and tying laterals for Summer PT. Therefore, we recommend Summer PT as the optimum cane management practice for blackberry production in Florida.

There is a growing demand for blackberries in recent years because of the high antioxidant contents and health benefits (Clark and Finn, 2014). Per capita consumption of caneberries has increased by 17% annually over the past decade, showing the highest growth rate among all fruits and nuts (Clark, 2019). The import of fresh blackberries increased from 43,363 tons in 2010 to 100,924 tons in 2018 (USDA, 2019). The average grower price of fresh blackberries increased from $2.47/kg in 2002 to $3.90/kg in 2017 (USDA, 2018). Although Oregon used to be the dominant blackberry producer in the U.S., blackberry production recently expanded to other states including southeastern states (Clark, 2019).

Proper cane management is critical for successful long-term production of floricane-fruiting blackberry cultivars (Pritts, 2009). In this type of cultivar, first-year canes that grow until plants enter dormancy are called primocanes, and they have only vegetative growth. After dormancy is broken, the same canes are called floricanes, as they develop flower laterals and produce berries. During the time of fruiting on floricanes, new primocanes can emerge. The coexistence of primocanes and floricanes causes the competition of resources such as water, nutrients, and sunlight (Bell et al., 1995; Bryla and Strik, 2008; Mohadjer et al., 2001; Strik and Bryla, 2015). Floricanes should be pruned once harvesting is completed.

In Florida, subtropical climates with short and warm winter extend the growth period of primocanes by inducing their early initiation and delaying the onset of dormancy. Excessive primocane growth makes cane management practices laborious and time-consuming, while negatively affecting light interception, disease management, and nutrient partition (Fernandaz and Krewer, 2008; Pritts, 2009). Despite the increased interest in commercial blackberry production in Florida, cane management recommendations under subtropical climates have not been developed.

Different cane management practices are adopted depending on cultivars and production areas. In Brazil, maintaining all primocanes in summer and pruning to four main canes per plant in winter achieved the highest yield with highest number of berries without affecting total soluble solids (TSS) content for 'BRS-Tupy' (Lugaresi et al., 2018). Tullio and Ayub (2013) found that increasing the number of main canes to maintain in winter to up to eight per plant resulted in the highest yield for 'Tupy'. In addition to main canes, number of lateral canes is also important to achieve high blackberry yields. Number of lateral canes can be controlled by the height of tipping of main canes. Himelrick et al. (2001) compared three different tipping heights (91, 122, and 152 cm) on 'Navaho', and they found that the highest yield was obtained from primocanes tipped at 122 cm.

In this study, we compared three cane management practices used in three production locations: North Carolina, Mexico, and Brazil. We evaluated cane growth, yield, berry size, and TSS content of three erect floricane-fruiting cultivars (Natchez, Navaho, and Ouachita) grown using the three cane management practices. Our goal is to develop optimum cane management practices for blackberry production in Florida.

*Corresponding author. Email: sagehara@ufl.edu

Materials and Methods

Plant materials. Three erect floricane-fruiting cultivars, Natchez, Navaho, and Ouachita, were used as experimental materials. Each plant was grown in a 189-L black plastic pot (60-cm-diameter with 30-cm-height). The pots were spaced 0.9 m within a row and 1.8 m between rows under a single-bay plastic-covered high tunnel (45 m in length × 7.8 m in width × 4.5 m in height). Sidewall curtains were open at 1.5 m height to facilitate cross-ventilation. A 1.5-m tall T-trellis system made with pressure-treated lumber and five metal wires were used for trellising blackberry canes. Standard production practices recommended for southeast bramble production guide were applied (Fernandaz and Krewer, 2008).

Experimental site. This study was conducted at the Gulf Coast Research and Education Center, University of Florida in Balm, FL during the 2017–18 season. The accumulation of chill hour (air temperature < 7.2 °C) was 254 h during the winter.

Cane management. All canes were pruned to the base on 18 July 2017 to induce new primocane growth after the end of the 2016–17 harvest season. Tested cane management practices were: 1) summer pruning and tipping (Summer PT)—prune to five main canes per plant in August and tip canes when they reach the top wire; 2) summer pruning without tipping (Summer P)—prune to five main canes per plant in August, and train canes to grow downward when they reach the top wire; 3) winter pruning and tipping (Winter PT)—allow all main canes to grow before dormancy, and prune to five main canes per plant and tip them in late-February. Summer PT, Summer P, and Winter PT were methods used in North Carolina, Mexico, and Brazil, respectively, with some modifications. Pruning for Summer PT, Summer P, and Winter PT was performed on 11 Aug. 2017, 23 Aug. 2017, and 26 Feb. 2018, respectively. Tipping for Summer PT and Winter PT was performed when pruning was done.

Cane growth. The number of main canes and lateral canes were recorded on 26 Sept. and 18 Oct. 2017, respectively. The maximum lateral cane length was measured from the bottom of primocanes to the tip by a tape measure.

Yield Data Collection. All plots were harvested weekly from 4 May to 2 July 2018. Berries were graded based on USDA standard (USDA, 1997). Berries were classified as large berries when weighing ≥ 5 g and small berries when weighing < 5 g. Marketable yield included both large and small berries, which external quality met the requirements for the U.S. No. 1 grade. Unmarketable berries included misshapen, overripe, and insect damaged berries. Number and fresh weight were recorded for each category of berries.

Berry size and Brix. At each harvest, the four largest berries per plot were sampled to measure the length and width of a berry by a digital caliper. The total soluble solids content were measured by using a digital refractometer (PAL-1; ATAGO, Tokyo, Japan).

Experimental design. There were four replicate plots for each treatment arranged in a split-plot design with cultivar as a main plot and cane management practice as a subplot. Each replicate plot consisted of 2 potted plants.

Statistical analysis. All data were analyzed by using generalized linear mixed model procedures (PROC GLIMMIX) in SAS (version 9.4; SAS Institute Inc., Cary, NC), in which cultivars and cane management practices and their interaction were considered as fixed effect, while the experimental repeats and the interaction effects between experimental repeat and cultivar were considered as random effect. The data of marketable yield, berry size and Brix, and maximum length of canes were modeled with a lognormal distribution with the option ddfm = kr. The count data, cane, and berry number, were modeled with Poisson and a negative binomial distribution, respectively, with Gauss-Hermite quadrature rule (method = quad). Means modeled with a lognormal distribution were back-transformed by using the Delta rule; while means modeled with a Poisson and a negative binomial distribution were rescaled to their equivalence on the data scale by using the inverse link option (ilink) in the LSMEANS statement. All data are reported on the data scale in the tables and figures. Least square means comparisons were performed using the Tukey–Kramer test. Unless otherwise noted, P values < 0.05 were considered statistically significant.

Results

Cane growth. Cane growth data presented in Table 1 and discussed below were pooled by each main effect, as they were not significantly affected by the cultivar × cane management interaction. Number of main canes per plant during the peak vegetative growth was significantly affected by both cultivars and cane management practices. 'Ouachita' produced 103% (6.7 vs. 13.6) and 46% (9.3 vs. 13.6) more main canes than 'Natchez' and 'Navaho', respectively, although only the difference between 'Ouachita' and 'Natchez' was statistically significant. Winter PT, in which pruning was not yet performed prior to the measurement, had 13.4 main canes per plant, which was 52% to 81% more canes than Summer PT (7.4 canes/plant) and Summer P (8.8 canes/plant). Contrasting results were observed in lateral cane number. 'Ouachita' produced 63% (17.2 vs. 6.3 canes/plant) and 46% (11.7 vs. 6.3 canes/plant) fewer lateral canes than 'Natchez' and 'Navaho', respectively. Winter PT (7.8 canes/plant) had 36% to 41% fewer lateral canes per plant than Summer PT (13.3 canes/plant) and Summer P (12.2 canes/plant), although these differences were not statistically significant. The maximum lateral cane length showed somewhat similar cultivar and cane management effects as lateral cane number. 'Ouachita' and 'Navaho' developed 50% (108.1 vs. 54.1 cm) and 36% (108.1 vs. 68.7 cm) shorter lateral canes than 'Natchez', respectively. Winter PT (62.6 cm) had 21% to 23% shorter lateral canes than Summer PT (79.5 cm) and Summer P (80.9 cm).

Pruned main cane data for Winter PT showed significant cultivar effects (Fig. 1). Both number and dry weight of pruned main canes were greatest in 'Ouachita' (15.3 canes and 126.2 g) followed by 'Navaho' (6.5 canes and 68.2 g) and 'Natchez' (1.6 canes and 17.5 g). Differences in number and dry weight of pruned main canes among cultivars were all statistically significant.

Yield. Marketable yield and berry number data presented in Table 2 and discussed below were pooled by each main effect, as they were not significantly affected by the cultivar × cane management interaction. 'Natchez' produced 15 (117 vs. 1812 g/plant) and 11 times (159 vs. 1812 g/plant) greater marketable yield than 'Navaho' and 'Ouachita', respectively. The main effect of cane management practices on marketable yield was statistically significant only at $P < 0.1$. Summer PT and Winter PT had 40% and 34% higher marketable yield than Summer P, although these differences were not statistically significant according to the Tukey-Kramer test.

'Natchez' produced 8 times more berries than 'Navaho' (42.2 vs. 340.3 berries/plant) and 'Ouachita' (42.1 vs. 340.3 berries/

Table 1. Main cane number and lateral maximum (max.) cane length and number of blackberry grown in central Florida as affected by cultivars and cane management practices.[z]

Cultivar	Cane management[y]	Main cane number per plant[x]	Lateral cane[w] no./plant	Lateral cane[w] Max. length (cm)
Natchez		6.7 b[v]	17.2 a	108.1 a
Navaho		9.3 ab	11.7 a	68.7 b
Ouachita		13.6 a	6.3 b	54.1 b
	Summer PT	7.4 b	13.3	79.5 a
	Summer P	8.8 b	12.2	80.9 a
	Winter PT	13.4 a	7.8	62.6 b
		P value		
Cultivar		0.0014	0.0047	0.0018
Cane management		0.0003	0.1749	0.0133
Cultivar × Cane management		0.1264	0.8448	0.1232

[z]Because cultivar × cane management interaction was nonsignificant, data were pooled by each main effect.

[y]Tested cane management practices were: 1) summer pruning and tipping (Summer PT)—prune to five primocanes per plant in August and tip canes when they reach the top wire; 2) summer pruning without tipping (Summer P)—prune to five primocanes per plant in August, and train canes to grow downward when they reach the top wire; 3) winter pruning and tipping (Winter PT)—allow all primocanes to grow before dormancy, and prune to five primocanes per plant and tip them in late-February.

[x]Main cane number per plant was recorded on 26 Sept. 2017 and lateral cane maximum length and number were recorded on 18 Oct. 2017.

[w]The maximum lateral cane length was measured from the tip of the cane to the attachment point to the main cane.

[v]For each main effect, means (n = 4) in a column with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

plant), respectively. Summer PT had 13% (88.2 vs. 99.9 berries/plant) and 37% (72.9 vs. 99.9 berries/plant) more berry number than Summer P and Winter PT, respectively, although these differences were not statistically significant.

Regardless of cane management practices, average berry size was greatest for 'Natchez' (5.10–5.58 g) followed by 'Ouachita' (3.69–4.15 g) and 'Navaho' (2.82–3.28 g) (Table 2). Although the cultivar × cane management interaction was significant, mean separation by the Tukey–Kramer test did not detect any significant differences in average berry size.

Berry size and brix. Berry size and Brix data presented in Table 3 and discussed below were pooled by each main effect, as they were not significantly affected by the cultivar × cane management interaction. All data were collected on four largest

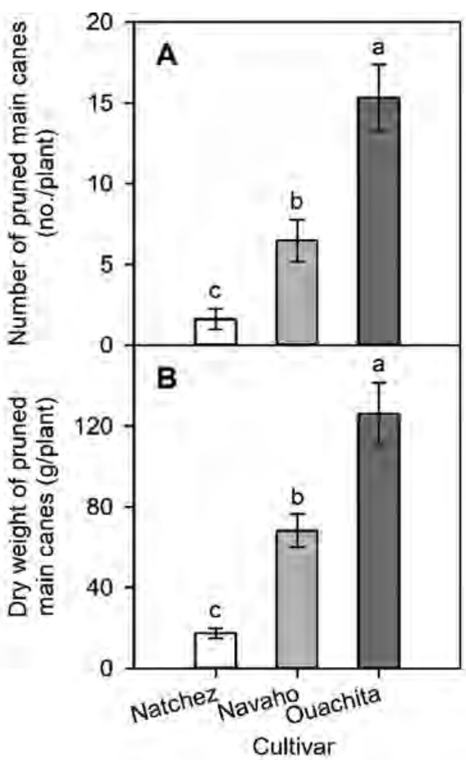

Fig. 1. Number and dry weight of pruned main canes for Winter PT of blackberry grown in central Florida as affected by cultivars and cane management practices. Means (n =4) followed by the same letter are not significantly different by Tukey–Kramer test at $P < 0.05$.

Table 2. Marketable yield, berry number, and size of blackberry grown in central Florida as affected by cultivars and cane management practices.

Cultivar	Cane management[z]	Yield (g/plant)	Berry number (no./plant)	Berry size (g)
Natchez	Summer PT	2036	366.8	5.58 a[y]
	Summer P	1565	313.7	5.10 ab
	Winter PT	1867	342.4	5.47 a
Navaho	Summer PT	127	46.3	2.82 e
	Summer P	115	42.1	3.28 de
	Winter PT	110	38.5	2.95 de
Ouachita	Summer PT	191	48.8	4.14 bc
	Summer P	100	29.4	3.69 cd
	Winter PT	212	52.1	4.15 bc
		Pooled data		
Natchez		1812 a	340.3 a	5.38
Navaho		117 b	42.2 b	3.01
Ouachita		159 b	42.1 b	3.99
	Summer PT	367	99.9	4.03
	Summer P	262	88.2	3.95
	Winter PT	352	72.9	4.06
		P value		
Cultivar		< 0.0001	< 0.0001	< 0.0001
Cane management		0.0997	0.4538	0.7060
Cultivar × Cane management		0.3652	0.6183	0.0281

[z]Tested cane management practices were as described in Table 1: 1) summer pruning and tipping (Summer PT); 2) summer pruning without tipping (Summer P); 3) winter pruning and tipping (Winter PT).

[y]Means (n = 4) in a column with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

Table 3. Berry length, width, fresh weight (FW) and total soluble solids (TSS) content of blackberry grown in central Florida as affected by cultivars and cane management practices.[z]

Cultivar	Cane management[y]	Berry length[x] (cm)	Berry width (cm)	Berry FW (g)	TSS (°Brix)
Natchez		2.84 a[x]	2.36 a	8.76 a	8.56 b
Navaho		2.14 b	1.95 b	4.49 b	12.10 a
Ouachita		2.27 b	2.07 ab	5.69 b	11.84 a
	Summer PT	2.42	2.11	5.98	10.43
	Summer P	2.40	2.11	6.10	10.88
	Winter PT	2.39	2.13	6.12	10.83
		P value			
Cultivar		0.0002	0.0015	0.0002	< 0.0001
Cane management		0.7467	0.8814	0.8907	0.6026
Cultivar × Cane management		0.5369	0.5139	0.2641	0.2374

[z]Because cultivar × cane management interaction was nonsignificant, data were pooled by each main effect. Data are the averages across harvests between 15 May and 2 July 2018.

[y]Tested cane management methods were as described in Table 1: 1) summer pruning and tipping (Summer PT); 2) summer pruning without tipping (Summer P); 3) winter pruning and tipping (Winter PT).

[x]Means (n = 4) in a column with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

berries per plot, and they were significantly affected only by the main effect of cultivars. ‘Natchez’ produced 25% to 32% longer (2.14–2.27 vs. 2.84 cm), and 14% to 21% wider (1.95–2.07 vs. 2.36 cm) berries than ‘Navaho’ and ‘Ouachita’. These differences were statistically significant, except the difference in berry width between ‘Natchez’ and ‘Ouachita’. Consequently, berry fresh weight was 54% to 95% greater in ‘Natchez’ than ‘Navaho’ and ‘Ouachita’ (4.49–5.69 vs. 8.76 g). However, TSS content of ‘Natchez’ was 28% to 29% lower (11.84–12.10 vs. 8.56 °Brix) compared to ‘Navaho’ and ‘Ouachita’.

Discussion

Floricane-fruiting blackberry cultivars have biennial canes, which are called primocanes until plants enter dormancy, but are called floricanes after dormancy is broken (Strik, 2018). The accumulation of photosynthase by primocanes is important for the subsequent fruit production by floricanes (Salgado and Clark, 2015). Proper management of primocanes is critical for successful long-term blackberry production, as it affects many aspects of crop production, including light interception, disease management, nutrient partitioning, yield, and fruit quality (Fernandaz and Krewer, 2008; Pritts, 2009). In our study, although tested cane management practices significantly altered growth of both main and lateral primocanes, they did not affect marketable yield and fruit quality regardless of cultivar. Therefore, the optimum cane management practice can be selected based on the ease of management and labor. Winter PT was not practical because of increased difficulties to manage vigorously-developed canes at the time of pruning, particularly for ‘Ouachita’. Training canes to grow downward for Summer P was more labor-intensive than tipping and tying laterals for Summer PT. Furthermore, Summer PT produced, although not statistically significant, 40% higher marketable yield than Summer P. These results suggest Summer PT is the optimum cane management practice for blackberry production in Florida.

Different cane management practices are adopted in different production areas. For example, Summer PT is the standard practice recommended for commercial blackberry production in North Carolina (Fernandez and Krewer, 2008). Summer P is one of the cane management practices used in Mexico. In Brazil, Lugaresi, et al. (2018) demonstrated that Winter PT can maximize the marketable yield of ‘BRS-Tupy’. In Florida, subtropical climates with short and warm winter extend the growth period of primocanes by inducing their early initiation and delaying the onset of dormancy. This prolonged growth period of primocanes is probably the reason why Winter PT is not suitable in Florida. Our results show that it is critical to maintain a manageable primocane density by constant pruning, especially for ‘Ouachita’.

In addition to main canes, lateral canes can also grow excessively, resulting in increased difficulties and labor to train canes. The timing of lateral cane development is controlled by the apical dominance (Takeda et al., 2003). Growers may be able to improve the efficiency of lateral cane management by delaying the timing to tip main canes. The optimum lateral number and length should be investigated in future studies.

Literature Cited

Bell, N.C., B.C. Strik, and L.W. Martin. 1995. Effect of primocane suppression date on ‘Marion’ trailing blackberry. I. Yield components. J. Amer. Soc. Hort. Sci. 120:21–24.

Bryla, D.R. and B.C. Strik. 2008. Do primocanes and floricanes compete for soil water in blackberry? Acta Hort. 777:477–482.

Clark, J.R. 2019. NARBA conference highlights strong market for berries. Growing Produce. Willoughby, OH. 17 May 2019. <https://www.growingproduce.com/fruits/berries/narba-conference-highlights-strong-market-for-berries/>.

Clark, J.R. and C.E. Finn. 2014. Blackberry cultivation in the World. Rev. Bras. Frutic. 36: 46–57.

Fernandez, G.E. and G. Krewer. 2008. 2008 Southeast bramble production guide. 28 Apr. 2015. <http://www.smallfruits.org/SmallFruitsRegGuide/%0AGuides/2008/08BrambleguideMay22.pdf>.

Himelrick, D.G., R.C. Ebel, F.M. Woods, B.S. Wilkins, and J.A. Pitts. 2001. Effect of primocane topping height and lateral length on yield of ‘Navaho’ blackberry. Small Fruits Rev. 1:95–101.

Lugaresi, A., A. Uberti, C.L. Giacobbo, M. Lovatto, M., Girardi, and A. Wagner Junior. 2018. Management of pruning and evaluation in blackberry cultivars in relation to productive characteristics and bioactive compounds. Anais da Academia Brasileira de Ciências. 90:3879–3885 (in Portuguese with English abstract).

Mohadjer, P., B.C. Strik, B.J. Zebarth, and T.L. Righetti. 2001. Nitrogen uptake, partitioning and remobilization in ‘Kotata’ blackberry in alternate-year production. J. Hort. Sci. Biotechnol. 76:700–708.

Pritts, M. 2009. Bramble pruning and trellising. New York Berry News. 8:11–15.

Salgado, A.A. and J.R. Clark. 2015. Blackberry growth cycle and new varieties from the University of Arkansas. University of Arkansas. Fayetteville, AR. 18 May 2019. <http://www.vsuag.net/wp-content/uploads/2015/03/Salgado-Virginia-2015.pdf>.

Strik, B.C. and D.R. Bryla. 2015. Uptake and partitioning of nutrients in blackberry and raspberry and evaluating plant nutrient status for accurate assessment of fertilizer requirements. HortTechnology 25:452–459.

Strik, B.C. 2018. Growth and development, p. 17–34. In: H.K Hall. and R.C. Funt (eds.). 2018. Blackberries and their hybrids. CABI, Wallingford, UK.

Takeda, F., A.K. Hummell, and D.L Peterson. 2003. Primocane growth in 'Chester Thornless' blackberry trained to the rota cross-arm trellis. HortScience: 38:373–376.

Tullio, L. and R.A. Ayub. 2013. Produção da amora-preta cv tupy, em função da intensidade da poda. Semin. Agrar. 34:1147–1152 (in Portuguese with English abstract).

U.S. Department of Agriculture. 1997. United States standards for grades of dewberries and blackberries. USDA, Agricultural Marketing Service. Washington D.C. 26 Aug. 2015. <https://www.ams.usda.gov/sites/default/files/media/DewberriesBlackberriesStandard.pdf>.

U.S. Department of Agriculture. 2018. Berries commercial acreage, yield per acre, production, and grower price. USDA, Economy Research Service. Washington D.C. 7 May 2019. <https://www.ers.usda.gov/webdocs/DataFiles/90438/FruitYearbookBerries_Ds.xlsx?v=3994.8>.

U.S. Department of Agriculture. 2019. Fruit and tree nut data . USDA, Economy Research Service. Washington D.C. 17 May 2019. <https://data.ers.usda.gov/reports.aspx?programArea=fruit&stat_year=2009&top=5&HardCopy=True&RowsPerPage=25&groupName=Noncitrus&commodityName=Blackberries&ID=17851>.

Proc. Fla. State Hort. Soc. 132:29–31. 2019.

Characterization of Papaya Accessions in South Florida

SARAH E. BREWER*AND ALAN H. CHAMBERS

University of Florida/IFAS, Tropical Research and Education Center, 18905 SW 180th St., Homestead, FL 33031

ADDITIONAL INDEX WORDS. *Carica papaya*, fruit quality, yield, germplasm evaluation

***Carica papaya* is one of many species comprising South Florida's diverse tropical fruit industry. Increasing domestic consumption, markets for both green and ripe fruits, and a short juvenile phase make papaya an attractive option for growers. To date, two of the most significant impediments to profitable papaya production in Miami-Dade are: 1) The widespread presence of papaya ringspot virus (PRSV), which can decimate yields and diminish fruit quality and 2) Competition with imported papayas, especially large 'Maradol' or 'Tainung' type fruits. The papaya breeding program at TREC aims to develop new cultivars that will address both of these issues by introgressing PRSV resistance into "solo-type" accessions, which are renowned for excellent fruit quality and that command a premium price. Toward this end, we have conducted a preliminary evaluation of twenty-one papaya accessions obtained from the USDA-ARS germplasm repository in Hilo, Hawai'i. A field trial was established at the TREC in Homestead, Florida, with traits characterized including plant height and fruit number, as well as fruit weight, brix, and acidity. We observed significant variation among accessions for many of these traits, and have utilized this data to identify promising parents for our breeding program. In the near-term, these findings may be useful to growers when deciding which accessions to plant.**

The United States is the world's leading importer of papaya, purchasing more than 200,000 tons of fruit in 2018 (USDA-ERS, 2019). Large-fruited varieties (> 1 kg) including 'Maradol' and various 'Tainung' types account for a majority of these imports. Domestic production consists mainly of pear-shaped, small-fruited varieties such as 'Rainbow', 'Kapoho', and 'Sunrise', selected and grown in the Hawaiian Islands. These "Hawaiian" or "solo-type" papayas are renowned for their excellent fruit quality, and command a higher price at retail markets. Between 2015 and 2018, solo papayas sold for an average of $1.39 per lb—more than 30% higher than the price of 'Maradol' and 'Tainung' fruits ($0.95 per lb) over the same period (USDA–AMS, 2019). Papayas are grown on approximately 300 acres in South Florida. Growers in this region primarily produce large papayas, and face steep competition from imports. Papaya ringspot virus (PRSV) also limits the productive life of papaya plantings thus increasing costs. Researchers at the Tropical Research and Education Center (TREC) in Homestead, FL, have developed PRSV-resistant lines, which should be available to growers in the near future. However, none of the forthcoming varieties may be categorized as solo-types. Development of additional varieties combining strong agronomic performance, PRSV resistance, and solo-type fruit quality is the current focus of the papaya breeding program at TREC. In order to identify promising accessions for use in the breeding program and, possibly, cultivation by South Florida growers or home gardeners, we evaluated twenty-one papaya accessions for traits including fruit number, fruit weight, plant height, acidity, and brix.

Materials and Methods

TRIAL ESTABLISHMENT AND MAINTENANCE. Seeds of all available *Carica papaya* accessions were obtained from USDA–ARS-Tropical Plant Genetic Resources and Disease Research (TPGRDR) in Hilo, HI. In April 2017, seeds were prepared for germination by soaking in 0.5 M potassium nitrate for 24 hours prior to planting in soilless mix. Seedlings were genotyped to favor hermaphrodites as described by Liao et al. (2017), and transplanted in the field in July. The field plot consisted of 5 plants per accession × 21 accessions arranged in raised rows covered with plastic mulch. Spacing was 5 ft within row, 6 ft between rows. Plants were fertigated daily via drip tape with 3–0–10 fertilizer at a rate of 1 lb nitrogen per acre per day. Botanigard and Keyplex 350 were applied as needed.

FIELD DATA. Plant height to first fruit was recorded and defined as the distance in cm from the ground to the top of the peduncle attaching the first harvestable fruit (peel beginning to yellow). Fruits were harvested at the color-break stage on a bi-weekly basis over a six-month period (June–Nov. 2018). The number of fruit from each plant was recorded. Yield was estimated by multiplying the number of fruit per plant by the average fruit weight of the accession.

FRUIT QUALITY DATA. For each accession, the first three plants to bear fruit were selected for fruit quality analysis. After harvest, five fruit per plant were weighed then allowed to ripen at room temperature (~75 °F) until the peels were fully yellow. Approximately 50 g mesocarp tissue was saved from each fruit and stored at –20 °C for later processing. At the conclusion of the field trial, all fruit samples from the same plant were pooled and homogenized. Clarified juice samples were obtained by centrifuging 40 g aliquots of fruit puree for 15 min. at 12,100 × g. Brix and titratable acidity (TA) were assessed with two technical replicates per sample. Brix was measured using an Atago PAL-1 pocket refractometer. TA samples were prepared by adding 50 mL distilled water to 10 g papaya juice. End-point titration to a pH of 8.1 was performed using a Mettler Toledo automated titrator with titrant 0.02 M NaOH.

Results

YIELD. Estimated yield per plant in kilograms ranged from 4.14 ('Kapoho 2') to 39.59 (N07-23). Six yellow-fleshed solo-

*Corresponding author. Email: sarah.brewer@ufl.edu

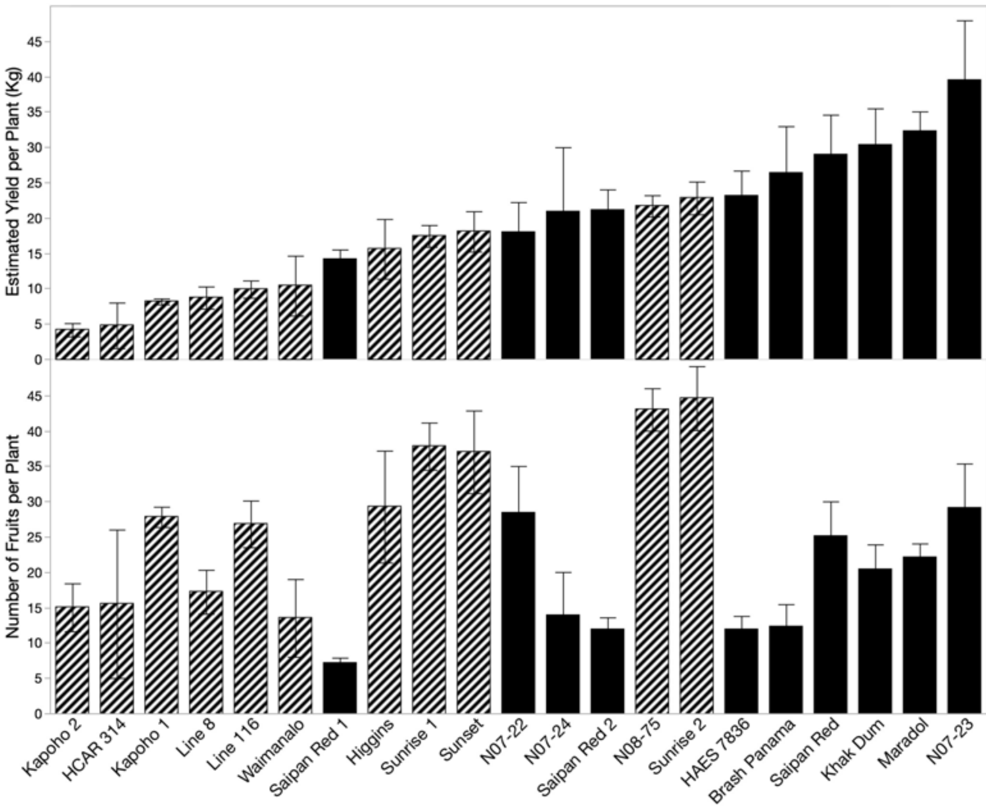

Fig. 1. Per-plant yield by accession from June 2018 to Nov. 2018. Top: Estimated kg fruit per plant. Bottom: Number of fruit per plant. Solo-type fruits denoted by striped bars. Error bars represent standard error.

type accessions were the lowest yielding. The four accessions yielding the highest number of fruits were red-fleshed solo-types: 'Sunrise 2', N08-75, 'Sunset', and 'Sunrise 1' (Fig. 1).

Plant height. The height at which the first fruits were borne was measured for each accession. The eight tallest accessions were solo-types: 'Kapoho 1', 'Sunrise 1', 'Sunrise 2', 'Kapoho 2', 'Sunset', Line 116, HCAR 314, and Line 8; all of which yielded their first fruit more than 110 cm from the ground. 'Saipan Red', a large-fruited accession from the Mariana Islands, had the lowest bearing height at 78 cm (Fig. 2).

Fruit quality. A wide range of average fruit sizes was observed: 276 g ('Kapoho 2') to 2.1 kg ('Brash Panama'). Total

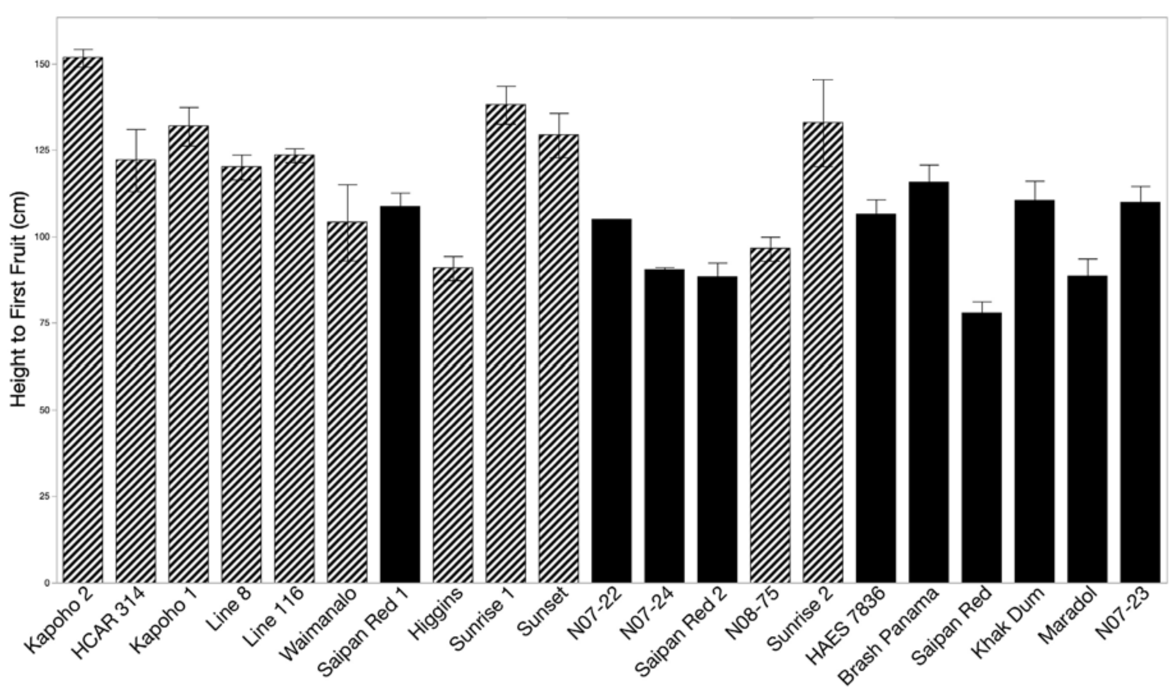

Fig. 2. Height to first fruit by accession. Solo-type fruits denoted by striped bars. Error bars represent standard error.

Table 1. Fruit quality parameters by accession (standard error in parentheses).

Accession	HCAR ID[z]	Color	Hermaphrodite or female	Average fruit wt (g)	% Citric acid	% TSS[y]
Brash Panama	14	Yellow	Hermaphrodite	2133.54 (156.80)	0.1097 (0.0055)	10.82 (0.14)
HAES 7836	16	Yellow	Hermaphrodite	1934.79 (236.35)	0.1174 (0.0116)	11.72 (0.27)
HCAR 314	314	Yellow	Hermaphrodite	307.20 (21.73)	0.1647 (0.0214)	14.50 (1.00)
Higgins	196	Yellow	Female	533.00 (93.82)	0.1528 (0.0129)	12.60 (0.41)
Kapoho 1	158	Yellow	Hermaphrodite	293.33 (25.24)	0.1318 (0.0076)	14.20 (0.20)
Kapoho 2	27	Yellow	Hermaphrodite	276.00 (17.55)	0.1118 (0.0051)	12.57 (0.39)
Line 8	302	Yellow	Hermaphrodite	505.44 (54.06)	0.1294 (0.0152)	10.62 (0.94)
Line 116	11	Yellow	Hermaphrodite	369.00 (25.72)	0.1336 (0.0045)	14.28 (0.26)
Maradol	313	Red	Hermaphrodite	1457.93 (162.11)	0.1349 (0.0104)	9.80 (0.40)
N07-22	320	Yellow	Female	634.33 (112.03)	0.2293 (NA)	12.65 (1.20)
N07-23	321	Yellow	Hermaphrodite	1355.73 (126.56)	0.1940 (0.0024)	11.35 (0.33)
N07-24	322	Red	Hermaphrodite	1498.67 (160.19)	0.1636 (NA)	9.38 (0.13)
N08-75	323	Red	Hermaphrodite	503.80 (44.34)	0.1207 (0.0057)	11.25 (0.03)
Saipan Red	17	Red	Hermaphrodite	1152.71 (98.01)	0.1006 (0.0035)	8.85 (0.98)
Saipan Red 1	319	Red	Hermaphrodite	1966.67 (88.66)	0.1369 (0.0104)	10.77 (0.37)
Saipan Red 2	318	Red	Hermaphrodite	1767.27 (113.25)	0.1332 (0.0147)	10.50 (0.81)
Sunrise 1	312	Red	Hermaphrodite	460.73 (47.10)	0.1370 (0.0043)	12.55 (0.41)
Sunrise 2	315	Red	Hermaphrodite	511.13 (36.37)	0.1390 (0.0053)	12.32 (0.06)
Sunset	311	Red	Hermaphrodite	487.93 (41.62)	0.1492 (0.0152)	12.13 (0.73)
Waimanalo	310	Yellow	Hermaphrodite	769.33 (139.81)	0.1295 (0.0003)	9.95 (0.55)

[z]HCAR ID represents a unique accession identifier assigned by the USDA-ARS germplasm repository in Hilo, HI.

[y]TSS = total soluble solids.

NA = not applicable.

soluble solids (TSS) content for all accessions was above 8%. The yellow-fleshed solo-types HCAR 314, Line 116, and 'Kapoho 1' had the highest % TSS, above 14%. Titratable acidity for all accessions was less than 0.25% citric acid (Table 1).

Conclusions

We observed substantial phenotypic variation among the twenty-one papaya accessions included in this study. In general, plants which are low-bearing and high-yielding, with fruits high in TSS are desirable for the dessert fruit market. Solo-type fruits which are small, pear-shaped, and sweet with a pleasant, musk-free aroma may represent an under-exploited niche for South Florida growers. Among these, N08-75 stood out due to its combination of short stature and higher than average yield for a solo-type accession. 'Sunrise 2', the globally popular solo-type variety, boasted the highest small-fruited yield, in combination with TSS exceeding 12%. Under-performing accessions were also identified, such as the large-fruited 'Khak Dum', a Thai variety. Despite bearing many kilograms of fruit, each succumbed to decay before ripening. Preliminary results from this study were used to select several accessions with good agronomic performance and diverse fruit quality traits for further characterization in a replicated, multi-site field trial, and for use in ongoing plant breeding efforts at the TREC.

Literature Cited

Liao, Z., Q. Yu, and R. Ming. 2017. Development of male-specific markers and identification of sex reversal mutants in papaya. Euphytica, 213(2):53.

USDA-AMS. 2019. Specialty Crop News Custom Average Tool. U.S. Department of Agriculture–Agricultural Marketing Service, Washington, D.C. 7 June 2019. <https://cat.ams.usda.gov>.

USDA-ERS. 2019. Fruit and Tree Nuts Data by Commodity, Papaya. U.S. Department of Agriculture–Economic Research Service, Washington, D.C. 7 June 2019. <https://data.ers.usda.gov/reports.aspx?programArea=fruit&stat_year=2009&top=5&HardCopy=True&RowsPerPage=25&groupName=Noncitrus&commodityName=Papayas&ID=17851#P4969fb6a6b504f67bd32621cb0755026_3_292>.

Proc. Fla. State Hort. Soc. 132:32–36. 2019.

Growing Bananas in the Everglades Agricultural Area

Nicholas A. Larsen*

NK Lago Farms, PO Box 983, Canal Point, FL 33438

Additional index words. plantains, histosols

In 2009, bananas and plantains were planted on histosols and mineral soils in the Everglades Agricultural Area (EAA). Thirty-five cultivars have been planted for evaluation over the past decade. Observations on yield, economics, mat-lifespan, pest resistance, and required horticultural practices have been made. Observations on plant nutrition, weed control, and pest control are discussed. Three cultivars are recommended for growing in the EAA—FHIA-1, Dwarf Puerto Rican Plantain, and Dwarf Nam Wah.

Bananas, (*Musa spp*.), are an important crop in Florida not only for their fruit production, but also as landscape plants. According to the most recent Census of Agriculture (2017), Commercial banana farms currently occupy over 385 ha in the state, primarily in Miami-Dade County. Approximately 60 ha of that total is in Palm Beach County, with about a third in the Everglades Agricultural Area (EAA). Bananas have been part of the cropping mix in the EAA since the area was settled. Photos of bananas being grown in the EAA during the 1920s and 1930s exist in the State Library and Archives of Florida (Snyder, 2004). Despite the long history, the crop has never been a major crop in the area and very little research has been done on the cultivation of bananas in the area. This paper will summarize observations that have been made by the author during his ten years of cultivating bananas and plantains in the EAA. Observations on land preparation, choice of propagation material, fertility management, water management, cultivar selection, pest control, harvesting and handling, and economics. While none of these observations were the result of a controlled experiment, the observations will provide information to future researchers and other growers.

The Everglades Agricultural Area

The EAA is a 280,000-ha region in South Central Florida that was developed for agricultural use. The unique feature of the area is the high organic matter histosols, which are some of the world's most productive soils (Snyder, 2005).

Lake Okeechobee modifies the local climate. A northwest wind during the winter must travel approximately 40 km over the relatively warm lake before it hits the southeastern shore. Temperatures below freezing within a mile of the lake are rare. Temperatures in Canal Point (0.2 km from the lakeshore) are sometimes 5 °C warmer than in Belle Glade (14k m from the lakeshore) during cold winter nights. An example of this occurred during a cold weather event on 15 Dec. 2010, when it was –2.8 °C in Belle Glade, but 1.7 °C in Canal Point. As bananas and plantains require year-round above freezing temperatures, the area near the lake provides adequate frost and freeze protection. (Table 1)

The histosols near the lake are very productive. The dominant soil type is Torry Muck (Euic, hyperthermic Typic Haplosaprists), which has a mineral composition of 40 to 70%, is rich in nitrogen and basic cations and has a pH from 6.5 to 7.5. The soils can be variable in terms of fertility and water holding capacity but are generally suitable for cultivating bananas and plantains. The results reported in Table 2 respresent a 1600-m transect 125-m inland, parallel to the shore of Lake Okeechobee near Canal Point, FL.

Propagation Material

There are three primary methods of obtaining material for planting bananas and plantains—tissue culture, micropropagation, and off-shoots called suckers. Each type has merits and limitations. Published literature and university extension recommendations recommend using tissue cultured plants and have documented increased yield, increased crop uniformity, and fewer problems with disease (Fonsah et al., 2007). The author started his farm using tissue culture plants, but found some issues: not every variety is available and sometimes the quantity available is insufficient; plants must be hardened and grown out prior to field planting, which requires infrastructure; and plants tend to produce off-types. The author has had particular problems with off-types for 'Hua Moa', 'Dwarf Nam Wah', and 'Dwarf Puerto Rican Plantain'. Due their small size at field planting (30–50 cm), weed control can be difficult.

Macropropagation can be very useful for multiplying cultivars that are either not available as tissue culture plants or are not available in sufficient quantity. Using this method, plants are dug out of the field, the pseudostems removed, roots removed, corms cleaned and cut up into "bits" with at least one growing point, treated with hot (43.3 °C) water to control nematodes and insects, and then planted (Gettman et al., 1992). Planting can then be made into containers or into a field nursery. For a field nursery, it is important to choose a site that will not re-infest the plants with disease, nematodes, and insects. Bits can be planted into the ground on a 1 m × 0.5 m spacing, allowed to sprout and grow for a few months, dug up and replanted elsewhere. Flaws in the micropropagation method include the high requirement for labor and the inability to completely eliminate insect and nematode problems. As the plants tend to be bigger when planted to the field, weed control is marginally easier than with tissue culture plants.

*Corresponding author. Email: nklagofarms@aol.com

Table 1. Monthly average temperatures, minimum temperatures, and maximum temperatures measured at the USDA/ARS Sugarcane Field Station, Canal Point, FL 1997–2017.

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Min.	40.1	42.8	49.9	53.7	61.2	67.8	70.5	71.3	70.0	61.0	51.4	46.1
Max.	84.5	85.2	88.1	91.4	93.4	94.7	95.5	95.4	93.8	91.1	87.1	85.9
Mean	63.5	65.9	69.8	73.9	77.8	81.2	82.3	82.6	81.2	77.3	70.7	67.1

Using suckers to plant is by far the quickest and easiest method of propagating bananas and plantains. It is also the riskiest method as there are no controls for insects, nematodes, and diseases. Sword suckers can be selected from healthy plants dug up and replanted. One way to reduce the risk is to use designated nursery blocks where the mother plants are derived from tissue-culture and managed to limit disease and insect infestation. Since suckers tend to be tall, and grow quickly, weed control is easiest when using suckers. Chemical weed control is more easily directed so that it does not drift onto the plants and the relatively quick growth starts to shade out vegetation sooner.

Land Preparation and Planting

Fields in the EAA tend to have a water management infrastructure in place. Water levels in the ditches can be modified either by allowing water into the ditches or pumping it out. Some banana plantings have wells that can be used for surface irrigation. Bananas require about 2.5–4 cm of water per week (Crane and Balerdi, 2016). Given the normal rain fall pattern in the EAA, supplemental irrigation should be supplied from Mid-October to June.

The type of planting pattern is another decision. The literature shows maximum yields and greater net returns with relatively high densities. Cavendish bananas should be planted at 1666 plants/ha (Robinson and Nel, 1989) and dwarf plantains at 3500 plants/ha (Irizarry et al., 1981). The most common spacing in the EAA is 3.8 m × 3.8 m, which allows for a more relaxed strategy for managing the number of suckers in a mat and more easily using a mower and tractor in the middles. At a relatively low density, the individual bunches tend to be heavier. There may be 2–3 bunches at the same time on a mat. For high density plantings, the author has employed the double line system. For dwarf plantains there is a double row with rows 1.5 m apart and with a 3 m wide road between double roads. Plants are 1.5 m apart in the row and staggered so that a plant in one row is between two plants in the next row. For bananas the set-up is the same except with 2.1 m between plants in a row.

The final step is whether to prepare a clean planting area through cultivation or to plant no-till; the former is easiest, but it quickly becomes infested with weeds. For a high-density double row system, a furrowing rig is advisable to use since it will facilitate the planting process. When using small tissue culture plants and a clean planting bed, weed management is critical and can be difficult due to the limited number of herbicides available for bananas in the United States.

Planting in a no-till situation is the author's preferred method. First a roughly 2-m wide band is killed with herbicides and then the plants are planted into the ground. The alleys between the killed areas are maintained by mowing and the bands are maintained with herbicides. The grassed alleys provide a more stable surface for driving equipment and maintaining the 2-m killed band is more economical than trying to keep the whole area free of weeds. The decreased growth of weeds is especially noteworthy when growing taller cultivars such as 'Giant Plantain' or 'Kandrian'.

Cultivar Selection

The author has grown and evaluated 35 cultivars from 2009-2019 (Table 3). Each cultivar has its own merits, but the ultimate goal was to find cultivars that are easy to manage, has few pests, and are profitable.

The highest observed yields were from 'FHIA-1' ('Goldfinger'), 'FHIA-17', and 'Williams'. Bunches of these varieties frequently exceed 18 kg. The author has observed bunches of FHIA-17 in excess of 40 kg with 17 hands of fruit.

For easy management, 'Giant Plantain', 'Dwarf Puerto Rican Plantain', and 'Orinoco' are the least demanding in terms of time and effort required for marketable fruit. 'Njalipoovan', 'Dwarf Namwah' and 'Williams' require extensive management efforts. 'Njalipoovan' and 'Dwarf Namwah' produces prodigious amounts of suckers that have to be destroyed. 'Dwarf Namwah' and 'Williams' require removal of the male flower and floral remnants and bunch pruning to properly fill out.

'Hua Moa', 'FHIA-17', and 'African Rhino Horn' have prob-

Table 2. Soil test results for NK Lago Farms Canal Point, FL.[z,y]

Site	CEC	pH	P	K	Ca	Mg	S	B	Zn	Fe	Mn
1	25.96	7.32	216.2	84.6	8980.3	661.8	47.3	7.0	30.3	186.2	40.2
2	19.48	7.07	169.5	110.9	6455.6	498.4	38.7	5.7	36.0	245.5	26.7
3	10.66	6.97	147.5	99.7	3428.0	297.4	43.2	4.7	21.3	219.9	25.6
4	25.55	7.22	189.9	284.2	9347.7	263.0	38.8	2.1	28.3	177.6	28.0
5	41.69	7.29	183.9	377.9	15644.1	330.6	56.0	2.1	30.6	150.8	21.9
6	5.91	6.89	100.2	87.1	1869.2	97.5	20.5	3.6	18.7	172.6	10.8
7	62.26	7.49	19.1	36.2	27113.6	284.7	32.5	0.2	12.0	69.1	7.3
8	22.62	7.42	72.9	65.2	8749.7	119.6	2.4	0.0	15.1	97.3	9.1
9	7.79	7.10	95.0	96.9	2640.8	158.1	7.6	2.6	22.5	176.3	10.3
10	6.12	6.73	128.5	167.5	1724.3	152.4	32.4	1.7	28.1	261.8	15.5

[z]Nutrient values reported as ppm.

[y]Chemical analyses performed by Waters Agricultural Laboratory, Camilla, GA.

Table 3. Cultivars grown, number of locations observed, and observations on height, yield, diseases, insects, and notes on special uses.

Cultivar	Locations observed (no.)	Height (ft)	Yield	Yield limiting diseases	Insects	Uses
Diploids (AB)						
Njalipoovan	2	12–15	Low	None Observed		
Veinte Cohol	1	7–8	Low	None Observed		Short cycle dessert banana
Dessert Bananas (AAA)						
Williams	2	8–9	High	Sigatoka		Dessert banana
Grande Naine	1	7–8	High	Sigatoka, Cigar End Rot		Dessert banana
Baloy	2	5	Medium	Sigatoka, Cigar End Rot		Dessert banana
Gros Michel	2	12–15	Medium	Panama Disease, Sigatoka		Dessert banana
Paggi	1	10–12	Medium	None Observed		Dessert banana
Morado	2	10–12	Low	None Observed		Dessert banana
Dessert Bananas (AAB)						
Manzano	1	12–15	Low	Panama disease	*Metamasius hemipterus*	Dessert banana
Mysore	3	12–15	High	Banana streak virus		Dessert banana
Dwarf Brazillian	3	10–12	Medium	None observed		Dessert banana
SH-3640	1	10–12	High	None observed		Dessert banana
Pisang Raja	2	12–15	Low	None observed		Dessert banana
Plantains (AAB)						
Giant	4	14–16	High	Sigatoka	*Metamasius hemipterus*	Plantain
Giant French	2	14–16	High	Sigatoka	*Metamasius hemipterus*	Plantain
Dominican Red	1	12–14	High	Sigatoka	*Metamasius hemipterus*	Plantain
African Rhino Horn	1	12–14	Low	Sigatoka, Cigar End Rot	*Metamasius hemipterus*	Plantain
Hartón Común	2	10–12	High	Sigatoka	*Metamasius hemipterus*	Plantain
Dwarf Puerto Rican	5	7–9	High	Sigatoka, Cigar End Rot	*Metamasius hemipterus*	Plantain
Dwarf Superplantain	2	6–8	High	Sigatoka, Cigar End Rot	*Metamasius hemipterus*	Plantain
Cooking Bananas (AAB)						
Hua Moa	4	10–12	High	Panama disease, sigatoka	*Metamasius hemipterus*	Tostones, when ripe can be eaten out of hand.
Dessert Bananas (ABB)						
Nam Wah	3	10–12	High	Panama Disease	Flower thrips	Cooked or eaten ripe
Dwarf Nam Wah	4	6–8	High	Panama Disease	Flower thrips	Cooked or eaten ripe
Blue Java	1	8–10	Medium	None Observed		Dessert Banana
Cooking Bananas (ABB)						
Orinoco	2	10–12	Medium	None Observed		Cooked or eaten ripe
Saba	1	15–18	Medium	None Observed		Cooking banana
Kandrian	1	15–18	High	None Observed		Cooking banana
Praying Hands	1	12–15	Low	None Observed		
Tetraploids						
FHIA-1 (AAAB)	4	10–12	Very High	None Observed		Dessert banana
FHIA-2 (AAAA)	1	6–8	Low	None Observed		Dessert banana
FHIA-3 (AABB)	1	8–10	Medium	None Observed		Cooking or eaten ripe
FHIA-17 (AAAA)	4	10–12	Very High	None Observed		Dessert banana
FHIA-18 (AAAB)	4	10–12	High	None Observed		Dessert banana
FHIA-21 (AAAB)	1	10–12	High	None Observed		Plantain
FHAI-23 (AAAA)	1	10–12	Very High	None Observed		Dessert banana

lems with longevity in the field. 'African Rhino Horn' seems to be "floating mat"; after the second or third crop, the plants have trouble holding bunches and uproot easily in the wind. Tne author does not fully understand why 'Hua Moa' and FHIA-17 decline, but after the third crop the bunches tend to be progressively smaller. 'Orinoco', the plantains, and 'Mysore' maintain productivity in the field; the author has observed adequate crops ten years after the original planting.

Fertility

Soils in the EAA tend to be very fertile with a few exceptions. A histosol may mineralize in excess of 300 kg N per hectare. Phosphorus in the soils near the lake is usually adequate for bananas and plantains. Potassium can be limiting since bananas and plantains require large quantities of potassium (Lahav and Turner, 1989) which can be readily leached and must be managed. 0.5 kg K per mat should be applied over several applications to ensure an adequate potassium supply.

Micronutrient availability tends to be problematic. Deficiencies of manganese and boron have both been observed in the field. These deficiencies seem to be related to climactic conditions. Boron deficiency was observed during a period of rapid growth in the late spring and the manganese deficiency occurred during a dry period. Irrigation was via seepage so there was adequate moisture deeper in the root zone, but the top several inches of soil was very dry. Once the rainy season began, the manganese deficiency symptoms vanished.

Pest Control

Bananas and plantains have relatively few insect and disease problems in the EAA, but weed control requires a concentrated effort. Cultivars are noted in Table 3 for their susceptibility to certain insect and disease problems.

'Hua Moa', 'Manzano', 'Nam Wah', and 'Dwarf Nam Wah' are known to be susceptible to Panama Disease *Fusarium oxysporum cubense* in Florida (Crane and Balerdi, 2016). Using tissue culture plants, pathogen free potting soil, and controlling traffic and tools are effective means of limiting the possibility of infestation.

Black Sigatoka *Mycosphaerella fijiensis* is present in the EAA and occasionally requires fungicidal treatment. 'Williams', 'Grande Naine', 'Baloy', and 'Gros Michel' seem to be especially susceptible, sometimes causing nearly complete defoliation prior to harvest. The plantains, 'Hua Moa', and 'Dwarf Brazillian' seem to be slightly less susceptible. The ABB bananas and tetraploid bananas have high levels of observed resistance. Management of the disease requires an IPM program. Dead leaves must be removed from the pseudostem, and symptomatic leaves also must be removed. Sprays of azoxystrobin seem to be particularly effective, especially during particularly long wet periods.

Cigar end rot is a symptom of several pathogens. It is particularly problematic in fruits of the cavendish and plantain subgroups. Removal of the floral remnants and the male bud are strategies to reduce the likelihood of infection (Robinson and Galan-Sauco, 2010). Bunch sprays with copper and/or covering the bunch with a bunch cover can also reduce the likelihood of infection. The disease is especially problematic during the summer rainy season.

Flower thrips are a seasonal problem that are worst in the late spring. All cultivars are affected, but the symptoms are most pronounced on 'Dwarf Nam Wah' and 'Nam Wah'. Flower thrips will oviposit on the fruit and the ovipositional scars damage the fruit aesthetically. The symptom is easily identified as several raised dark spots on the surface of the banana or plantain fruit. While the damage does not impact the pulp, it can render the fruit unmarketable. The insect can be managed by removal of the flowers and the male bud and promptly covering the bunch.

Metamasius hemipterus can be a major pest of bananas in the EAA. The author has observed severe infestations in 'Hua Moa' and 'Dwarf Puerto Rican Plantain'. As the major crops in EAA is sugarcane, it is not surprising to find it infesting bananas and plantains. Fogain and Price (1994) observed that plantains were most susceptible to weevil damage. Damage occurs when larvae feed on the corm and pseudostem, which weakens the plants' ability to hold bunches of fruit. The problem may not be obvious until a windy day occurs and infested plants are blown over. Corms should be inspected prior to planting and grubs removed. Treating corms with hot water can help prevent the pest from being transported from field to field. Once a field is infested, there are few options available.

Mat and Bunch Management

Bananas and plantains must have mats and bunches managed for optimum production. Ideally, a mat should be kept to a mother plant, a mid-sized sucker, and a small sucker so that fruit can be harvested from each mat at six-month intervals. This guidance is less critical in low-density plantings, but even there it is advisable to reduce the number of suckers to allow for good airflow and room for bunches. At the very least, suckers below bunches should be cut down so that they do not damage the bunch. Also, it is not advisable to dig up suckers around a pseudostem that is close to flowering or carrying a bunch since that makes them susceptible to blowing down in the wind.

Bunch pruning should be done to produce bunches with fruit of a commercially acceptable length and diameter. Usually the last two hands of a bunch contain fruit that is too short. Once a bunch has finished revealing fruit, the male bud is removed, and the ultimate and sometimes penultimate hands are removed. At this point the bunch can be covered. The decision to remove one or two hands depends on the overall size of the bunch and the relative vigor of the plant. Generally, 'Dwarf Puerto Rican', 'Giant Plantain', 'Dwarf Super Plantain', and 'Hua Moa' will produce five marketable hands of fruit. 'Dwarf Super Plantain' can produce a bunch with ten hands, but if left with that many,

Table 4. Highly simplified economics of banana production in the Everglades Agricultural Area.[z]

Item	Cash price	Per plant	Per acre low density[y]	Per acre high density[x]
Plants	$3.00/plant	$3.00	$675.00	$2400.00
Labor	$12.00/h	$3.00	$675.00	$2400.00
Fertilizer	$0.25/lb	$0.40	$90.00	$320.00
Herbicide	$20.00/gal	$0.03–$0.09	$20.00	$20.00
Land Rent	$400.00/acre	$0.50–$1.78	$400.00	$400.00
Per plant cost year one		$6.93–$8.27		
Per plant costs subsequent years		$3.93–$5.27		
Per acre costs year one	$1860.00	$5540.00		
Per acre costs subsequent years			$1185.00	$3140.00
Harvesting	$12.00/h	$0.20	$45.00	$160.00
Packing	$12.00/h	$3.00	$675.00	$2400.00
Wholesale value	$0.75/lb	$30.00[w]	$6750.00	$24000.00
Retail value	$1.50/lb	$60.00[w]	$13500.00	$48000.00
3-year net cash return wholesale		61.59–$65.61	$15300.00	$68,320.00

[z]Assumption of a five acre leased farm with seepage irrigation managed by the drainage district.

[y]225 plants per acre.

[x]800 plants per acre.

[w]Assumes high yielding cultivar with 40 lb of marketable fruit per bunch.

the fruit will not fill out properly and it tends to be too heavy for the plant to support. For bananas such as 'FHIA-1', 'FHIA-17', 'FHIA-18', 'Dwarf Nam Wah', 'Williams', and 'Grande Naine', ten hands seems to be the upper limit. While vigorous plants of these cultivars may have 12–13 hands on a bunch, bunch pruning ensures that the fruit fills out and attains a marketable length (Robinson and Galan-Sauco, 2010).

Bunch covers are essential for blemish-free fruit (Robinson and Galan-Sauco, 2010). While poly-ethylene covers impregnated with insecticide are standard for bananas in Latin America, they are difficult to source in the United States. Bunch covers also can increase finger length and bunch weight (Robinson and Galan-Sauco, 2010). The author has made bunch covers out of lightweight frost protection fabric and found them to be effective in protecting the fruit from insect and physical damage. Frost cloth is sold in doubled-over rolls. This makes it easy to sew a seam down the open side to create a roll of bunch covers which can be installed from a ladder and cut to an appropriate length.

Economics

The economics of a crop are an important consideration before planting. As opposed to most tropical fruits, bananas offer the opportunity of a relatively rapid return as harvest begins approximately 12–15 months after planting. Table 4. Summarizes cash expenses. The data are based on a small farm, with minimal staffing and minimal equipment. On a cash basis, bananas and plantains can be grown profitably, fixed non-cash expenditures and depreciation may decrease profitability. It should be noted that since bananas and plantains are harvested year-round, it can be difficult to have a year of harvests without some type of weather event causing yield loss. A "good" year might see 10% of the bunches affected by cold spells, wind-storms, hail, or animals. Bad years with hurricanes may cause losses for over a year. As such, a reasonable assumption might be that only 3/4 of the bunches produced will actually make it to harvest. If selling the fruit retail, higher values can be expected, in the range of $1.50 to $2.00 per pound.

Conclusions

The Everglades Agricultural Area, especially near Lake Okeechobee, is an acceptable place to grow bananas and plantains. The warm microclimate provided by the lake limits cold weather. The soils have the ability to hold water and supply nutrients to the plants and approximately seven million potential customers live within 100 miles of the area. A properly developed market for high quality 'FHIA-1', 'FHIA-17', 'Grande Naine', and 'Williams' could prove quite profitable with adequate management. Given the large population of immigrants from the Caribbean in South Florida, plantains find a ready market that is not as demanding about the aesthetics of the fruit. Given the narrow band around the lake where production is suitable, it is expected that banana and plantain cultivation will remain a small part of the local agricultural landscape.

Literature Cited

Crane J.H. and C.F. Balerdi. 2016. Banana growing in the Florida home landscape. Univ. of Florida Bulletin HS 10.

Fogain, R. and N.S. Price. 1994. Varietal screening of some *Musa* cultivars for susceptibility to the banana borer weevil. Fruits. 49:247–251.

Fonsah, E.G., C.A. Adamu, B.N. Okole, and B.G. Mullinix. 2007. Field evaluation of Cavendish banana cultivars propagated either by suckers of by tissue culture, over six crop cycles in the tropics. Fruits. 62:205–212.

Gettman, A.D., W.C. Mitchel, L. Po-Yung, and R.F.L. Mau,. 1992. A hot water treatment for control of the banana root borer *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae), in banana planting stock. Proc., Hawaiian Entomol. Soc. 31:59-63

Irizarry, H., J. Rodriguez-Garcia, and N. Diaz. 1981. Effect of three population densities and fertilizer levels on yields of high-yielding clones at two localities. J. Agric. Univ. Puerto Rico. 65:395–400.

Lahav, E. and D.W. Turner. 1989. Banana nutrition, Bull. 12. Intl. Potash Institute, Worblaufen-Bern, Switzerland.

Robinson, J.C. and D.J. Nel. 1989. Plant density studies with banana (cv. Williams) in a subtropical climate. II. Components of yield and seasonal distribution of yield. J. Hort. Sci. 64:211–222.

Robinson, J.C. and V. Galan-Sauco. 2010. Bananas and Plantains. Second Edition. CAB International, Oxfordshire, UK.

Snyder, G.H. 2005. Everglades Agricultural Area soil subsidence and land use projections. Annu. Proc. Soil Crop Sci. Soc. Florida. 64:44–51.

Snyder, J. 2004. Black Gold and Silver Sands. Historical Society of Palm Beach County. Palm Beach, FL.

U.S. Department of Agriculture. 2019. 2017 Census of Agriculture. U.S. Dept. Agr., Washington, D.C.

Proc. Fla. State Hort. Soc. 132:37–39. 2019.

First Report on Yield Performance and Miraculin Content of Miracle Fruit (*Synsepalum Dulcificum*) in Homestead, Florida

Lynhe Demesyeux*[1], Maria Brym[1], Juan Li[2], Alan H. Chambers[1]

[1]*University of Florida/IFAS, Tropical REC, 18905 SW 280th St., Homestead, FL 33031*

[2]*Zhongkai University of Agriculture and Engineering, Guangzhou, China*

Additional index words. miracle fruit, miraculin, non-caloric sweetener, protein extraction, taste altering property, yield

Synsepalum dulcificum or miracle fruit is a novel crop with a promising future. Miraculin, a non-caloric protein sweetener, present in the ripe fruits has a singular property for turning acidic taste into sweet by interacting with the sweet receptors of the tongue in the presence of an acid (Yamamoto et al., 2006; Kurihara, 1992; Kurihara and Beidler, 1969). The overall sweetening sensory impact is ~400 times that of sugar on a molecular basis when stimulated by foods or beverages with an acidic pH (Gibbs et al., 1996; Kurihara and Beidler, 1968). The sweet flavor induced by this non-caloric, natural sweetener is closer to sugar than to other natural sweeteners (Rodrigues, et al., 2016; Hellekant et al., 1998).

Miracle fruit is a perennial shrub originating from west Africa, from Ghana to the Congo, and belongs to the Sapotaceae family. Due to the growing number of people affected by chronic diseases associated with high sugar consumption, the demand for natural, non-caloric sweeteners such as miraculin is increasing, hence a rising interest in using miracle fruit within the medical and food industries. Although miraculin has been well-studied for its sweet inducing properties, there is a lack of information regarding the agronomic performance and variation in protein content among cultivars for this crop. The purpose of this study was to fill the critical knowledge gaps for yield and content of miracle fruit berries. We expect that the results from this study will further our understanding of this species and provide valuable information for those considering its cultivation.

Material and Methods

Plant material. The experiment was carried out in a private farm located in Homestead, FL from May 2018 to May 2019. Nine different morphological types of miracle fruit, namely: 'Typical', 'Wavy leaf', 'Columnar', 'Holly', 'Combo' 'Curly leaf', 'Plates', 'Weaping', and 'Christmas' were evaluated. For each type, weekly data were collected from 10 different plants, except for 'Christmas', 'Weaping', and 'Holly', for which only one, two, and three plants, respectively, were evaluated due to the availability of plants of these types for the study. All the plants were 5 years-old during the evaluation time and were maintained in 80-L containers in a shade house. Supplemental irrigation was provided to the plants when needed and a slow release fertilizer was applied once a year.

Yield parameters. Ripe fruit from each plant were harvested each week over a 52-week period. The total fruit weight/plant was recorded throughout the study and fruit number/tree/type were collected over the multiple harvest windows to calculate the average weight of a single fruit/type.

Protein extraction. Ripe fruit from each tree were smashed in plastic zip closure bags separately to extract the juice without the peel and the seed. Extracted juice from each tree was frozen at –80 °C until protein extraction. The miraculin crude extract was collected following a procedure previously described (He et al. (2015) with some modification. One gram of finely ground frozen juice tissue was used for this experiment. Two water extractions (1:10 ratio) were performed. Each time, the supernatant was discarded. The pellet obtained was resuspended in two mL of NaCl buffer. The solution was vortexed for 2 min, mixed on a Belly Button® shaker (IBI Scientific Corp, Dubuque, IA) for an hour at 4 °C and centrifuged at 12,000 rcf for 20 min. The supernatant was stored at –20 °C until protein purification.

Miraculin purification. The purified miraculin was obtained following a method previously described (He et al., 2015). A 500-µL Qiagen Ni-NTA Agarose (50% Ni-NTA slurry) was poured into a 6-mL chromatography column previously washed with 5-mL of biding buffer (20 mM Tris-HCl, 300 mM NaCl, pH 7.0). After it had completely settled, the Ni-NTA Agarose was washed with 5 mL of the same biding buffer. Two mL of the crude extract, (adjusted to pH 7.0) was added to the column and the flow through was applied to the column twice more. Five mL of the

*Corresponding author. Email: l.demesyeux@ufl.edu, (ac@ufl.edu)

biding buffer was once again added to the column. The bound protein was eluted and collected for quantification with 500 µL of elution buffer (300 mM imidazole, 200 mM Na2HPO4, 300 mM NaCl, pH 8).

Miraculin quantification. The miraculin content of the crude extract and elution samples was quantified using high performance liquid chromatography (HPLC, Infinity II, Agilent Biosystems, Santa Clara, CA). All the samples were filtered using a 4.5 µm PES syringe filter, collected into amber vials and run on the HPLC using an Agilent Poroshell 300SB-C18, 2.1x75 mm, 5 µm column. A wash bottle containing water was added to wash the HPLC needle in between samples. The absorbance was measured using UV-vis detector at 280 nm.

Table 1. Average yield for each accession in kg/tree/year for fruit fresh weight.

Accessions	Kg/tree/year[z]	g/fruit
Typical	2.76 a	1.23 a
Christmas	2.41 abc	1.22 b
Plates	2.25 ab	1.27 b
Wavy Leaf	2.21 ab	1.48 a
Columnar	2.01 ab	1.50 a
Curly Leaf	1.86 bc	1.40 b
Weeping	1.80 abc	1.54 a
Combo	1.68 bc	1.35 b
Holly	0.73 c	1.27 b

[z]Averages followed by the same letter are not significantly different using Tukey's honestly significant test ($\alpha = < 0.05$).

Results and Discussion

Yield evaluation. This is the first report on miracle fruit yield that is based on a structured experiment that has captured the total fruit production for a yearlong period. Weekly crop evaluations revealed that on average, 0.4 kg/tree per week and 2.1 kg/tree/year of mature fruits can be harvested under the conditions described above. This yield is ~6 times lower than a performance reported in 2015 (Achigan-Dako et al., 2015). However, since information regarding the plant materials and the data collection methods used for this research were not provided, comparison between findings is impossible.

The highest yielding tree type was 'Typical' with 2.76 kg/tree/year and was significantly higher than 'Curly leaf' (1.86 kg/tree/year), 'Combo' (1.68 kg/tree/year), and 'Holly' (0.73 kg/tree/year), the lowest yield value recorded. Yield value for 'Christmas', 'Plates', 'Wavy leaf', 'Columnar' and 'Weeping' with an average of 2.1kg/tree/year of fruit were not statistically different with the yield of the other studied types (Table 1).

It is important to mention that the yield for this species varies according to the branching, the habitat and the age of the plants (Achigan-Dako et al., 2015). In the case of this study, the morphology of the trees might be the major cause for the yield difference between types. Regarding the average weight for a single fruit, 'Curly Leaf', 'Typical', 'Wavy leaf', and 'Plates' were not significantly different and were bigger in size (average 1.5 g/fruit) than 'Columnar', 'Combo', 'Holly', 'Weeping', or 'Christmas' (~1.3 g/fruit, $P < 0.05$) (Table 1).

Seasonal yield. Six major production peaks were observed during the evaluation period and were used to identify different harvest windows for this species. The highest production for all the trees was recorded during the first harvest window between May 2018 to July 2018 while the lowest was recorded from March 2019 to May 2019, harvest window 6 (Fig. 1).

Protein extraction and quantification. The miraculin was successfully extracted, purified and quantified from frozen tissue collected throughout the study. The protein identity was confirmed through protein sequencing by the University of Florida Proteomics Core (data not shown). Figure 2 shows the absorbance of the different proteins of the crude extract (including the miraculin) and miraculin absorbance is seen in Fig. 3. Our ongoing research consists of extracting and comparing miraculin content across the nine morphological types.

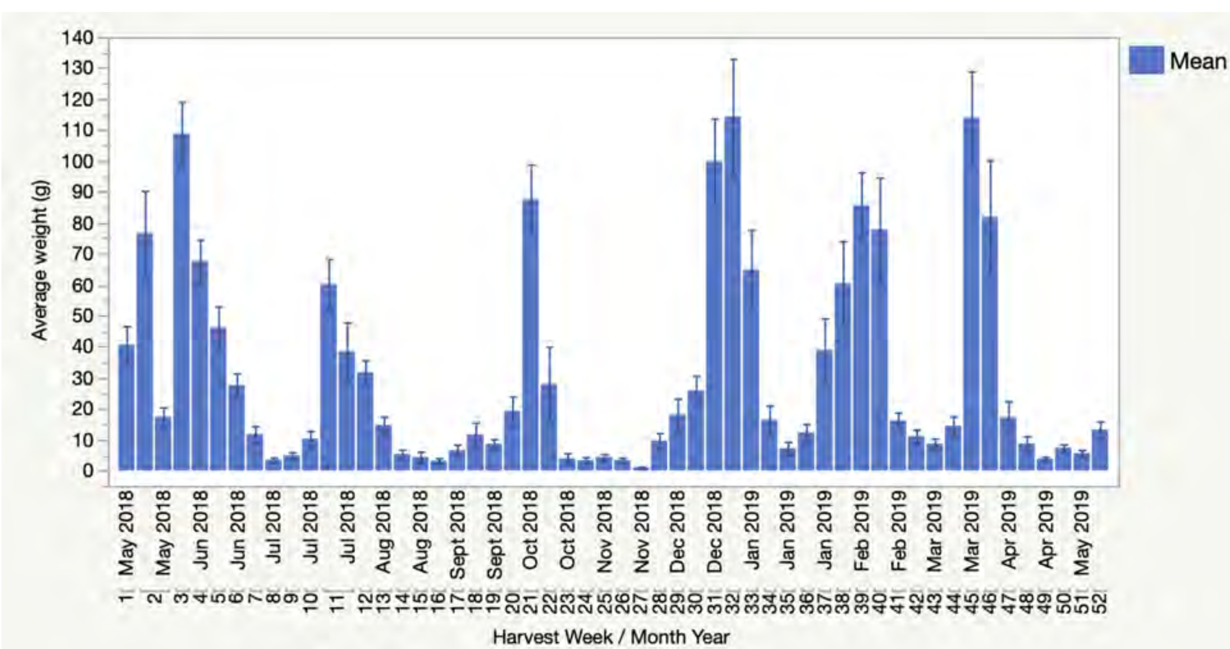

Fig. 1. Average fruit yield for all 66 accessions in grams per tree over 52 weeks. Error bars represent standard error for all accession yield data.

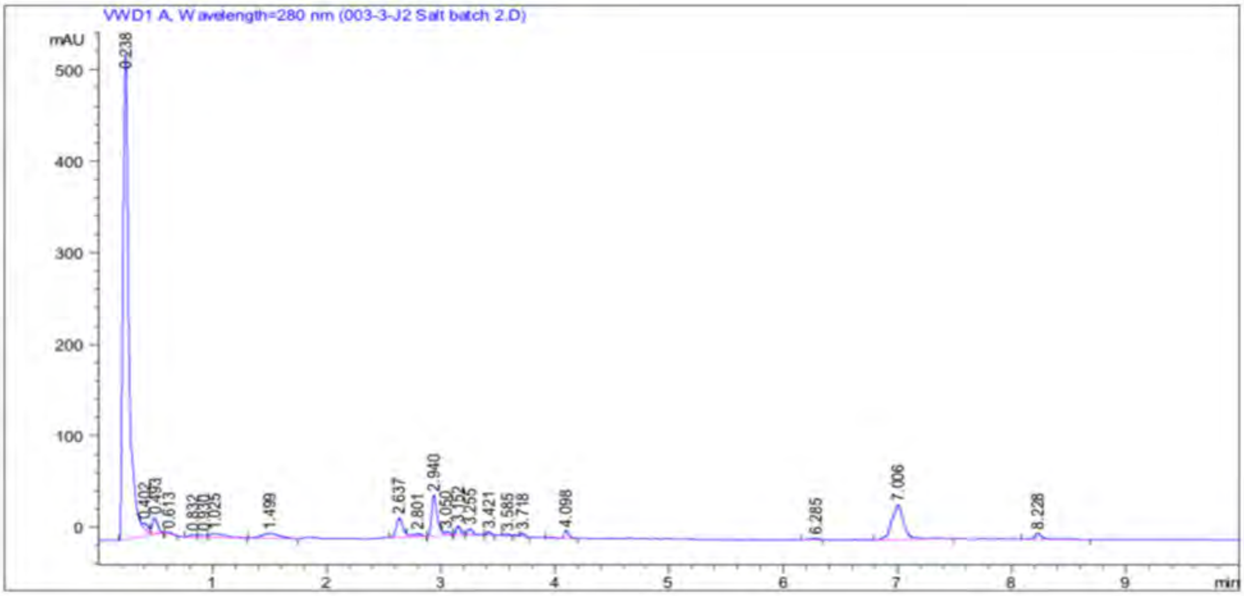

Fig. 2. Miracle fruit crude extract. Figure shows the total protein content of the sodium chloride solution. Y axis is for the absorbance while the X axis is the retention time. Miraculin elutes at 7 min.

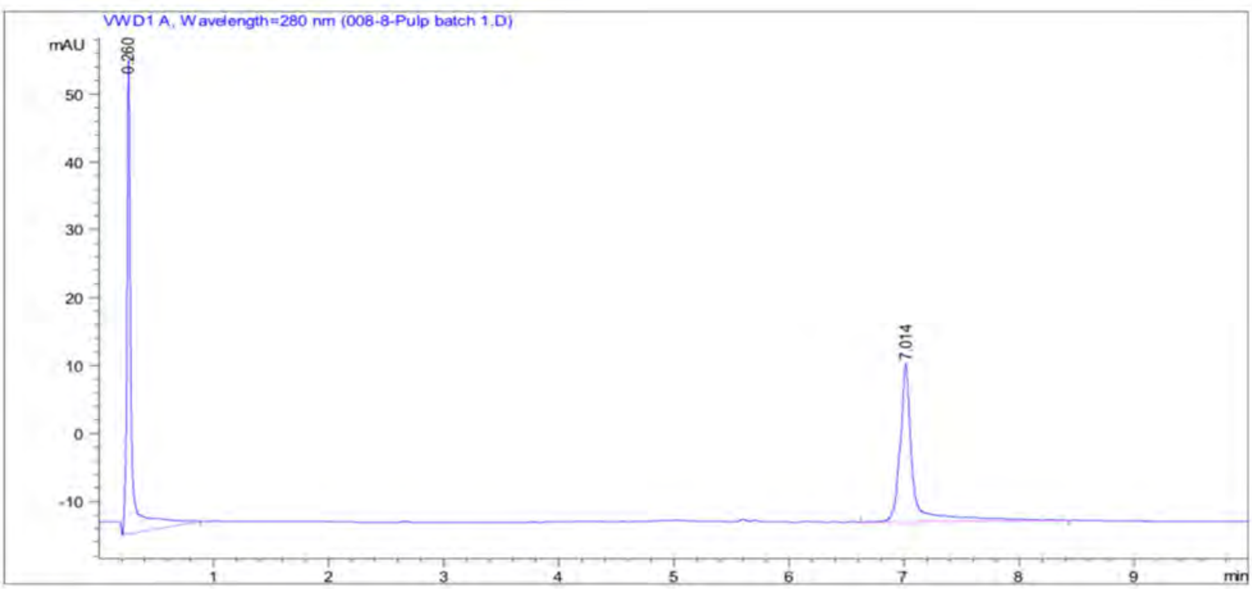

Fig. 3. Single peak of miraculin after purification with IMAC. Y axis is for the absorbance while the X axis is the retention time.

Literature Cited

Achigan-Dako, E.G., D.A. Tchokponhoué, S. N'Danikou, J. Gebauer, and R.S. Vodouhè. 2015. Current knowledge and breeding perspectives for the miracle plant *Synsepalum dulcificum* (Schum. et Thonn.) Daniell. Genetic Res. Crop Evol. 62(3):465–476.

Gibbs, B.F., I. Alli, and C. Mulligan. 1996. Sweet and taste-modifying proteins: A review. Nutrition Res. 16(9):1619–1630.

He, Z., J.S. Tan, O.M. Lai, and A.B. Ariff. 2015. Optimization of conditions for the single step IMAC purification of miraculin from *Synsepalum dulcificum*. Food Chem. 181:19–24.

Hellekant, G., Y. Ninomiya, and V. Danilova. 1998. Taste in chimpanzees. III: Labeled-line coding in sweet taste. Physiol. Behavior. 65(2):191–200.

Kurihara, K., and L.M. Beidler. 1968. Taste-modifying protein from miracle fruit. Science. 161(3847): 241–1243.

Kurihara, K., and L.M. Beidler. 1969. Mechanism of the action of taste-modifying protein. Nature. 222(5199):1176.

Kurihara, Y. 1992. Characteristics of antisweat substances, sweet proteins, and sweetness-inducing proteins. Critical Reviews in Food Science & Nutrition. 32(3):231–252.

Rodrigues, J.F., R. da Silva Andrade, S.C. Bastos, S.B. Coelho, and A.C.M. Pinheiro. 2016. Miracle fruit: An alternative sugar substitute in sour beverages. Appetite, 107, 645–653.

Yamamoto, C., H. Nagai, K. Takahashi, S. Nakagawa, M. Yamaguchi, M. Tonoike, and T. Yamamoto. 2006. Cortical representation of taste-modifying action of miracle fruit in humans. Neuroimage 33(4):1145–1151.

Proc. Fla. State Hort. Soc. 132:40–42. 2019.

Use of Sparkleberry as a Potential Rootstock in Commercial Blueberry Production

Jeff Williamson* and Rebecca Darnell

Horticultural Sciences, University of Florida/IFAS, P.O. Box 110690, Gainesville, FL 32611-0690

Additional index words. bacterial leaf scorch, grafting, *Vaccinium arboreum*, *Vaccinium corymbosum* interspecific hybrid,

Southern highbush blueberry (SHB) has specific soil requirements that include high soil organic matter (OM) content and low pH. Southern highbush blueberry is often grown in sandy soils that are acidified and heavily amended with OM. In the southeast United States, pine bark is the primary source of OM. Pine bark represents a major establishment cost and additional pine bark is needed every two to three years to maintain plant health. Blueberry plants grown on pine bark amended soils are shallow-rooted and subject to diurnal and periodic drought stresses during warm weather, which reduces yield. After establishment, the single greatest SHB production cost is hand harvesting. Although grower interest in machine harvesting of SHB is high, the wide, multi-caned crown of SHB is not conducive to efficient mechanical harvesting. *Vaccinium arboreum* (sparkleberry) is a small tree that is native to the southeastern U.S. and has the following beneficial traits for consideration as a blueberry rootstock: 1) graft compatibility with SHB; 2) single truck architecture; 3) deep, expansive root system; 4) tolerance to low soil OM; and 5) greater tolerance to high soil pH than SHB. Research with *V. arboreum* as a blueberry rootstock is ongoing. To date, our research shows increased fruit production for grafted SHB compared to non-grafted SHB under both amended (high OM) and non-amended (low OM) soil conditions.

Southern highbush blueberry (*Vaccinium corymbosum* interspecific hybrid) is the primary blueberry type grown commercially in Florida. The Florida blueberry crop ripens from March though the first half of May and has historically been the earliest ripening source for fresh blueberries in North America. While Florida production costs are high due to extensive soil inputs and hand harvesting labor, the crop has generally been profitable for Florida growers due to high demand and limited supply during the Florida market window. Recently, increased production and imports of Mexican blueberries into the U.S. during the traditional Florida production window have significantly reduced fruit prices for Florida growers. Mexican imports have increased from 0.8 million pounds in 2009 to 71 million pounds in 2018. The surge in Mexican imports has significantly reduced profits for Florida growers who must reduce production costs and increase production efficiencies to remain competitive in an expanding international market.

Southern highbush blueberry has specific soil requirements; namely low soil pH (4.5–5.5), high organic matter, and good internal drainage (Williamson, et al., 2018). Root systems tend to be fibrous and shallow, making SHB susceptible to drought stress during extreme summer heat, even when irrigated. Drought and other plant stresses contribute to the incidence of diseases such as blueberry stem blight, which can reduce the productive life of blueberry fields (Flor, et al., 2019). Addition of pine bark, the most common soil amendment, is a major establishment and recurring maintenance cost for Florida growers. For example, pine bark necessary for amending new fields can cost up to $6,000/acre, which represents 24% of the overall establishment costs (Singerman, et al., 2016). Furthermore, additional pine bark is required at 3- to 4-year intervals to maintain plant health. Ultimately, required soil amendments increase production costs and reduce farm profitability.

Florida blueberries are traditionally hand harvested for the fresh market. Labor for hand harvesting represents the single greatest annual production cost for Florida growers. Hand picking can represent 43% of total harvest costs in Florida (Singerman, et al., 2016). Machine harvesting can increase harvest efficiency by reducing harvest labor costs (Brown, et al., 1996). Estimated costs of mechanically harvesting blueberries range from 4 to 10 times less than hand harvesting costs (Safley et al., 2005; Takeda et al., 2008; Takeda et al., 2017). Therefore, there is great interest among Florida growers in transitioning to mechanical harvesting. While fruit characteristics of recently released cultivars are better suited for machine harvesting, the multi-cane architecture of blueberry results in excessive ground drops and does not lend itself to current machine harvester designs (Mainland, 1993; Strik and Muller, 2002). In machine harvesting studies conducted in the Pacific Northwest in 2018 and 2019, ground fruit losses were as high as 25% of total harvestable yield. Approximately 70% of those losses occurred in the middle of the row, where multi-cane plant architecture prevented harvester catch plates from closing tightly around the base of the plants (W. Yang, personal comm.).

Sparkleberry (*V. arboreum*) is a small native tree that is graft compatible with blueberry, adapted to mineral soils low in organic matter, tolerates higher soil pH than blueberry, is more drought tolerant than blueberry, and has a single trunk that is suited for machine harvesting (Lyrene, 1997). The objective of this proj-

This information is based on work supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2009-51181-06021 and by the Florida Department of Agriculture and Consumer Services award number 024045.

*Corresponding author. Email: jgrw@ufl.edu

ect was to evaluate the potential of sparkleberry as a potential rootstock for SHB.

Initial Grafted Planting. In 2010, sparkleberry seedlings were grown in a nursery on the University of Florida campus and grafted with 'Meadowlark' or 'Farthing', which were two commercially important cultivars at the time. In 2011, plantings were established at the Plant Research and Education Unit (PSREU), Citra, FL, and at Straughn Farms, Archer, FL using both grafted and own-rooted 'Meadowlark' and 'Farthing' plants. Each plant type was grown on pine bark amended and non-amended soils. The soil was a well-drained Arrendondo sand at both locations (pH ~ 6.0 and organic matter ~ 1.4%). Recommended production practices were followed for drip irrigation, fertigation, pest management and pruning (Williamson et al., 2018). Plant growth and survival, and fruit yield and quality were evaluated at both locations. Details on site preparation, experimental design, and data collection and statistical analyses can be found at Casamali, et al. (2016).

Initially, grafted plants were smaller and had lower yields than own-rooted plants. However, by the end of the third growing season (2014), canopy volume for grafted plants on non-amended soil was similar to own-rooted plants on amended soil (data not shown). General trends for greater vegetative vigor were observed for grafted plants compared to own-rooted throughout the remainder of the study. Figure 1 shows grafted 'Meadowlark' plants in 2013. Yield was also initially reduced by grafting, but grafted plants produced greater yields than own-rooted plants for both cultivars and both locations during 2016, 2017, and 2018 . Interestingly, yields of grafted plants on non-amended soils were usually not different from own-rooted plants on amended soils, suggesting that grafting SHB on sparkleberry may reduce the need for organic soil amendments, which are a significant establishment and maintenance cost. Mean berry weight tended to be greater for grafted plants than for own rooted plants. No consistent differences were observed for fruit acidity or soluble solids.

Over the course of the 7-year study, field survival was greater for grafted plants than for own-rooted plants. In 2016, symptoms of bacterial leaf scorch (BLS) (*Xylella fastidiosa*) were observed on own-rooted 'Meadowlark' (a susceptible cultivar) at the

Fig. 1. 'Meadowlark' grafted on sparkleberry in the original experiment during the third year after planting. Photo taken in 2013.

Fig. 2. Crown and roots of 'Meadowlark' southern highbush blueberry grafted on sparkleberry. Photo taken in 2016.

PSREU site. Further evaluation during 2016–2018 revealed much higher levels of bacterial leaf scorch incidence and severity for own-rooted 'Meadowlark' than for grafted 'Meadowlark'. These preliminary results suggest that using sparkleberry as a rootstock may impart increased BLS tolerance to the grafted plant. However, other factors may have increased field survival of grafted plants since plant longevity was also greater for grafted plants at the Archer site where BLS was not detected. Excavation of a grafted 'Meadowlark' plant at the PSREU revealed a deep, spreading root system, uncharacteristic of SHB (Fig. 2).

Evaluation of *V. arboreum* selections. During the 7-year study period from 2011 to 2018, grafted plants were evaluated for field survival, scion vigor, graft compatibility, and the tendency for the plants to produce sprouts from below the graft union. Approximately 30 *V. arboreum* selections were made from the original seedling rootstock population based on the above criteria. These selections were propagated by root cutting and grafted with 'Optimus', 'Patricia', and 'Keecrisp' SHB. The grafted plants were planted in spring of 2018 at the PSREU and are being evaluated for survival, vigor, sprouting below the graft union, and yield. From these 30 *V. arboreum* selections, seven clones were selected for propagation in tissue culture. The selection criteria were: 1) high transplant survival and good overall plant vigor as non-grafted plants; 2) graft compatibility with blueberry; 3) straight, up-right trunks as grafted plants; 4) high scion vigor; and 5) low sprout production below the graft union. Figure 3 shows an example of a low-sprouting, grafted 'Optimus' plant. Additionally, four non-grafted plants of each of the sparkleberry selections were planted at the PSREU for observation as non-grafted plants and as a source of future propagation material. Our goal is to establish larger plantings of grafted SHB to evaluate the performance of these advanced sparkleberry selections as rootstocks for SHB cultivars that have potential for mechanical harvesting.

Conclusions

The recent influx of blueberries from Mexico during the March-May market window has created a financial crisis for Florida blueberry growers. To remain competitive, Florida blueberry growers must reduce production costs. Use of soil amendments (pine bark) to make soils suitable for production represents significant establishment and maintenance costs, and labor for hand harvesting berries is the single largest annual production cost

Fig. 3. Young grafted ‘Optimus’ plant currently under evaluation. Photo taken in 2019.

for current Florida production systems. Use of sparkleberry as a rootstock for blueberry presents potential cost-savings by reducing the need for pine bark and producing plants with improved architecture for machine harvesting. There is also preliminary evidence that grafted plants may have greater longevity due to increased tolerance to BLS and/or other stresses. However, grafting represents a significant increase in nursery plant production costs. Moreover, grafted plants would require some changes in cultural practices (i.e. sprout removal). More research is needed to determine if the potential benefits of grafted plants would outweigh the added costs of nursery plants and the necessary adjustments to cultural practices.

Literature Cited

Brown, G.K., N.L. Schulte, E.J. Timm, R.M. Beaudry, D.L. Peterson, J.F. Hancock, and F. Takeda. 1996. Estimates of mechanization effects on fresh blueberry quality. Applied Engineering in Agriculture 12:21–26.

Casamali, B., R.L. Darnell, A.P. Kovaleski, J.W. Olmstead, and J.G. Williamson. 2016. Vegetative and reproductive traits of two southern highbush blueberry cultivars grafted onto *Vaccinium arboreum* rootstocks. HortScience. 51:1503–1510.

Flor, N.C., D.A. Phillips, and P.F. Harmon. 2019. Botryosphaeria stem blight on southern highbush blueberry in Florida. (PP-347). University of Florida Coop. Extension Service, Gainesville, FL.

Lyrene, P.M. 1997. Value of various taxa in breeding tetraploid blueberries in Florida. Euphytica. 94:15–22.

Mainland, C.M. 1993. Blueberry production strategies. Acta Hortic. 346:111–116.

Safley, C.D., W.O. Cline, and C.M. Mainland. 2005. Estimated costs of producing harvesting and marketing blueberries in the southeastern United States. Proc. 12th Biennial Southeast Blueberry Conf. Savannah, GA. 33–49.

Singerman, A., M. Burani-Arouca, J.G. Williamson, and G.K. England. 2016. Establishment and production costs for southern highbush blueberry orchards in Florida: Enterprise budget and profitability analysis. (FE-1002). University of Florida Coop. Extension Service, Gainesville, FL.

Strik, B. and G. Muller. 2002. Improving yield and machine harvest efficiency of ‘Bluecrop’ through high density planting and trellising. Acta Hortic. 574:227–231.

Takeda, F., G. Krewer, E.L. Andrews, B. Mullinix Jr., and D.L. Peterson. 2008. Assessment of the V45 blueberry harvester on rabbiteye blueberry and southern highbush blueberry pruned to v-shaped canopy. HortTechnology 18:130–138.

Takeda, F., W.Q. Yang, C. Li, A. Freivalds, K. Sung, R. Xu, B. Hu, J. Williamson, and S. Sargent. 2017. Applying new technologies to transform blueberry harvesting. Agronomy. 7:33. doi:10.3390/agronomy/7020033

Williamson, J.G., J.W. Olmstead, and P.M. Lyrene. 2018. Florida’s commercial blueberry industry (HS-742). University of Florida Coop. Extension Service, Gainesville, FL.

Proc. Fla. State Hort. Soc. 132:43–46. 2019.

Muscadine Grape Production in Florida: A Potential Alternative Fruit Crop

Ali Sarkhosh*[1], Yuru Chang[1], and John Peterson[2]

[1]*Horticultural Sciences Department, University of Florida, Gainesville FL 32611*

[2]*Environmental Horticulture Department, University of Florida, Gainesville FL 32611*

Additional index words. fruit quality, production technologies and strategies, management practices

The commercial acreage of muscadine grape production in Florida is estimated to be about 1000 acres. Over 85% of production is sold to wineries, however, some growers sell their fresh market crop through u-pick operations, local sales, and other direct-to-consumer marketing avenues. As the second largest wine consuming state in the nation, Florida has a distinct advantage in marketing wine. Tourists, as well as new residents, enhance market growth potential for grape products as either fresh produce or value-added products (juice, wine, jellies, candies, etc.). Circumstances and factors seem to indicate that Florida has a high potential to become the top major producer of muscadine grapes in the United States, and that the current industry will expand. Muscadine grape is a fruit crop that is tolerant to insect and disease pests. Commercial growers and homeowners can both successfully grow this crop with minimal use of pesticides and fungicides. While many traditional perennial fruit crops are highly sensitive to domestic competition and in need of extensive disease and pest control measures, muscadine grapes do not seem to face the same market challenges or intense disease and pest control requirements as other crops. Increased production and enhanced profitability are likely if producers are provided with new and improved best management practices for this crop. Consistent production of high-quality muscadine grapes with good berry size and color for both fresh and value-added markets can be challenging for producers in Florida due to the lack of information about optimal cultural practices, including irrigation, plant nutrition, and canopy management.

Origin and Adoptability

Muscadine grape (*Vitis rotundifolia*. L., *Vitaceae*) (Fig.1) is the predominant grape variety commonly grown in the southeastern United States, with current markets existing for juice, wine, and fresh fruit. As shown in Fig. 2, muscadine grape is originally from the southeast United States and is widely cultivated throughout 17 states (Wan et al., 2013). The *Vitis* genus has two subgenera: *Euvitis* (the European, *Vitis vinifera* L. grapes and the American bunch grapes, *Vitis labrusca* L.) and *Muscadinia* (the Muscadine grapes *Vitis rotundifolia* L.) (Fig. 3) (Comeaux et al., 1987). Muscadine grapes are round, having either bronze- or purple-colored leather-like thick skin. They are well-adapted to warm, humid climates that are not suitable for *Vitis vinifera* (Pastrana-Bonilla et al., 2003).

Muscadines have some different characteristics compared with bunch grapes (*Vitis vinifera* L.), they have thicker skins, unique flavor, and a distinctive aroma. They grow in small clusters instead of large and tight bunches. Unlike other grapes, their thick skins tend to slip away from the pulp, and the berries detach from stems one-by-one as they mature. *Euvitis* has 38 chromosomes while *Muscadinia* has 40. Thus, it can be sometimes challenging to do crossbreeding and hybridization between muscadines and bunch grapes (Olien, 1990).

As a native grape species, muscadines have many apparent benefits compared to bunch grapes (*V. vinifera* L.). First, Mus-

Fig. 1. Muscadine berries. Photo credit: Yuru Chang.

*Corresponding author. Email: sarkhosha@ufl.edu

Fig. 2. Native states of muscadine grapes (USDA, and Hopkins, 1989).

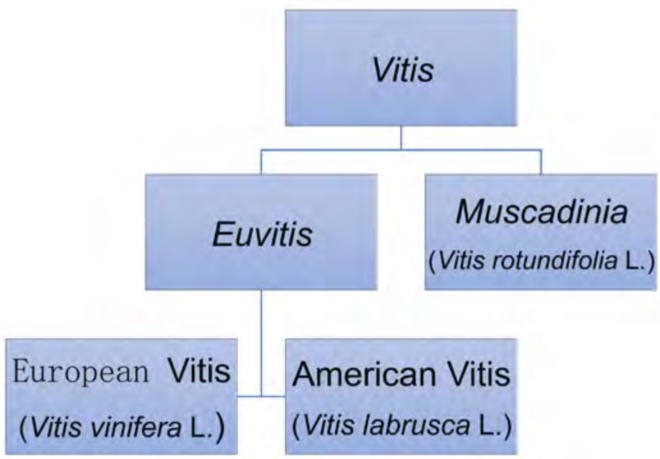

Fig. 3. Grape classification (Wan et al., 2013).

cadines need fewer chilling hours than other grapes so that they can thrive in the summer heat (Luo et al., 2017). They require 100 days to fruit and can tolerate temperatures down to about 10 °F. In addition, they show incredibly dynamic and regional adaptation to warm, humid climates in Florida, where it is difficult to grow most other grapes. Due to its warmer climate, Florida can produce the earliest fruit; thus, growers face much less competition in the market. Second, muscadines can adapt well to Florida soils. Vines can grow in a wide range of soils, from loams to clays. The soil pH should be between 5.5 and 7.5. As seen in Fig. 4, well-drained sandy soils are preferred by muscadines whose performance is poor in calcareous soils or those with poor drainage. Typical Florida soils are moderately-well drained sands and upland soils with underlying clay at about three feet, which is very suitable for muscadine cultivation. Third, muscadines have natural resistance to Pierce's disease (PD), caused by the bacterial pathogen *Xylella fastidiosa* which is spread by the invasive insect vector *Homalodisca vitripennis*. PD is a prevalent and lethal disease for bunch grapes throughout the United States, from Florida to California, leading to approximately 3 billion dollar wine industry losses a year (Hopkins, 1989; Kyrkou et al., 2018; Sanscartier et al., 2012). This pathogen limits and even prevents extensive cultivation of *V. vinifera* in Florida. A characteristic PD symptom is leaf-scorch, which begins with chlorosis at the leaf margins (Nascimento et al., 2016). The symptoms look

Fig. 4. Muscadine vineyard in Clermont, FL. Photo credit: Yuru Chang.

very similar to drought stress, but a yellow or red-brown band between green and scorched areas is absent in vines suffering from drought stress. However, commercial muscadine varieties have preserved most of their resistance genes, making them a valuable crop with excellent potential for further expansion (Talcott et al., 2003).

Muscadine grapes are notable for the high pigment and polyphenols residing mostly in their thick skin. These polyphenols include anthocyanins, tannins, quercetin, flacan-3-ols, gallic acid, ellagic acid, ellagic acid glycosides, ellagitannins, myricetin, and kaempferol (Talcott and Lee, 2002). Muscadine seeds have a higher content of total phenolic compounds than either the skin or the flesh (Pastrana-Bonilla et al., 2003; Sandhu and Gu, 2010; Xu et al., 2014). They are rich in dietary fiber, which can help control cholesterol and blood sugar levels (Huang et al., 2009). From previous research, consumption of muscadine grape and wine phytochemicals in the diet can contribute to health and prevent obesity-related metabolic complications as well as cardiovascular diseases and cancers (Burton et al., 2015; Gourineni et al., 2012; Huang et al., 2009; Hudson et al., 2007; Paller et al., 2015).

Muscadine grapes can be served fresh, processed into wine, juice, jam, jelly, and other products; seeds can also be pressed for oil. In 2010, a consumer consumption study about muscadine grapes reported that 56.6% of customers were familiar with muscadine wine and 48.7% were familiar with muscadine jelly, but only 16.4% familiar with fresh muscadine grapes (Duarte Alonso and O'Neill, 2012). That is because most muscadine varieties have small berries, large seeds, and thick skins. During the past decade, more muscadine breeders have been working on developing new cultivars. Based on function, muscadine cultivars can be divided into two categories: fresh market cultivars (Table 1) and juice cultivars (Table 2). Usually, the cultivars with larger fruit are used for the fresh market while those with smaller fruit are used for processing. Juice and wine from *Muscadinia* are sweeter than those from *Vinifera*. Compared to bunch grape wine, muscadine wines have unique characteristics, including a high concentration of individual phenolic compounds, the presence of ellagic acid, and phenolic composition (Basha et al, 2004). Mechanical harvesting of muscadine can be used for juice and wine production (Figs. 5 and 6). However, fresh market is a very labor-intensive process because hand harvesting is necessary.

Table 1. Varieties for fresh market, reproduced from <http://muscadines.caes.uga.edu/cultivars/juice-cultivars.html>.

Cultivar	Flower Type	Berry Color	Harvest Period	Berry Size	Productivity
Alachua	Self-fertile	Purple	Midseason	Medium	100%
Cowart	Self-fertile	Purple	Midseason	Medium	40%
Daelene	Female	Bronze	Midseason	Very Large	40%
Delicious	Self-fertile	Purple	Early-Mid	Large	130%
Dixieland	Female	Bronze	Late	Large	90%
Early Fry	Female	Bronze	Early	Very Large	80%
Eudora	Female	Purple	Midseason	Med/Large	100%
Fry	Female	Bronze	Midseason	Large	70%
Granny Val	Self-fertile	Bronze	Very Late	Large	110%
Higgins	Female	Bronze	Midseason	Large	70%
Hall	Self-fertile	Bronze	Early	Large	100%
Ison	Self-fertile	Purple	Late	Large	120%
Janet	Self-fertile	Bronze	Late	Large	100%
Jumbo	Female	Purple	Midseason	Large	80%
Lane	Self-fertile	Black	Early	Large	60%
Late Fry	Self-fertile	Bronze	Late	Very Large	80%
Loomis	Female	Red	Late	Medium	20%
Magoon	Self-fertile	Purple	Midseason	Small	90%
Nesbitt	Self-fertile	Purple	Midseason/Late	Large	100%
Pam	Female	Bronze	Late	Very Large	60%
Pineapple	Self-fertile	Bronze	Midseason	Medium	130%
Polyanna	Self-fertile	Purple	Late	Large	80%
Pride	Female	Purple	Midseason	Large	90%
Scarlett	Female	Pink	Midseason	Large	30%
Scuppernong	Female	Bronze	Late	Small	40%
Southern Home	Self-fertile	Purple	Midseason/Late	Medium	80%
Southland	Self-fertile	Purple	Late	Small	90%
Southern Jewel	Self-fertile	Purple	Early	Large	/
Sugargate	Female	Purple	Early	Large	40%
Summit	Female	Bronze	Midseason	Large	80%
Supreme	Female	Purple	Midseason	Very Large	90%
Sweet Jenny	Female	Bronze	Midseason	Very Large	50%
Tara	Self-fertile	Bronze	Early	Large	90%
Triumph	Self-fertile	Bronze/Pink	Early	Medium	100%

Table 2. Varieties for juice and wine production. Table reproduced from <http://muscadines.caes.uga.edu/cultivars/juice-cultivars.html>.

Cultivar	Flower Type	Berry Color	Harvest Period	Berry Size	Productivity
Carlos	Self-fertile	Bronze	Midseason	Small	90%
Doreen	Self-fertile	Bronze	Late	Small	90%
Golden Isles	Self-fertile	Bronze	Late	Large	110%
Magnolia	Self-fertile	Bronze	Midseason	Small	90%
Noble	Self-fertile	Purple	Midseason	Small	100%
Regale	Self-fertile	Purple	Midseason	Medium	110%
Sterling	Self-fertile	Bronze	Midseason	Medium	100%
Welder	Self-fertile	Bronze	Midseason	Small	90%

Fig. 5. Mechanical harvesting of wine grapes. Photo recognition: <https://grapes.extension.org>.

Fig. 6. Mechanically-harvested muscadine berries for juice production. Photo recognition: <https://site.extension.uga.edu/viticulture>.

Literature Cited

Basha, S.M., M. Musingo, and V.S. Colova. 2004. Compositional differences in the phenolics compounds of muscadine and bunch grape wines. African J. Biotechnol. 3(10):523–528.

Burton, L J., B.A. Smith, B.N. Smith, Q. Loyd, P. Nagappan, D. McKeithen, C.L. Wilder, M.O. Platt, T. Hudson, and V.A. Odero–Marah. 2015. Muscadine grape skin extract can antagonize Snail–cathepsin L–mediated invasion, migration and osteoclastogenesis in prostate and breast cancer cells. Carcinogenesis. 36(9):1019–1027.

Comeaux, B.L., W.B. Nesbitt, and P.R. Fantz. 1987. Taxonomy of the native grapes of North Carolina. Castanea. 52(3):197–215.

Duarte Alonso, A. and M.A. O'Neill. 2012. Consumption of muscadine grape by-products: An exploration among Southern US consumers. British Food J. 114(3):400–415.

Gourineni, V., N.F. Shay, S. Chung, A.K. Sandhu, and L. Gu. 2012. Muscadinegrape (*Vitis rotundifolia*) and wine phytochemicals prevented obesity–associated metabolic complications in C57BL/6J Mice. J. Agr. Food Chem. 60(31):7674–7681.

Hopkins, D. 1989. *Xylella fastidiosa*: Xylem-limited bacterial pathogen of plants. Ann. Rev. Phytopathology. 27(1):271–290.

Huang, Z., B. Wang, P. Williams, and R.D. Pace. 2009. Identification of anthocyanins in muscadine grapes with HPLC–ESI–MS. Lwt–Food Science and Technol. 42(4):819–824.

Hudson, T.S., D.K. Hartle, S.D. Hursting, N.P. Nunez, T.T.Y. Wang, H.A. Young, P. Arany, and J. E. Green. 2007. Inhibition of prostate cancer growth by muscadine grape skin extract and resveratrol through distinct mechanisms. Cancer Res. 67(17):8396–8405.

Kyrkou, I., T. Pusa, L. Ellegaard-Jensen, M.-F. Sagot, and L.H. Hansen. 2018. Pierce's Disease of Grapevines: A review of control strategies and an outline of an epidemiological model. Front. Microbiol. 9. https://doi.org/10.3389/fmicb.2018.02141

Luo, J., S. Song, Z. Wei, Y. Huang, Y. Zhang, and J. Lu. 2017. The comparative study among different fractions of muscadine grape 'Noble' pomace extracts regarding anti-oxidative activities, cell cycle arrest and apoptosis in breast cancer. Food Nutr. Res. 61(1):1412795.

Nascimento, R., H. Gouran, S. Chakraborty, H.W. Gillespie, H.O. Almeida–Souza, A. Tu, B.J. Rao, P.A. Feldstein, G. Bruening, L.R. Goulart and A.M. Dandekar. 2016. The type II secreted lipase/esterase LesA is a key virulence factor required for *Xylella fastidiosa* pathogenesis in grapevines. Scientific Reports. 6:18598.

Olien, W.C., 1990. The muscadine grape: Botany, viticulture, history, and current industry. HortScience, 25(7):732–739.

Paller, C.J., M.A. Rudek, X.C. Zhou, W.D. Wagner, T.S. Hudson, N. Anders, H.J. Hammers, D. Dowling, S. King, and E.S. Antonarakis. 2015. A phase I study of muscadine grape skin extract in men with biochemically recurrent prostate cancer: Safety, tolerability, and dose determination. The Prostate. 75(14):1518–1525.

Pastrana–Bonilla, E., Akoh, C.C., S. Sellappan, and G. Krewer. 2003. Phenolic content and antioxidant capacity of muscadine grapes. J. Agr. Food Chem. 51(18):5497–5503.

Sandhu, A.K. and L. Gu. 2010. Antioxidant capacity, phenolic content, and profiling of phenolic compounds in the seeds, skin, and pulp of *Vitis rotundifolia* (muscadine grapes) as determined by HPLC–DAD–ESI–MS n. J. Agr. Food Chem. 58(8):4681–4692.

Sanscartier, C.A., A.K. Arora, G.M. Tulgetske, and T.A. Miller. 2012. Glassy-winged sharpshooter population survey and *Xylella fastidiosa* detection. Undergraduate Res. J. 6:31.

Talcott, S.T., C.H. Brenes, D.M. Pires, and D. Del Pozo–Insfran. 2003. Phytochemical stability and color retention of copigmented and processed muscadine grape juice. J. Agr. Food Chem. 51(4):957–963.

Talcott, S.T. and J.H. Lee. 2002. Ellagic acid and flavonoid antioxidant content of muscadine wine and juice. J. Agr. Food Chem. 50(11):3186–3192.

Wan, Y.Z., H.R. Schwaninger, A.M. Baldo, J.A. Labate, G.Y. Zhong, and C.J. Simon. 2013. A phylogenetic analysis of the grape genus (*Vitis* L.) reveals broad reticulation and concurrent diversification during neogene and quaternary climate change. Bmc Evolutionary Biology. 13.

Xu, C., Y. Yagiz, W.–Y. Hsu, A. Simonne, J. Lu, and M.R. Marshall. 2014. Antioxidant, antibacterial, and antibiofilm properties of polyphenols from muscadine grape (*Vitis rotundifolia* Michx.) pomace against selected foodborne pathogens. J. Agr. Food Chem. 62(28):6640–6649.

Proc. Fla. State Hort. Soc. 132:47–49. 2019.

'O21-4-1', A Potential Breeding Selection for Table Muscadine Grape Industry

Zhongbo Ren*, Violet Tsolova[1], and Islam El-Sharkawy

Center for Viticulture & Small Fruit Research, College of Agriculture and Food Sciences, Florida A&M University, Tallahassee, FL 32317

Additional index words. Self-fertile flower, large fruit, spur productivity, loose clusters

'O21-4-1' is a self-fertile muscadine breeding line developed at the Center for Viticulture, Florida A&M University, by a cross of 'Supreme' × 'Triumph' in 2011. This breeding line produced 14.8 g fruits with 15.4% sugar content (SSC), and has good yield potential. The dark red fruits are round shaped with conspicuous lenticels. High dry scar rate, small and semi-loose fruit clusters, uniform fruit size, and moderate vine vigor are the additional advantages of the hybrid as a potential table grape selection.

Muscadine grapes (*Vitis rotundifolia*) are indigenous to the southeastern United States. They are well adapted to the hot-humid environment of the region and are resistant to the prevailing of Pierce's Disease (PD), a lethal epidemic to bunch (Euvitis) grapes. These grapes are widely grown in the region for fresh market and processing products, such as wine and juice, with different industry-destined cultivars. Table muscadine grapes, or grapes for the fresh market, are different from grapes for processing in that they must be large. Large fruit are more attractive to consumers than the small fruit for the fresh market. Grapes ~10 g or more are always required by the market, that is why only large fruit cultivars are recommended for the table muscadine grape market. Muscadine grape fruit are produced by either female (pistillate) flowers or self-fertile (perfect, hermaphrodite) flowers. While large grape fruit are overwhelmingly produced on vines with female flowers, and large fruits produced on vines with self-fertile flowers are rare. Compared with self-fertile flowers, the stamens of female flowers are not completely developed and are nonfunctional, pollens from other pollinizers are necessary for fruit set. This process could be easily affected by environmental conditions. Unfavorable environments, such as cool and wet weather during blooming, may reduce the activities of pollen transferring agents, which would prevent the stigmas of female flowers from receiving pollen, thereby causing poor fruit set, resulting in low yield. In addition, calyptra drying, or cap sticking is common among female muscadine flowers but rare among self-fertile flowers, in which calyptras dry down and do not fall off the pistils, this prevents pollen from reaching the stigma, thus the flowers are not pollinated, and there is no fruit set. Therefore, vines with female flowers tend to have lower and more inconsistent yields than vines with self-fertile flowers, due to these physiological behaviors, but they produce larger fruit. Self-fertile flower cultivars, on the other hand, may complete the pollination/fertilization within their own flowers, pollen transfer from a pollinizer is not essential, environmental influences are minimized, and yields are stabilized. In addition, vineyard management could be simplified without the need for a pollinizer, which is an advantage for commercial production. This means grape vines with self-fertile flowers are always preferred by the grape industry, but self-fertile flowers vines have rarely produced the market-preferred large fruits. This is a paradox for the muscadine grape industry: female flower cultivars produce market required large fruits while possessing some production disadvantages to growers, on the other hand, self-fertile flower cultivars are preferred by growers but rarely produce the large fruit demanded by the market. Therefore, a self-fertile cultivar with large berry size, which offer big fruits for consumers and satisfactory production for growers, would benefit the table muscadine industry significantly, and work on this is necessary.

To promote the production and appreciation of premium grapes and fine wines from Florida, Florida A&M University's (FAMU) grape breeding program has been working intensively on table muscadine grapes, especially those with self-fertile flowers, over the past 20 years. 'O21-4-1' is a breeding line we obtained recently. It possesses two key traits as table grapes: self-fertile and large fruit. Together with other preferred horticultural characteristics, this breeding line shows high potential for the table muscadine grape industry.

Origin

'O21-4-1' originated from the grape breeding program at the Center for Viticulture and Small Fruit Research, FAMU, Tallahassee, FL. It is a hybrid of 'Supreme' × 'Triumph' in 2011 and was selected in 2015.

'Supreme' (Ison, 1990) is a muscadine grape cultivar (U.S. Plant Pat. No. 7267) introduced in the late 1980s for the fresh fruit market in the Southeastern United States. It was developed from a cross between the female 'Black Fry' (U.S. Plant Pat. No. 5824) and the pollen parent 'Dixieland' made by Mr. W.G. Ison. The distinguishing features of 'Supreme' were that it was an improved cultivar of muscadine grape at the time of its release and its large-sized black berry.

'Triumph' (Lane, 1980) was released in 1980 by R. Lane of the University of Georgia, it was selected from the cross of 'Fry' × Ga. 29-49. 'Triumph' is a self-fertile cultivar, producing medium large fruits with a bronze skin, nice flavor and sweet taste. Early

*Corresponding author. Email: zhongbo.ren@famu.edu

Table 1. Horticultural characteristics of 'O21-4-1' and leading table muscadine cultivars in Tallahassee, FL.

Culivar	Flower	Vigor	Internode length (cm)	Internode circle (cm)	Lateral shoot/ primary shoot[y]	Spur fruiting rate[z] (%)	Fruit cluster/ spur[z]	Fruit # per cluster[z]	Fruit/ spur	Spur productivity[z] (g fruit/spur)	Dry scar rate[z] (%)	Ripe rot rate[z] (%)
Fry	female	m-w	4.0	1.5	0.63	81	2.1	7.2	14	162.4	84.5	15.8
Supreme	female	m-w	3.8	1.5	0.22	91	2.0	6.0	12	182.4	83.1	4.7
Ison	perfect	m-h	4.5	1.7	0.48	94	2.8	10.8	29	263.4	85.0	5.1
O21-4-1	perfect	m	3.9	1.7	0.31	94	2.6	5.7	15	222.0	84.8	4.8

[z]Average of 20 random spurs from 2017 and 2018.
[y]2018 season.

Table 2. Fruit characteristics of 'O21-4-1' and leading table muscadine cultivars in Tallahassee, FL.

Cultivar	Color	Shape (L/D)	Size (g)	SSC[z] (%)	TA[y] (%)	pH	Seeds/fruit
Fry	bronze	0.99	11.6	17.2	0.40	3.36	3.5
Supreme	black red	1.04	15.2	15.1	0.40	3.24	3.6
Ison	black red	1.10	9.1	16.0	0.41	3.27	3.8
O21-4-1	black red	0.98	14.8	15.4	0.39	3.41	3.5

[z]SSC = sugar content.
[y]TA = titratable acidity.

ripening is a distinguishing characteristic of 'Triumph', its fruit ripens in mid-August in Tallahassee, FL.

The self-fertile flower and large fruit produced by 'O21-4-1' were first observed in 2015, and evaluation started in 2016. 'Supreme', 'Fry' and 'Ison' have been compared with 'O21-4-1' on major horticultural characteristics during evaluation (Table 1).

Evaluations were conducted at the vineyard in the Viticulture Center, FAMU, Tallahassee, FL. (30°47'67.87'N, 84°17'21,82'W). Vines were planted at density of 10 feet within rows and 12 feet between rows. Vines were trained into single-wire bilateral cordon system and were pruned with 3~4 buds spur-pruning technics annually. Commercial vineyard management was applied to the vines.

Spur productivity, based on total fruit weight of a spur, rather than the mostly used vine yields, were used to evaluate productivity in the evaluations. Spurs are the basic production unit of a vine, and total yield is the sum of fruit production of each individual spur. Spur productivity would not only reflect the overall productivity of a vine, it may also reveal more detailed information about the vine, and eliminate the influences of ages, sizes, and spur number among the vines on vine yields. Twenty random spurs were labeled around bud breaking for the purpose, data of fruits and lateral shoots were collected during fruit ripening.

Major Characteristics

Flowers. 'O21-4-1' bears self-fertile (perfect, hermaphroditic) flowers, it is self-fertility has been proven. Flower clusters (inflorescences) typically grow at the 3rd and 4th nodes. There are an average of 70 individual flowers per flower cluster.

Fruit Characteristics

Large fruit. 'O21-4-1' produces 14.8 g fruit, which is obviously larger than the fruit produced by 'Ison' (self-fertile), and 'Fry'(female), both of which are primary choices for table muscadine grapes, but slightly smaller than the fruit of 'Supreme' (female) (Fig. 1, Table 2), the largest fruit on the market today.

Fruit SSC. The fruit SSC of 'O21-4-1' was 15.4%, which is similar to other cultivars grown in Tallahassee, FL. (Table 2).

Pleasant taste. 'O21-4-1' fruits have very good characteristics such as firm flesh texture and aromatic flavor, which give an overall pleasant taste.

Uniform size. Uniform ripening and uniform fruit size have been consistent during the evaluation period from 2016 to 2018 (Figs. 1–3).

Fruit appearance. The color of 'O21-4-1' fruit is dark purple to near black at peak ripeness with inconspicuous lenticels, these inconspicuous lenticels occasionally give the skin some degree of roughness (Fig. 1). The fruits of 'O21-4-1' are round shaped (0.98 L/D).

Fruit dry scar. The average dry scar rate of 'O21-4-1' in 2017 and 2018 were 84.8%, had a similar dry scar fruit rate to 'Fry', 'Ison', and 'Supreme' (Table 1), which are generally are considered as high dry scar rates.

Smaller sized and semi compact clusters. The clusters of 'O21-4-1' are small and not compact with about 3~9 fruit (Fig. 2, Table 1), these clusters have the potential for better air flow than

Fig. 1. Fruit of 'O214-1' at harvest.

Fig. 2. Leaf, shoot tip, and fruit cluster of 'O214-1' during ripening.

Fig. 4. The canopy of 8-year-old 'O214-1' in mid-summer.

Fig. 3. Productivity of 'O214-1' during ripening.

the compact or very compact clusters found with most muscadine grapes, fruit disease incident may be reduced by such a cluster and is a preferred trait for grape growers.

Ripe rot. The ripe rot of 'O21-4-1' was 4.8%, obviously lower than that of 'Fry', and similar to 'Ison' and 'Supreme' (Table 1). The smaller cluster may contribute to this low ripe rot rate.

Productivity

'O21-4-1' produced 222g fruit per spur, which seems higher than that of 'Fry' and 'Supreme', but lower than the total fruits produced per spur by 'Ison' (Table 1). This indicates a satisfactory production potential (Fig. 3) of 'O21-4-1', as the yield of a vine consists of all fruit produced by every spur.

Growth Habit

'O21-4-1' has a moderately vigorous vine. Shoot internode length and circle are similar to those of 'Ison' (Table 1). Shoots tend to grow horizontally to semi-erectly (Fig. 4). 'O21-4-1' has a light to moderate lateral shoot growth habit; an average of 0.3 lateral shoot was observed for each primary shoot under commercial vineyard management practices (Table 1). The microclimate within the side canopy may be better for a vine with fewer lateral shoots than for an over-crowded canopy which has excessive lateral shoot growth.

Annual Growth Circle

In Tallahassee, FL, 'O21-4-1' bud break is the end March–early April, it blooms in late May, fruit veraison starts in mid-late July, fruits ripen in early September, and leaves fall in late December.

Symptoms of Disease

Pierce's disease (PD) symptoms has not been observed on 'O21-4-1' during the evaluation period, but a few black rot symptoms on leaves were observed during or after harvest.

In brief, 'O21-4-1' has been able to produce very large muscadine grape fruits with self-fertile flowers, and its productivity has been satisfactory. Together with several other preferred horticultural traits, this breeding line has demonstrated promising potential for the table muscadine industry.

Literature Cited

Ison, W. 1990.'Supreme'. U.S. Plant Pat. No. 7267 Filed 11 Mar. 1988. Issued 10 July 1990.

Lane, R.P. 1980. Triumph' Muscadine Grape. HortScience 15:322.

Proc. Fla. State Hort. Soc. 132:50–52. 2019.

Subtropical Stone Fruit Production in Florida: A Review

Ali Sarkhosh*, Trequan M. McGee, and José X. Chaparro

Horticultural Sciences Department, University of Florida/IFAS, P.O. Box 110690, Gainesville FL 32611

Additional index words. Fruit quality, production technologies and strategies, stone fruit, management practices

The stone fruit industry in Florida once had over 4000 acres in production in north-central Florida. However, challenges in marketing and a series of freezes affected the industry. As a result, by 2008 the industry was significantly reduced to fewer than 400 acres. A successful stone fruit breeding program at the University of Florida has released varieties well-adapted to sub-tropical production regions. Some of these varieties have formed the basis of low chill stone fruit industries in subtropical countries. Since 2003, the breeding program has shifted to breed varieties with even lower chilling requirements. Growing these varieties in central and southern Florida not only reduced the risk of production failures, but also allowed growers to target a crucial market gap from late March to early May when neither international exports nor domestic production could satisfy U.S. demand. All these breeding programs, accompanied by extensive research and extension programs on low chill stone fruit production, helped the Florida peach industry produce over seven million pounds fruit in 2017 on an estimated 1800 acres. This indicates a resurgence of interest in low chill stone fruit production, especially peaches in Florida as growers are looking for a profitable substitute fruit crop to diversify their production systems. Producing high-quality stone fruit while reducing production costs will allow sustainable development of an industry in Florida. Orchard management or pre-harvest practices can significantly affect fruit characteristics such as fruit size, sweetness, firmness, etc., which can directly affect consumers' preferences and buying patterns. To improve fruit quality in low-chill stone fruit in Florida, research on the adoption of superior management practices as well as strategies for the extension of these practices to growers is needed. Although, most of these technologies and strategies already exist for growing stone fruit in temperate zones. Success and challenges are presented and discussed in this review.

Past and Current Production

Florida stone fruit production was once located in the northern-central region and consisted of medium-chill peach cultivars (Ferguson et al., 2006; Olmstead, 2010). Cultivars grown in this area could expect to receive between 400 to 700 chilling units (Fig. 1). The industry lacked overhead irrigation during this time and was subsequently set back by several freeze events. The freeze events, accompanied by maketing challenges and domestic competition, resulted in a lack of support for stone fruit production, followed by a drop in acreage in stone fruit production.

The University of Florida (UF) stone fruit breeding program has been successful in producing varities suited for sub-tropical production with chilling requirements of 350 to 450 chilling units but needed to shift focus if it wanted to make a reemergence (Andersen et al., 2001). Varities produced from the UF stone fruit breeding program are grown in subtropical areas of the world, but after the challenges with freezes in northern Florida the majority of varieties are now low-chill (less than 200 chill units) cultivars that could be grown in the central and southern regions of Florida. Growers in these areas are able to avoid damage caused by freezes with proper site selection and overhead irrigation (Sherman and Lyrene, 2003). This shift to central and southern Florida allows growers to ship peaches to the market between late-March and early May, a period where foreign and domestic peaches do not meet the demand (Olmstead, 2010).

The United States (U.S.) marketing season peaks from 20 May 20 to 30 Sept., thus Florida peaches could dominate the market for several weeks prior to peak domestic production, allowing Florida growers to capture higher price margins. As of 2017, there were 20 states producing peaches with the top producers being

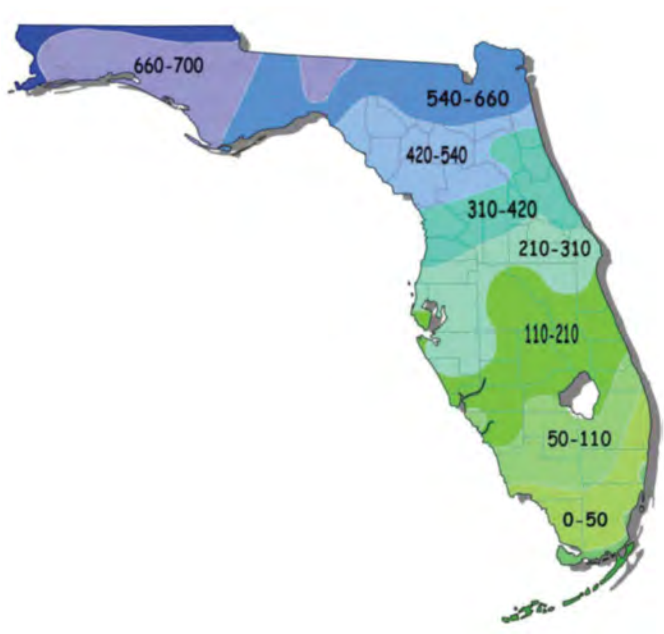

Fig. 1. Estimated chill unit accumulation for Florida. Photo: University of Florida/IFAS.

*Corresponding author. Email: sarkhosha@ufl.edu

California, South Carolina, Georgia, and New Jersey, but the shift in production for Florida alleviates the domestic competition as no other state gets peaches to the market as early (Agricultural Marketing Service, 2019).

The breeding program has produced several low-chill cultivars that are performing well in Florida and other places with similar climates. UF varities 'UFBeauty', 'UFGold', 'TropicSnow', 'UFO', and 'Flordabest' require 200–300 chill units and represent the cultivars suited for production in central Florida (Sarkhosh et al., 2018). UF varities 'UFSun', 'UFBest', 'UFGem', 'Flordaprince', 'Flordaglo', and 'TropicBeauty' require 100–200 chill units and represent the cultivars suited for production in southern Florida (Sarkhosh et al., 2018). The industry has successfully rebounded to approxiamtely 2000 acres of low-chill peaches up from 400 acres in 2008. Another success of the stone fruit program was the development of the rootstock 'Flordaguard'. This rootstock was an important development because Florida is home to the peach root-knot nematode, *Medloidogyne floridensis*, and 'Flordaguard' has resistance to this species unlike other popular root-stocks (Handoo, et al., 2004; Sherman et al 1991). Most of the acreage is now planted with 'UFSun', 'UFOne', 'UFBest', and 'TropicBeauty' graphed onto 'Flordaguard'. Growers also now have access to overhead irrigation and, in the event of a radiation freeze, they can now properly protect the crop unlike in the 1980s. Now that Florida peaches have overcome the major marketing challenges that once plagued the industry, they can be found in major grocery stores as well as at pick your own operations.

Fig. 2. Budbreak in peach cv. Tropicbeauty when (left) chilling requirements have been met, and (right) when they have not. Photo: Ali Sarkhosh.

Current Research and Extension

Although the stone fruit industry has addressed the original concerns of marketing and freeze protection, challenges continue to arise. The stone fruit extension program's research emphasis is now focused on three major components of production: climate change; fruit quality and size; and labor costs. Climate change brings the concern of not being able to attain chilling hour requirements, high temperature stress, and abiotic stresses like insects, disease, and flooding. We will go further into details on climate change later. Fruit quality and size bring the concern of experiencing undesireable fruit size and the need to increase shelf life. Peaches typically have a fruit development period of about 120 d, but with early ripening culitvars like the UF varities, it may be as short as 60–90 d (Sarkhosh et al., 2018). The fruit development period is critical for fruit size as the longer the period the larger potentional fruit could be. A shorter development period results in small fruit, and since retailers only accept peaches with a diameter of at least 2.5 inches, thinning may be required to produce larger fruit size and better flavor (Chang et al., 2018). Labor costs include hand labor related to training systems, prune, and fruit thinning. Peaches require more work than citrus, a major tree fruit in Florida, as manual labor is needed for pruning (twice a year), thinning, and harvesting. These practices are especially evident in Florida where the climate is conducive for trees to grow vigoursly through November (Sarkhosh and Ferguson, 2018). Studies to make management processes more effective and efficient are underway in hopes of reducing the hand labor associated with growing stone fruit.

Climate change is one of a main concern since chill unit acculmulation has been decling over recent seasons. A single chill unit is defined as one hour between 0 °C to 7.2 °C (Weinberger, 1956; Richardson et al., 1974; Sharpe et al., 1990). Failure to achieve chill unit requirements results in delayed and prolonged bud break and uneven shoot development, flowering and fruit maturity. This makes management strategies difficult because different parts of a tree are at different growth stages. This also requires a grower to pay labor for longer periods of time to harvest (Fig. 2). Abnormal growth can also occur, for example apical dominance on scaffold branches imparting paradormancy on fruiting wood. The threat of not achieving chilling unit requirements is a growing concern as over the last couple of years as most of the major production counties have not been reaching their historic average chilling unit.

Recently, flooding has become a bigger threat to Florida production as reports indicate that over 1 billon dollars in total crop loss occurred due to flooding between 2008–11 (Pimentel et al., 2014). Florida growers reported major peach loss due to flooding during hurricane Irma. With peach being deemed a hypoxia sensitive crop combined with the high amounts of rainfall typically experienced in Florida during hurricane season, flood resistant root-stocks would be a great addition to the tools Florida growers can currently access. Studies are underway to investigate several root-stocks and their ability to endure prolonged flooding of up to 1 week (Fig. 3). In addition to flooding, another major issue is the excess vegetative growth experienced by Florida growers. Conditions favorable for vegetative growth are present longer in Florida than in more temperatre states, thus Florida orchards tend to experience excessive vegetative growth that can compete with reproductive growth. Studies are underway to find ways

Fig. 3. Wood samples of scion 'UFSun' (top) and rootstock 'Flordaguard' (bottom) when not flooded (left 3 samples) and flooded (right 5 samples). Tissue necrosis can be seen in the rootstock tissue of the flooded treatments while there is some minor necrosis in scion tissue after 7 d of flooded conditions. Photo: Trequan McGee.

to reduce the amount of vegetative growth and promote greater reproductive growth.

The stone fruit industry in Florida has had its ups and downs but there is certainly hope for the industry to continue to grow and for stone fruit to serve as alternative crops for growers looking to diversify their crop portfolio. The industry has come a long way and has things in the pipeline that will make it even more robust.

Literature Cited

Agricultural Marketing Resource Center-AMRC. 2019. Peaches. 30 Sept. 2019. <https://www.agmrc.org/commodities-products/fruits/peaches>

Andersen, P.C., W.B. Sherman, and J.G. Williamson. 2001. Low chill peach and nectarine cultivars from the University of Florida breeding program: 50 years of progress. Proc. Fla. State Hort. Soc. 114:33–36.

Ferguson, J.J., J.X. Chaparro, D.M. O'Malley, and L. Harrison. 2006. Options for subtropical peach production in Florida. Proc. Fla. State Hort. Soc. 119:29–31.

Chang, Y., A. Sarkhosh, J. Brecht, P. Andersen. 2018. Thinning Florida peaches for larger fruit. HS1324, UF/IFAS Extension. 6 p.

Handoo, Z.A., A.P. Nyczepir, D. Esmenjaud, J.G. v.d. Beek, P. Castagnone-Sereno, L.K. Carta, A.M. Skantar, and J.A. Higgins. 2004. Morphological, molecular, and differential-host characterization of *Meloidogyne floridensis* (Nematoda: meloidogynidae), a root-knot nematode parasitizing peach in Florida. J. Nematology. 36(1):20–35.

Olmstead, M.A., 2010. The role of research and extension in establishment of a Florida stone fruit industry. Proc. Fla. Hort. Soc. 123:11–13.

Pimentel, P., R.D. Almada, A. Salvatierra, G. Toro, M.J. Arismendi, M.T. Pino, B. Sagredo, and M. Pinto. 2014. Physiological and morphological responses of *Prunus* species with different degree of tolerance to long-term root hypoxia. Scientia Hort., 180:14–23.

Richardson, E.A., S.D. Seeley, and D.R. Walker. 1974. A model for estimating the completion of rest for 'Redhaven' and 'Elberta' peach trees. HortScience 9(4):331–332.

Sarkhosh, A., and J. Ferguson. 2018. Training and pruning Florida peaches, nectarines, and plums. HS1111, UF/IFAS Extension. 5 p.

Sarkhosh, A., M. Olmstead, J. Chaparro, P. Andersen, J. Williamson. 2018. Florida peach and nectarine varieties. Cir 1159, UF IFAS Extension. 17 p.

Sharpe, R.H., W.B. Sherman, and J.D. Martsolf. 1990. Peach cultivars in Florida and their chilling requirements. Acta Hortic. 279:191–197.

Sherman, W.B. and P.M. Lyrene. 2003. Low chill breeding of deciduous fruits at the University of Florida. Acta Hort. 622:599–605.

Sherman, W.B., P.M. Lyrene, and R.H. Sharpe. 1991. Flordaguard peach rootstock. HortScience, 26(4):427–428.

Weinberger, J.H., 1956. Prolonged dormancy trouble in peaches in the southeast in relation to winter temperatures. Proc. Amer. Soc. Hort. Sci. 67:107–112.

Proc. Fla. State Hort. Soc. 132:53. 2019.

—Scientific Note—

Parasitic Nematodes on Sugarcane in Florida

Stewart Swanson*[1], William Crow[2], and Benjamin Waldo[2]

[1]*University of Florida/IFAS, Hendry County Extension, P.O. Box 68, LaBelle, FL 33975*

[2]*Entomology and Nematology, University of Florida/IFAS, PO Box 110620, Gainesville, FL 32611*

Additional index words. nematodes, sugarcane, Florida

The diversity of plant-parasitic nematodes is higher in sugarcane than most other crops, with over 310 species of 48 genera having been reported from sugarcane production areas around the world. (Cadet. and Spaull, 2005). Yield losses due to nematodes of 10 to 20% have been documented in many countries producing sugarcane. (Blair and Stirling, 2007; Cadet and Spaull, 1985; Shakeel et.al., 2009; Machado, 2014; Bond et al., 2000).

In the 1960s, pre-plant treatment with available nematicides in Florida was shown to increase both sugarcane tonnage and brix (Winchester, 1964). Nematode surveys conducted in Florida have shown the presence of a wide diversity of nematode species (Hall and Irey, 1992; Swanson, 2016).

With no registered nematicides available there has been no further work done on sugarcane in Florida. The lack of knowledge in Florida about what constitutes a significant economic population for various species of nematodes, and what the yield loss potential may be is an important agronomic issue for Florida sugarcane producers. Adama Corporation has a nematicide, Nimitz (fluensulfone), which was labeled for sugarcane in 2018.

Two replicated efficacy trials for Nimitz were instituted in 2017, however, initial high nematode populations disappeared after the production area in South Florida received over twelve inches of rain from hurricane Irma in Sept. 2017. No efficacy data was generated when the nematode population crashed.

Presently there are four separate tests sites for Nimitz efficacy. Two sites used an injection rig to apply the chemical at three rates on each side of the stubble cane following harvest. On one site, three rates of the pesticide were sprayed in one foot bands on each side of the row and then irrigated in with 1 inch of water. The fourth site consists of one rate of Nimitz applied under three different irrigation regimes: sub-surface seep, overhead, and drip. So far horticultural data, stand counts, plant height, and chlorophyll readings between treatments have shown no statistical differences. Nematode populations are being monitored and early results show some efficacy of Nimitz in some of the treatments. Yield and quality parameters will be measured next fall at harvest and will be the true test of what impact nematodes may pose for Florida sugarcane producers.

The goal of this project to generate information that will help sugarcane growers in Florida make management decisions with respect to the need for chemical control of nematodes. It will serve as a first step in evaluating economic thresholds for nematode populations and document the degree of yield loss that nematodes may be causing.

Literature Cited

Blair, B.L. and G.R. Stirling. 2007. The role of plant-parasitic nematodes in reducing yield of sugarcane in fine-textured soils in Queensland, Australia. Australian J. Expt. Agric. 47(5):620–634

Bond, J.P., E.C. McGawley, and J.W. Hoy. 2000. Distribution of plant-parasitic nematodes on sugarcane in Louisiana and efficacy of Nematicides. Suppl. J. Nematol. 32(4S):493–501.

Cadet, P. and V.W. Spaull, 1985. Studies on the relationship between nematodes and sugarcane in South and West Africa: Plant cane. Revue Nematol. 8(2):131–142.

Cadet, P. and V.W. Spaull. 2005. Nematode parasites of sugarcane. p. 645-671. In: R. Sikora (ed.). Plant parasitic nematodes in subtropical and tropical agriculture, 2nd Ed. Chap. 17, CABI Pub., UK.

Hall, D.G. and M.S. Irey. 1992. Population levels of plant-parasitic nematodes associated with sugarcane in Florida, J.Amer. Soc. Sugarcane Technologists. 12:38–46.

Machado, C. 2014. Current nematode threats to Brazilian agriculture. Current Agric. Sci. and Technol. 20:26–35.

Shakeel, Q. S.A. Anwar, J. Nazir, and M. Shahid. 2009. Incidence of root knot nematode, *Meloidogyne javanica* infecting sugarcane, Jhang, Punjab, Pakistan. Pakistan J. Phytopath. 21(1):66–70.

Swanson, S. 2016. Nematode survey of sugarcane fields in the Clewiston, Fla. area. Unpublished data.

Winchester, J.A. 1964. Sugarcane nematodes and their control in Florida. Soil and Crop Sci, Soci. Fla. Proc. 24:454–457

*Corresponding author. Email: stew@ufl.edu

Proc. Fla. State Hort. Soc. 132:54–61. 2019.

Influence of Rootstock and Soil Environment on Citrus Rhizosphere Composition

John M. Santiago, Ute Albrecht, and Sarah L. Strauss*

Southwest Florida Research and Education Center, University of Florida Institute of Food and Agriculture Sciences, 2685 State Rd. 29N, Immokalee, FL 34142

Additional index words. citrus, microbiology, rhizosphere, rootstock, soil

Rootstocks are critical components for tree-fruit production due to their influence on vigor, fruit quality, harvestable yield, resistance to pests, and tolerance against environmental stresses. While it is recognized that rhizosphere microbial composition can significantly impact plant growth and nutrient uptake, the influence of the surrounding soil on the developmental process of rhizosphere microbial communities remains unclear. This may be particularly important for citrus, as trees are grown in potting medium in enclosed greenhouses prior to planting in the field, making the initial rhizosphere development critical for later plant growth. A greenhouse experiment was conducted to determine the influence of soil environment on citrus microbial community development. The experiment used the citrus rootstock US-802 [‘Siamese’ pummelo (*Citrus grandis* Osbeck) × ‘Gotha Road’ trifoliate orange (*Poncirus trifoliata*)] planted in four potting media: a sterile sand control, coir-perlite, peat-perlite, and plant-based compost. Rhizosphere samples were collected immediately after transplant, and 1 month, 2 months, 4 months, and 6 months after transplant. Rhizosphere DNA was extracted and sequenced using Illumina high-throughput amplicon sequencing, and data were analyzed using QIIME2 and R. The compost had significantly greater nitrogen and phosphorus concentrations compared to the other potting media. Compared to plants grown in other potting media, those grown in compost were larger and had a greater above and belowground biomass and leaf chlorophyll concentration. The rhizosphere of plants grown in compost had significantly greater bacterial diversity compared to other potting media.

Citrus and other fruit and nut tree crops are commonly grown by grafting a scion onto a rootstock (Alves et al., 2017). The rootstock functions as the root system that supplies nutrients, water, and hormones to the scion (Liu et al., 2017), whereas the scion is the portion of the plant that produces the fruit or nuts (Albacete et al., 2015). Rootstocks are bred for improved tree growth and disease resistance (Harrison et al., 2016), including tree vigor and size, fruit quality, harvestable yield, resistance to pests, and tolerance of adverse environmental conditions (Nawaz et al., 2016). Additionally, rootstocks are bred to enhance a crop’s ability to endure abiotic stresses, which can include salinity, pH, and temperature (Salis et al., 2017).

The health and yield of citrus crops are not only influenced by rootstock genotype, but also by the soil environment, notably the rhizosphere (Bakker et al., 2015). The rhizosphere is the area in the soil that surrounds the roots of plants and is characterized by plant-microbe interactions that potentially impact plant health (Schreiter et al., 2014). For example, plant growth promoting rhizobacteria (PGPR) that colonize the rhizosphere can form mutualistic relationships with their plant host, aiding in the acquisition of nutrients and host defenses prior to infection (Vejan et al., 2016). Rhizosphere microbial community composition can be shaped by characteristics of the soil (Ragot et al., 2016), including soil texture, organic matter content, and pH (Dijkstra and Cheng, 2007). Soil organic matter (SOM) serves as a carbon (C) source for heterotrophic microbes (Lambers et al., 2009). Soil pH affects the availability of nutrients for both plants and microbes by modifying adsorption and desorption reactions (Ragot et al. 2016). Soil texture, or particle size, is based on mineralogical composition, which corresponds to stages of weathering with primary minerals (Zhang et al., 2015). Differences in soil texture can affect pore size, impacting the relative magnitude of capillary and gravitational forces, thereby affecting the water available within a pore space (Chau et al., 2011).

While the overall rhizosphere composition is influenced by plant exudates (Tsuno et al., 2018) and the soil environment (Peiffer et al., 2013), the development of rhizosphere microbial communities under different soil environmental characteristics over time is still not well understood. Previous studies of the rhizosphere of *Arabidopsis thaliana* found that variation in soil type (Urbina et al., 2018) and plant genetics (Micallef et al., 2009), even within the same species, can result in distinct rhizosphere communities.

In this study, we examined the influence of the soil environment on the recruitment of the citrus rhizosphere community. The experiment used four different potting media—compost, peat/perlite, coir, and sterile sand—that vary in their nutrient and water holding capacity, texture, and microbial community structure and composition. Compost typically has high SOM content and microbial diversity (Wickramatilake et al., 2011). Peat perlite is a commercial potting mix that provides contrast in both organic matter concentration and texture between soil treatments and likely differs with respect to microbial community structure and composition due to the nature of the organic matter and nutrient-retention capabilities. Coir is extracted from coconut husks and is sometimes used in citrus nurseries because of its high-water holding capacity. Sterile sand was chosen to determine if the microbial community component of the soil environment is

We thank Rachel Berner and Bryce Meyering of the University of Florida, IFAS (UF/IFAS) Southwest Florida Research and Education Center for their help with experimental set-up, sample collection, and soil DNA extractions. This work was supported by the UF/IFAS Early Career Scientist Seed Fund.

*Corresponding author. Email: strauss@ufl.edu

critical to rhizosphere development. It was hypothesized that the soil environment with the greatest microbial diversity and nutrient concentrations [nitrogen (N) and phosphorus (P)] would result in the most diverse citrus rhizosphere microbiome.

Material and Methods

Experimental design and growth conditions. The study was conducted at the University of Florida, IFAS Southwest Florida Research and Education Center (SWFREC), Immokalee, Fla. Citrus were planted into four potting media: a plant-based compost (R&D Soil Builders, Inc., Immokalee, FL); a peat perlite mix with 70 peat:10 perlite:20 vermiculite (Sun Gro Horticulture Ltd., Orlando, FL); an 80:20 coir perlite mix (Southeast Soils Inc., Okahumpka, FL); and an Immokalee fine sand collected from the SWFREC. The sand was sterilized to serve as a control using a PRO-GROW Model SS-60 (Pro-Grow Supply Corp, WI) at 77 °C in two one-hour intervals, 24 hours apart. Citrus rootstock US-802 ['Siamese' pummelo (*C. grandis* Osbeck) × 'Gotha Road' trifoliate orange (*P. trifoliata*)] were grown from seed. To aid in germination, the seed coats were removed. Seeds were incubated in 1 L of water at 37 °C for 1.5 h. The pH of the water was adjusted to 11.5–12.5, and the seeds were incubated at 37 °C in for an additional 1.5 h. Seeds were then removed from solution, rinsed, and dried overnight in a shady, well ventilated area prior to planting. All seeds were germinated in the peat perlite mix (164-mL containers) for eight weeks prior to transplanting into 4-L plastic pots (Stuewe and Sons Inc., Tangent, OR) containing the different potting media.

Plants were grown in a greenhouse with a temperature ranging from 24 °C to 32 °C. A total of 6 replicates were used for each treatment. Plants were destructively sampled immediately after transplanting and 1 month, 2 months, 4 months, and 6 months after transplanting. A drip irrigation system was used to provide fertilizer and water. Tracite 20–20–20 fertilizer [20% N, 20% phosphate (P_2O_5), 20% potash (K_2O), Helena Agri-Enterprises, LLC, TN] was supplied at 2% dilution with a concentration of 400 ppm N. The total N of the fertilizer was composed of 4% ammonia 6% nitrate, and 10% urea. Fertilizer was applied twice a week during the first 2 months. During the dormant growth period (4- and 6-month time points) applications were reduced to once every two weeks. Foliar symptoms of iron deficiency became apparent in the 2-month-old plants grown in the coir and sterile sand treatments, manifested as yellowing of the leaves. Sequestrene 138 (BASF AG Products Inc., NC) was applied once a week to aid iron deprived plants, notably the coir. Symptoms of iron limitation subsided after 2 weeks.

Soil nutrient concentrations. Soil nutrient concentrations of potting media were analyzed by the soil testing lab at the University of Florida, IFAS Everglades Research and Education Center, Belle Glade, FL, prior to planting and 6 months after planting. Total P was measured by ashing soil samples in a muffle furnace for 16 h, followed by extraction with 6M Hal and analysis with ICP-OES (Spectro Arcos, Kleve, Germany). Total Kjeldahl nitrogen (TKN) was determined by digesting ashed samples followed by colorimetric determination using EPA Method 365.4 (EPA, 1992). Organic matter content was determined based on loss on ignition (LOI) of soils (Myalavarapu et al., 2002).

Plant growth parameters. At each sample collection, plant height was measured from the bottom of the stem to the crown of the apical meristem. The total chlorophyll concentration of each plant was determined with a Soil-Plant Analyses Development (SPAD) meter (Konica Minolta Sensing, Inc., Sakai, Osaka, Japan). Plants were excavated and separated into aboveground (shoots and leaves) and belowground (roots) parts. Roots were washed to remove any adhering potting medium. Both the aboveground and belowground portions of plants were oven-dried at 53 °C for one week to determine dry weight. After 6 months, roots were separated into fibrous roots (< 2 mm in diameter), medium-sized roots (2–5 mm in diameter), and large roots (> 5 mm in diameter). Fibrous roots were scanned with a Cannon MG 3620 scanner using Assess 2.0 (Lobet et al., 2017) to determine root length. To determine specific root length, the root length was divided by the dry weight of the scanned roots.

DNA isolation. Rhizosphere sampling occurred prior to washing the roots by removing a subsample of fibrous roots and shaking off the attached potting medium. The portion of potting medium that remained attached to the root surface after shaking was considered the rhizosphere. Approximately 50 g of rhizosphere soil was collected from each plant. Samples were stored at –20 °C prior to DNA extraction. Approximately 15 mL of 1× sterile phosphate-buffered saline (800 mL distilled water, 8 g NaCl, 0.2 g KCl, 1.44 g Na_2PO_4, and 0.24 g KH_2PO_4) was added to the sample, which was then shaken by hand for 15 sec. Roots were removed with forceps and discarded, and the remaining soil was centrifuged at 3000 g for 15 minutes. The supernatant was discarded. Soil DNA was extracted from 0.25 g of the soil pellet using the DNeasy PowerSoil Kit (Qiagen Inc., Germantown, Md.) according to the manufacturer's instructions.

DNA quantification. A Qubit fluorometer (Thermofisher Scientific, Wilmington, N.C.) was used to immediately quantify the extracted DNA prior to storage at –20 °C. MiSeq Illumina high-throughput amplicon sequencing of bacterial 16S rRNA gene region was performed at the DNA Services Facility at the University of Illinois, Chicago. The universal primers 515Fa/926R (Caporaso et al. 2011) were used to amplify the V4 and V5 regions of the bacterial 16S rRNA gene.

Bioinformatics. After sequencing, bioinformatic data were processed using DADA2 (Callahan et al., 2016) within the Qiime2 (Caporaso et al., 2010) package. Raw sequences were demultiplexed prior to truncating paired sequences to uniform lengths. Forward and reverse reads were truncated at 289 bp and 222 bp, respectively. Only reads with a quality score < 27 were kept. Sequences were dereplicated and unique sequence pairs were denoised. DADA2 was then used to filter chimeras, primers, and adapters, as well as assemble pair-ended sequences. Taxonomy was assigned to Operational Taxonomic Units (OTUs) in the feature table produced by DADA2 with the SILVA 119 Naïve Bayes classifier at 97% sequence similarity cutoff. Additionally, chloroplast and mitochondrial 16S rRNA gene sequences were removed. Only OTUs comprised of over 1000 reads per sample were analyzed.

Statistical analysis. Statistical analyses were conducted using R software 3.6.0 (RStudio, Boston, MA). Bacterial alpha diversity was estimated for each sample using the Chao1, Shannon, and Simpson diversity indices. Plant growth parameters and bacterial 16S rRNA gene abundance were log-transformed to meet the assumptions for normal distribution and homogeneity of variance. Differences between potting media were determined by one-way analysis of variance (ANOVA) with the aov function. Significant effects observed between the treatments were further analyzed with Tukey's honestly significant difference (HSD). Differences were considered significant at $P < 0.05$.

A $\log_{10}$ (x+1) formula was used to transform and calculate OTU relative abundances. Differences in mean relative abundance of bacterial classes after transplant and after 6 months were determined using a Kruskal-Wallis test. Weighted UniFrac and Bray-Curtis dissimilarities between samples were calculated through the vegan package in R (Oksanen et al., 2017) and used for principal coordinates analysis (PCoA). A permutational multivariate analysis of variance (PERMANOVA) was later performed on weighted Unifrac dissimilarities to determine the effects of the treatments on rhizosphere microbial community composition.

Results

Soil Nutrient Concentrations. The organic matter content of each potting medium prior to the start of the experiment was significantly different. Coir had the greatest percent organic matter (84.8% ± 1.4), followed by peat perlite (66.9% ± 0.7), compost (21.3% ± 0.9), and sterile sand (0.9% ± 0.1, Fig. 1A). Compost had a significantly greater total N concentration (3630.5 mg/kg ± 341.8) compared to the coir (2413 mg/kg ± 287.4) and sterile sand (132.1 mg/kg ± 24.3, Fig. 1B). Compost also had significantly greater total P concentration (1636.8 mg/kg ± 66.3) compared to the peat perlite (841.5 mg/kg ± 48.5), coir (260.1 mg/kg ± 22.3), and sterile sand (92.3 mg/kg ± 7.2, Fig. 1C).

After 6 months, significant differences in organic matter content were still present between potting media. The coir treatment had the greatest organic matter content (74.4% ± 1.9) followed by peat perlite (58.8% ± 0.7), compost (23.5% ± 1.1), and sterile sand (0.6% ± 0.2, Fig. 1A). In addition, the compost treatment had a significantly greater total N concentration (6981.4 mg/kg ± 428.5) compared to the peat perlite (5514.1 mg/kg ± 133.5), coir (4683.6 mg/kg ± 145.4), and sterile sand (156.7 mg/kg ± 11.9, Fig. 1B). Significant differences in the total P concentrations were also present between potting media. Compost had the greatest total P concentration (1847 mg/kg ± 69.4), followed by peat perlite (995.1 mg/kg ± 78), coir (441.5 mg/kg ± 61.4), and sterile sand (135.6 mg/kg ± 13, Fig. 1C). Interestingly, coir, compost, and peat perlite media had significantly greater total N concentrations after 6 months compared to the start of the experiment prior to planting (Fig. 1B). Furthermore, coir, compost, and peat perlite had significantly greater total P concentrations after 6 months compared to before planting (Fig. 1C). Significantly less organic matter content was also observed in both the coir and peat perlite treatments after 6 months compared to before planting (Fig. 1A).

Plant growth parameters. After 6 months of growth, significant differences were present in the dry weight of aboveground plant biomass, total belowground plant biomass, biomass of fibrous roots, and biomass of large roots depending on the potting medium the plants were grown in (Fig. 2, A–D). Plants grown in compost had significantly greater aboveground plant biomass (37.6 g ± 1.7) compared to plants grown in peat perlite (11.8 g ± 1.8), sterile sand (8.9 g ± 1), and coir (2.1 g ± 0.3, Fig. 2A). Plants grown in compost also had significantly greater total belowground biomass (20.9 g ± 1.8) than plants grown in peat perlite (11.1g ±1), sterile sand (7.5 g ± 0.7), and coir (3.7 g ± 0.4, Fig. 2B). Biomass of fibrous roots was significantly greater in plants grown in compost (9.5 g ± 0.8) compared to plants grown in peat perlite (5.1 g ± 0.4), sterile sand (3.2 g ± 0.4), and coir (1.1 g ± 0.1, Fig. 2C). The biomass of large roots was significantly greater in plants grown in compost (7.9 g ± 2.4) than plants grown in peat perlite (3.4 g ± 0.7), sterile sand (2.7 g ± 0.4), and coir (0.9g ± 0.3, Fig. 2D).

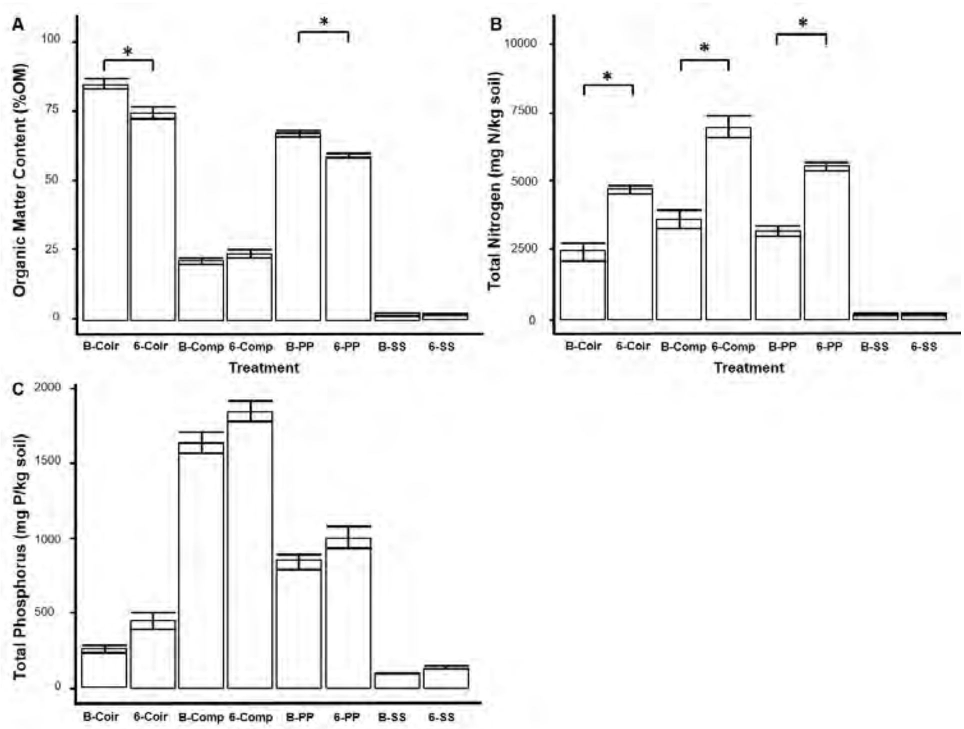

Fig. 1. Soil nutrient concentration of: **A)** organic matter, **B)** total nitrogen (mg/kg), and **C)** total phosphorus (mg/kg) of coir, compost (Comp), peat-perlite (PP), and sterile sand (SS) before planting (B) and 6 months after planting (6). Time points within a treatment with * were considered significantly different (*P*-values: * = < 0.05, ** = < 0.01, *** = < 0.001). Error bars represent standard errors of the mean.

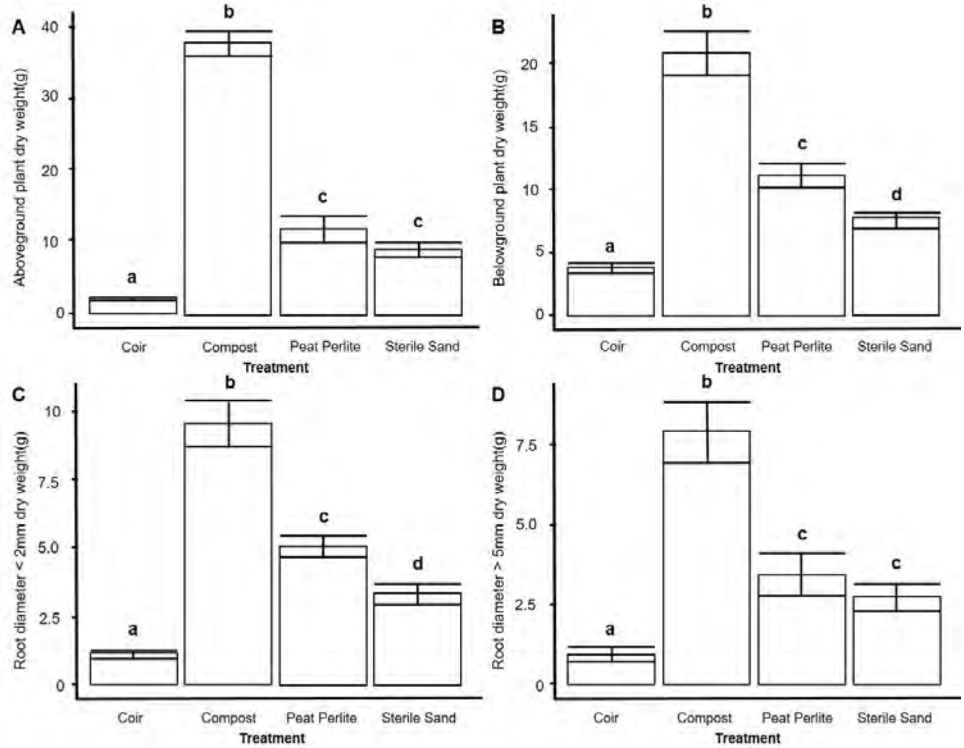

Fig. 2. Dry weight (g) of: **A**) aboveground plant biomass, **B**) belowground plant biomass, **C**) fibrous root biomass and **D**) large root biomass from citrus rootstock seedlings {US-802 ['Siamese' pummelo (*Citrus grandis* Osbeck) × 'Gotha Road' trifoliate orange (*Poncirus trifoliata*)]} grown in four different potting media. Different letters indicate significant differences between treatments. Error bars represent standard error of the mean.

Growing plants in coir resulted in significantly less aboveground, belowground, fibrous root, and larger root biomass compared to the other potting media (Fig. 2, A–D). There were no significant differences in the specific root length of plants grown in compost (0.65 m/g ± 0.02), peat perlite (0.61 m/g ± 0.02), sterile sand (0.6 m/g ± 0.04), and coir (0.69 m/g ± 0.02).

Leaf chlorophyll conce ntration and height of plants after 6 months were significantly different between treatments. Plants grown in compost had a significantly greater chlorophyll concentration (61.65 SPAD Units ± 2.2) than plants grown in peat perlite (35.43 SPAD Units ± 3.0), sterile sand (45.32 SPAD Units ± 2.5), and coir (16.36 SPAD Units ± 3.7). Plant height was significantly larger when plants were grown in compost (155.17 cm ± 1.55) than when they were grown in peat perlite (82.0 cm ± 2.0), sterile sand (87.58 cm ± 2.35), and coir (23.83 cm ± 0.53).

Rhizosphere microbial community changes in response to treatments. After the high-throughput amplicon sequence data were processed and quality filtered using DADA2, a total of 10,146,136 sequences were obtained from 120 samples with an average of 84,551 sequences per sample. There was a range of 28,442 to 186,962 reads across the 120 samples. Rhizosphere bacterial alpha diversity was significantly different between treatments and varied according to the Chao1 (richness), Shannon (richness and evenness; Fig. 3, A–E), and Simpson (richness and evenness) indices. In the compost, significantly greater rhizosphere bacterial Chao1, Shannon (Fig. 3A), and Simpson indices of alpha diversity were observed after transplant compared to the coir and sterile sand. After 1 month, significantly greater rhizosphere bacterial alpha diversity for all three indices was found in compost compared with the other potting media. The rhizosphere bacterial Chao1 index of alpha diversity of coir was significantly greater than the rhizosphere of the sterile sand after 2 months; however, significant differences were not present in the Shannon ($P = 0.09$) and Simpson ($P = 0.23$) indices of alpha diversity. After 4 and 6 months, in both the compost and sterile sand, there was a significantly greater bacterial Shannon index of alpha diversity than in coir (Fig. 3D and E); however, these differences were not significant for both the Chao1 ($P = 0.35$) and Simpson ($P = 0.07$) indices of alpha diversity. The lowest bacterial Shannon index of diversity was found in coir after transplant and after 6 months (Fig. 3A and E).

The rhizosphere bacterial community composition of each treatment was significantly different, as determined through a PERMANOVA test on both weighted Unifrac (Fig. 4, A–E) and Bray-Curtis dissimilarities. Differences among treatments in both weighted Unifrac and Bray-Curtis dissimilarities were greater after transplant (1-month, 2-month, 4-month and 6-month). The weighted UniFrac distances explained greater total variance (after transplant: 63.4%, 1 month: 77.1%, 2 months: 76.7%, 4 months: 64.7%, 6 months: 60.1%) than Bray-Curtis matrices (after transplant: 18.9%, 1 month: 24.7%, 2 months: 30.9%, 4 months: 13.3%, 6 months: 12.8%) when estimating beta diversity between treatments at each sample time point.

After transplant and after 6 months, there were significant differences in the relative abundance of the 17 most abundant bacterial taxonomic classes between treatments (Table 1). The rhizosphere bacterial community composition of citrus grown in coir had a significantly greater abundance of *Bacillus* (50.1%) and Actinobacteria (34%) after 6 months (Table 1) compared to the other treatments. *Anaerolineae* (34%) and *Gammaproteobacteria* (43.4%) were significantly more abundant in the rhizospheres of citrus grown in compost than in the other potting media (Table 1).

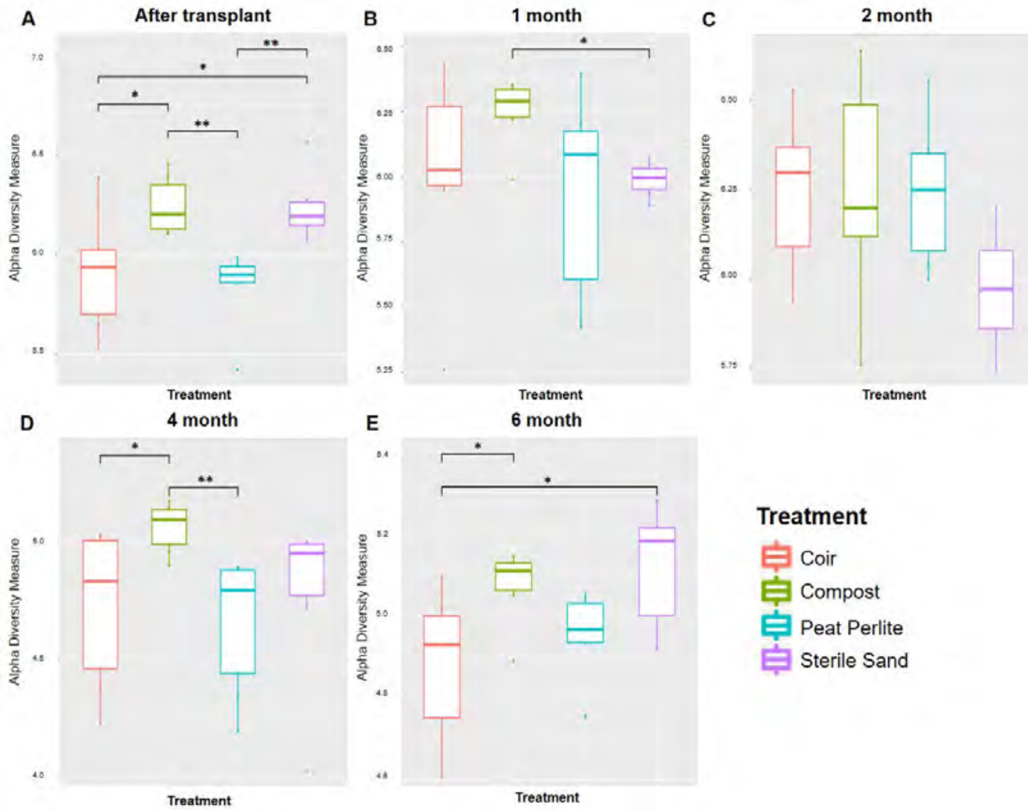

Fig. 3. Alpha-diversity measured by Shannon diversity index of rhizosphere bacteria between treatments at timepoints: **A**) after transplant, **B**) 1 month, **C**) 2 months, D) 4 months, and E) 6 months. Plotted in the figure are boxes (interquartile range) median (line within each box), and whiskers (lowest and greatest values). Treatments with * were considered significantly different (*P*-values: * = < 0.05, ** = < 0.01, and *** = < 0.001).

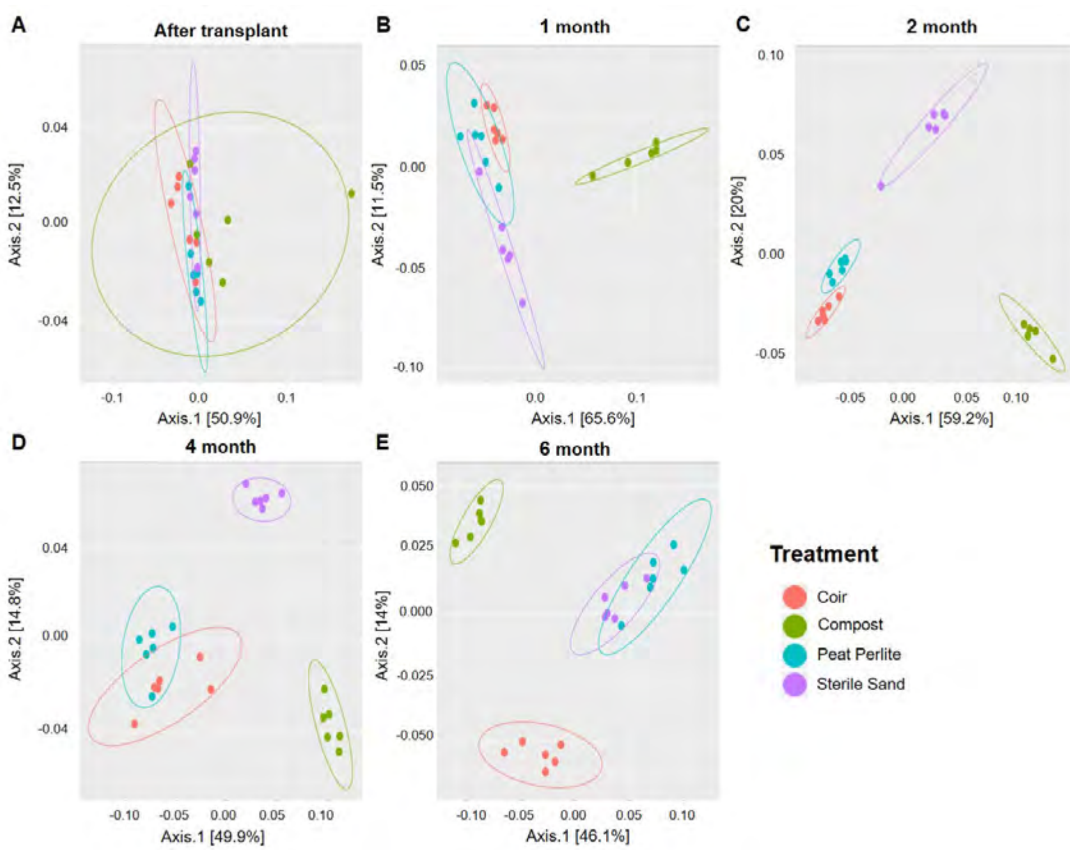

Fig. 4. Principal coordinates analysis (PCoA) based on Weighted UniFrac dissimilarity matrix of rhizosphere bacterial samples collected from time points: **A**) after transplant, **B**) 1 month, **C**) 2 months, **D**) 4 months, and **E**) 6 months. Ellipses indicate 95% confidence regions.

Table 1. Heat map illustrating changes in rhizosphere bacterial composition of four different potting media immediately after transplant and after 6 months of growth for the most abundant taxonomic bacterial classes. The relative abundances of taxa are indicated by color with red = high abundance, white = intermediate abundance, and blue = low abundance. Values are mean percentages of total sequences.

		Coir		Compost		Peat Perlite		Sterile Sand	
OTU	Bonferroni_P	After transplant	6 months	After transplant	6 months	After transplant	6 months	After transplant	6 months
Bacilli	1.60E-11	0.000	0.501	0.003	0.000	0.000	0.000	0.014	0.000
South African Gold Mine Gp 1	4.59E-11	0.022	0.000	0.033	0.000	0.023	0.000	0.017	0.000
Anaerolineae	5.88E-11	0.000	0.000	0.068	0.343	0.000	0.000	0.000	0.000
Sphingobacteriia	6.14E-11	0.065	0.000	0.094	0.000	0.067	0.159	0.088	0.000
Alphaproteobacteria	6.29E-10	0.285	0.000	0.246	0.057	0.243	0.491	0.260	0.000
Solibacteres	1.27E-09	0.025	0.000	0.032	0.000	0.033	0.125	0.031	0.000
Phycisphaerae	6.58E-09	0.026	0.000	0.026	0.000	0.027	0.025	0.023	0.000
OPB35 soil group	7.02E-09	0.022	0.000	0.015	0.000	0.014	0.000	0.023	0.000
Gammaproteobacteria	3.43E-08	0.043	0.000	0.077	0.434	0.041	0.002	0.050	0.000
Ktedonobacteria	6.06E-08	0.015	0.000	0.014	0.000	0.014	0.000	0.021	0.000
Opitutae	6.79E-07	0.021	0.000	0.033	0.000	0.020	0.072	0.019	0.000
Betaproteobacteria	4.33E-06	0.370	0.155	0.220	0.000	0.379	0.079	0.276	0.274
Subgroup 6	5.71E-06	0.021	0.000	0.026	0.000	0.028	0.021	0.023	0.000
Gemmatimonadetes	9.99E-06	0.004	0.000	0.011	0.000	0.009	0.000	0.011	0.000
Actinobacteria	3.33E-05	0.036	0.344	0.048	0.000	0.062	0.000	0.075	0.393
Thermoleophilia	5.95E-05	0.013	0.000	0.019	0.000	0.009	0.000	0.028	0.000
Acidobacteria	0.0001	0.012	0.000	0.006	0.000	0.005	0.021	0.009	0.000
TK10	0.0002	0.011	0.000	0.015	0.000	0.015	0.000	0.014	0.000
Acidimicrobiia	0.0003	0.008	0.000	0.008	0.000	0.010	0.000	0.013	0.000
Verrucomicrobia; Other	0.0027	0.003	0.000	0.007	0.000	0.001	0.003	0.007	0.000

Additionally, the rhizosphere bacterial community of plants grown in peat perlite had a significantly greater abundance of *Alphaproteobacteria* (49.1%) compared to the other treatments (Table 1). A significantly greater abundance of *Actinobacteria* (39.3%) was present within the rhizosphere bacterial community of plants grown in sterile sand compared with plants grown in the other potting media (Table 1).

Discussion

As expected, the compost treatment, which had the greatest N and P concentrations, had significantly greater diversity in the citrus rhizosphere microbiome compared to other treatments. Significant shifts in rhizosphere community composition have been observed after transferring plants into different soils (Lau and Lennon, 2012). However, when examining the impact of two soil types (grassland and woodland soils) and four plant species (onion, pea, sweet corn, and tomato) on rhizosphere bacterial communities, plant species had a greater impact on bacterial community composition compared to soil type (Matthews et al., 2019). Despite this, the rhizosphere bacterial community composition is likely limited to the accessible populations available from the bulk soil (de Boer et al., 2006).

This impact of the bulk soil composition on the rhizosphere was seen in this study, where the potting media had a significant effect on the relative abundance of specific rhizosphere bacteria. There was a 27.5% increase in *Anaerolineae* abundance in the rhizosphere of citrus plants from the compost treatment during the period beginning just after transplant to after 6 months (Table 1). *Anaerolineae* are present in anaerobic environments and can function as cellulose degraders (Xia et al., 2016). The greater water retention and degrading plant material present in the compost may have provided a suitable environment for *Anaerolineae*. Although the sterile sand had significantly less organic matter, total N, and total P than coir, plants grown in sterile sand had a significantly greater biomass than plants grown in coir. However, compared to coir, the citrus rhizosphere in the sterile sand had significantly greater abundance of *Actinobacteria* (39.3%), which may have affected plant biomass. Similar to other PGPR, *Actinobacteria* can affect plant growth through the production of growth hormones, including assistance in phosphate solubilization (Sathya et al., 2017). The significantly greater aboveground and belowground biomass of plants grown in compost compared to plants grown in the other three potting media may be related to the significantly greater concentrations of both total N and total P in the compost (Fig. 1, A and B) as well as the diversity of the bacterial community. Compost frequently has a positive impact on plant growth (Iverson and Maier, 2008), possibly due to the greater concentrations of C, N, and P (Verma et al., 2015), and changes in the microbial community. The use of compost as a soil medium for field-grown tomato var. Pusa Rohini (*Lycopersicon esculentum*) resulted in greater microbial diversity and plant biomass (Verma et al., 2015).

Soil microbial community compositions are closely related to nutrient concentrations (Jackson et al., 2019). The significant shifts observed in rhizosphere bacterial community composition of plants grown in sterile sand and coir from 4 to 6 months (Fig. 4D and E) are likely related to changes in the frequency of fertilizer applications during that time. The 4-month and 6-month time points occurred during October and December when citrus entered dormancy. Because citrus rootstock seedlings are usually less active at this time of the year, fertilizer rates were reduced from twice per week to once every 2 weeks. By decreasing the amount of nutrients, shifts in rhizosphere bacterial community composition of the coir and sterile sand treatments may have followed. Fertilizer may have also contributed to the significant increases in soil nutrient concentrations (total N and total P) of the coir, peat perlite, and compost treatments from the beginning to the end of the experiment. Because of the low nutrient retention, nutrient concentrations were not increased in the sand after 6 months.

Conclusion

In this study, soil composition significantly influenced rhizosphere bacterial community composition and diversity during the six months of this study. Citrus rootstock seedlings grown in compost had a significantly greater rhizosphere bacterial diversity compared to seedlings grown in coir, peat-perlite, and sand, likely due to the more diverse bacterial community in the soil treatment prior to planting. Using a soil or growing medium that is rich in nutrients and organic matter, such as compost, may improve rhizosphere microbial activity and potentially facilitate plant growth and yield. Further studies on other rootstock genotypes and growing media are essential to better understand the influence of the soil environment on rhizosphere recruitment and development.

Literature Cited

Albacete, A., C. Martínez-Andújar, A. Martínez-Pérez, A.J. Thompson, I.C. Dodd, and F. Pérez-Alfocea. 2015. Unravelling rootstock × scion interactions to improve food security. J. of Expt. Botany 66:2211–2226.

Alves, G. R., V.H. Beloti, K.M. Faggioni-Floriano, S.A. de Carvalho, R. deA. Moral, C.G.B. Demétrio, J.R.P. Parra, and P.T. Yamamoto. 2018. Does the scion or rootstock of *Citrus* sp. affect the feeding and biology of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae)? Arthropod-Plant Interactions 12:77–84.

Bakker, M.G., J.M. Chaparro, D.K. Manter, and J.M. Vivanco. 2015. Impacts of bulk soil microbial community structure on rhizosphere microbiomes of *Zea mays*. Plant and Soil 392:115–126.

Callahan, B.J., P.J. McMurdie, M.J. Rosen, A.W. Han, A.J.A Johnson, and S.P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods 13:581–583.

Caporaso, J.G., J. Kuczynski, J. Stombaugh, K. Bittinger, F.D. Bushman, E.K. Costello, N. Fierer, A.G. Peña, J.K. Goodrich, J.I. Gordon, G.A. Huttley, S.T. Kelly, D. Knights, J.E. Koenig, R.E. Ley, C.A. Lusopone, D. McDonald, B.D. Muegge, M. Pirrung, J. Reeder, J.R. Sevinsky, P.J. Turnbaugh, W.A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335–336.

Caporaso, J.G., C.L. Lauber, W. Walters, D. Berg-Lyons, C.A. Lozupone, P.J. Turnbaugh, N. Fierer, and R. Knight. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. of Sci. U.S.A. 108:4516–4522.

Chau, J.F., A.C. Bagtzoglou, and M.R. Willig. 2011. The effect of soil texture on richness and diversity of bacterial communities. Environ. Forensics 12:333–341.

de Boer, W., G.A. Kowalchuk, and J.A. van Veen. 2006. "Root-food" and the rhizosphere microbial community composition. New Phytologist 170:3–6.

Dijkstra, F.A. and W. Cheng. 2007. Moisture modulates rhizosphere effects on C decomposition in two different soil types. Soil Biol. and Biochem. 39:2264–2274.

Environmental Protection Agency (EPA). 1992. Method 365.4 Phosphorus, Total (Chlorimetric, Automated, Block Digester AA II). Methods for Chemical Analysis of Water and Wastes Environmental Monitoring and Support Laboratory, Cincinnati, OH.

Harrison, J.G., C.S. Philbin, Z. Gompert, G.W. Forister, L. Hernandez-Espinoza, B.W. Sullivan, I.S. Wallace, L. Bertran, C.D. Dodson, J.S. Francis, A. Schlageter, O. Shelef, S.A. Yoon, and M.L. Forister. 2018. Deconstruction of a plant-arthropod community reveals influential plant traits with nonlinear effects on arthropod assemblages. Functional Ecol. 32:1317–1328.

Iverson, S. L. and R.M. Maier. 2009. Effects of compost on colonization of roots of plants grown in metalliferous mine tailings, as examined by fluorescence in situ hybridization. Applied and Environmental Microbiology 75:842–847.

Jackson, O., R.S. Quilliam, A. Stott, H. Grant, and J.A. Subke. 2019. Rhizosphere carbon supply accelerates soil organic matter decomposition in the presence of fresh organic substrates. Plant and Soil 440:473–490.

Lambers, H., C. Mougel, B. Jaillard, and P. Hinsinger. 2009. Plant-microbe-soil interactions in the rhizosphere: an evolutionary perspective. Plant and Soil 321:83–115.

Lau, J.A. and J.T. Lennon. 2012. Rapid responses of soil microorganisms improve plant fitness in novel environments. Proc. Nat. Acad. Sci. 109:14058–14062.

Liu, X., J.W. Zhang, L.X. Guo, Y.Z. Liu, L.F. Jin, S.B. Hussain, W. Du, Z. Deng, and S.A. Peng. 2017. Transcriptome changes associated with boron deficiency in leaves of two citrus scion-rootstock combinations. Frontiers in Plant Sci. 8:1–10.

Lobet, G. 2017. Image analysis in plant sciences: Publish then perish. Trends in Plant Sci. 22:559–566.

Matthews, A., S. Pierce, H. Hipperson, and B. Raymond. 2019. Rhizobacterial community assembly patterns vary between crop species. Frontiers in Microbiology 10:1–13.

Micallef, S.A., S. Channer, M.P. Shiaris, and A. Colón-Carmona. 2009. Plant age and genotype impact the progression of bacterial community succession in the Arabidopsis rhizosphere. Plant Signaling and Behavior 4:777–780.

Myalavarapu, R.S., W. D'Angelo, N. Wilkinson and D. Moon. 2002. UF/IFAS Extension Soil Testing Laboratory (ESTL) Analytical Procedures and Training Manual. Soil and Water Science Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL.

Nawaz, M.A., M. Imtiaz, Q. Kong, F. Cheng, W. Ahmed, Y. Huang, and Z. Bie. 2016. Grafting: A technique to modify ion accumulation in horticultural crops. Frontiers in Plant Sci. 7:1–15.

Oksanen, J., F.G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. Mcglinn, P.R. Minchin, R.B. O'Hara, G.L. Simpson, P. Solymos, M.H.H. Stevens, E. Szoecs, and H. Wagner. 2017. Package "vegan": Community Ecology Package. R Package Version 2.4–4.

Peiffer, J.A., A. Spor, O. Koren, Z. Jin, S.G. Tringe, J.L. Dangl, E.S. Buckler, and R.E. Ley. 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proc. Natl. Acad. of Sci. U.S. 110:6548–6553.

Ragot, S.A., O. Huguenin-Elie, M.A. Kertesz, E. Frossard, and E.K. Bünemann. 2016. Total and active microbial communities and phoD as affected by phosphate depletion and pH in soil. Plant and Soil 408:15–30.

Salis, C., I.E. Papadakis, S. Kintzios, and M. Hagidimitriou. 2017. In vitro propagation and assessment of genetic relationships of citrus rootstocks using ISSR molecular markers. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 45:383–391.

Sathya, A., R. Vijayabharathi, and S. Gopalakrishnan. 2017. Plant growth-promoting actinobacteria: a new strategy for enhancing sustainable production and protection of grain legumes. 3 Biotech 7:1–10.

Schreiter, S., G.C. Ding, H. Heuer, G. Neumann, M. Sandmann, R. Grosch, S. Kropf, and K. Smalla. 2014. Effect of the soil type on the microbiome in the rhizosphere of field-grown lettuce. Frontiers in Microbiology 5:1–13.

Tsuno, Y., T. Fujimatsu, K. Endo, A. Sugiyama, and K. Yazaki. 2018. Soyasaponins: A new class of root exudates in soybean (*Glycine max*). Plant and Cell Physiol 59:366–375.

Urbina, H., M.F. Breed, W. Zhao, K. Lakshmi Gurrala, S.G.E. Andersson, J. Ågren, S. Baldauf, and R. Rosling. 2018. Specificity in *Arabidopsis thaliana* recruitment of root fungal communities from soil and rhizosphere. Fungal Biol. 122:231–240.

Vejan, P., R. Abdullah, T. Khadiran, S. Ismail, and A. Nasrulhaq Boyce. 2016. Role of plant growth promoting rhizobacteria in agricultural sustainability-A review. Molecules 21:1–17.

Verma, S., A. Sharma, R. Kumar, C. Kaur, A. Arora, R. Shah, and L. Nain. 2015. Improvement of antioxidant and defense properties of tomato (var. Pusa Rohini) by application of bioaugmented compost. Saudi J. of Biol. Sci. 22:256–264.

Wickramatilake, A.R.P., R. Munehiro, T. Nagaoka, J. Wasaki, and K. Kouno. 2011. Compost amendment enhances population and composition of phosphate solubilizing bacteria and improves phosphorus availability in granitic regosols. Soil Sci. and Plant Nutrition 57:529–540.

Xia, Y., Y. Wang, Y. Wang, F.Y.L. Chin, and T. Zhang. 2016. Cellular adhesiveness and cellulolytic capacity in Anaerolineae revealed by omics-based genome interpretation. Biotechnol. for Biofuels. 9:1–13.

Zhang, Z.S., X.J. Dong, B.X. Xu, Y.L. Chen, Y. Zhao, Y.H. Gao, Y.G. Hu, and L. Huang. 2015. Soil respiration sensitivities to water and temperature in a revegetated desert. J. of Geophysical Res.: Biogeosciences 120:773–787.

Proc. Fla. State Hort. Soc. 132:62–67. 2019.

Response of Huanglongbing-affected Citrus Trees to Zinc Fertilization under Microsprinkler Irrigation

Qudus O. Uthman[1,2], Davie M. Kadyampakeni*[1], Peter Nkedi-Kizza[2], and Christopher I. Vincent[3]

[1]*Soil and Water Sciences Department, University of Florida, IFAS, 2181 McCarty Hall, Gainesville, FL 32611*

[2]*Soil and Water Sciences Department, University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Rd., Lake Alfred, FL 33850*

[3]*Horticultural Sciences Department, University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Rd, Lake Alfred, FL 33850*

Additional index words. *Citrus sinensis*, Huanglongbing, sorption isotherm, zinc

Zinc plays a key role in structural, regulatory and catalytic cofactor of many enzyme reactions. Confused symptoms of citrus greening (also called Huanglongbing, HLB) disease with zinc (Zn) include small yellow leaves and mottling. Although enough Zn may be present in the soil, deficiency symptoms can develop due to soil depletion or formation of insoluble Zn compounds. This study investigates the response of HLB affected citrus trees to zinc fertilization using various application method and rates. Treatments include three nitrogen (N) rates at 168, 224, and 280 kg·ha^{-1} N as main plots and 4 subplots including control (no Zn fertilization), foliar applied Zn at = 5.6 and 11.2 kg·ha^{-1} Zn and soil applied Zn at 5.6 kg·ha^{-1} Zn. Results of a 2-year study showed that Zn concentration of the plot supplied with highest nitrogen (N) rate was significantly higher ($P < 0.0001$) than the plots supplied with the two lower N rates. Foliar treatment showed 110% significant increase in concentration of Zn in the leaf compared to soil treatment. High sorption coefficients (K_D) were observed at 0–15 cm and 15–30 cm soil depth while 30–45 cm and 45–60-cm soil depths showed no or very negligible sorption. Zn concentration in the leaf tissue was significantly higher in summer compared to fall. Thus, foliar application of Zn at any of the rates improved the Zn concentration in the leaves above the deficiency level and low critical concentration level while soil application optimized Zn availability in the soil for root uptake.

Florida citrus production declined from over 300,000 hain 2000 to around 166,000 ha in 2018, a reduction of 45% (USDA, 2018). This decline was primarily due to citrus greening disease (also called Huanglongbing, or HLB), which was detected in 2005 (Hodges et al. 2014). As a result of HLB, the tree loses productivity due to twig dieback and subsequent tree death (Alvarez et al., 2016). If the disease is widespread, citrus trees may live for up to 10 years and never produce usable fruit (Roistacher, 1996; Alvarez et al., 2016).

Nutrients appear to be important in disease control as they influence plant resistance, immunity and pathogen growth (Handique et al., 2012; Pustika et al., 2008). Zinc (Zn) plays a key role in structural, regulatory and catalytic cofactor of many enzyme reactions in plants (Hansch and Mendel, 2009). Although Zn may be present in the soil, deficiency can develop due to soil depletion or formation of insoluble Zn compounds (Obreza and Collins, 2002). The relationship between mineral nutrients and plant disease eventually influences the severity of symptoms (Dordas, 2008). In a two-year study to understand the effect of micronutrients on overall health of trees affected by HLB, we hypothesized that foliar compared to soil application method at the current Zn recommended rate and foliar application at single and double the current Zn recommended rate should improve the performance of HLB-affected trees. Further, we hypothesized that a synergistic relationship between nitrogen (N) and Zn exists to improve the health of HLB-affected trees. Overall, the study sought to: 1) determine performance of HLB-affected citrus trees through modified Zn fertilizer application methods and rates and 2) evaluate the fate of Zn in the HLB affected groves.

Materials and Methods

Location and background of the study site. This study was done 6-year-old HLB-affected *Citrus sinensis* 'Valencia' trees planted at 4.6 m between rows and 1.8 m between trees, and approximately 1111 trees/ha near Citrus Research and Education Center, Lake Alfred, FL at 28.108203°N, 81.682803°W. The soil is classified as Candler fine sand with a taxonomic class of Hyperthermic, uncoated Lamellic Quartzipsamments formed from eolian or sandy marine deposits. Soil characteristics for the Candler fine sand are listed in Table 1. The trees were supplied with a fertilizer at the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) recommendation rate containing a known amount of nutrient in four splits per year as: 9.75% N, 2% phosphorus pentoxide (P_2O_5), 13%image result for K_2O, symbol

The authors thank the University of Florida, IFAS Citrus Initiative funding and U.S. Department of Agriculture NIFA Hatch Project No. FLA-CRC-005593. The Water and Nutrient Management Lab Team at the Citrus Research and Education Center (CREC) Lake Alfred, FL. is thanked for help with data collection and processing.

*Corresponding author. Email: dkadyampakeni@ufl.edu

Table 1. Soil texture and chemical properties at a research plot near the Citrus Research and Education Center, Lake Alfred, FL.

Soil depth (cm)	Soil texture Sand (%)	Silt (%)	Clay (%)	Bulk density ($g \cdot cm^{-3}$)	Soil pH	Organic matter (%)
0–15	95.9	0.5	3.7	1.56 ± 0.03	6.27 ± 0.08	0.55 ± 0.04
15–30	96.8	0.7	2.5	1.67 ± 0.05	6.30 ± 0.12	0.30 ± 0.02
30–45	97.1	0.4	2.5	1.61 ± 0.02	6.31 ± 0.13	0.21 ± 0.02
45–60	97.4	0.6	2.0	1.63 ± 0.02	6.30 ± 0.14	0.19 ± 0.01

potassium oxide (K_2O), 2.28% calcium (Ca), 2.5% magnesium (Mg), 11.69% sulpher (S), 0.03% boron (B), 0.272% iron (Fe), 0.55% manganese (Mn) and 0.19% Zn.

Experimental Design and Treatment application. Treatments were assigned in a split plot arrangement where N rates formed the main plots and Zn application methods and rates formed the subplots. Each main plot had 4 sub-plots with 10 trees per plot where the middle 8 trees were used for measurements. Each main plot was replicated 3 times and received three N rates of 168, 224 and 280 $kg \cdot ha^{-1}$. The N composition of the fertilizer blend was adjusted (increased by additional of urea, over and above the minimum fertilization rate of 168 $kg \cdot ha^{-1}$ N). The subplots in each main plot received micronutrients (Zn) applications in three splits per year (February, June and October): 1) standard soil Zn applied (control); 2) foliar applied Zn based at 1× UF/IFAS recommendations + 1× standard soil nutrient application; 3) 2×foliar applied Zn UF/IFAS recommendations+ 1× standard Zn application; and 4) 2× soil applied UF/IFAS recommendations (1× = 5.60 $kg \cdot ha^{-1}$ Zn). All trees were irrigated by 40 $L \cdot h^{-1}$ emitters, with one emitter per tree having a wetting radius of about 1.5 m, with each irrigation applied at 8 am and 1 pm daily.

Soil samples and analysis. Soil samples were collected with an auger at two perpendicular points away from the tree at 30 cm (irrigated zone) and 60 cm (non-irrigated zone) at 0–15, 15–30, 30–45 and 45–60 cm depth. Soil samples collected were analyzed for Zn using Mehlich III solution extraction procedure (Mehlich, 1984) and determined using Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) (Hanlon et al., 1997) with a detection wavelength of 214 nm (Reed and Martens, 1996).

Leaf collection and analysis. Citrus leaf sampling technique by Obreza and Morgan (2008) was adopted in collecting the leaf tissue samples. After collection, the leaf tissues were transported from the field to the laboratory in an iced-cooler container and placed in the refrigerator to protect them from heat and keeping them dry. To remove contaminants, leaves were washed with soap and rinsed with deionized water. Then, leaves were placed in a ventilated oven at 70 °C for 72 h. Dried leaves were ground in a laboratory-size stainless steel Wiley mill to pass a 20-mesh (1-mm) screen before acid digestion. About 1.5 g of oven-dry plant tissue was placed inside high-form, glazed, porcelain crucibles, and then placed in a muffle furnace at 500 °C for 12 h. The furnace door was opened at a temperature below 200 °C so that the samples are not oxidized or disturbed by the rapid influx of air. The ash was moistened with five drops of pure water using eye dropper. About 5 mL of 0.5M HCl solution using an adjustable macro-pipettor was used to digest the ashes and allowed to stand for 30 min. The digested ashes were diluted with pure water up to 50-mL mark and filtered into labeled vials using Whatman no. 42 filter paper. Zn concentration was determined using ICP-AES (Inductively coupled plasma–atomic emission spectrometry) with a detection wavelength of 214 nm (Reed and Martens, 1996).

Sorption study. One-point sorption isotherms were determined to estimate the sorption coefficient of Zn as a function of soil depth. The amount of initial concentration of 10 $mg \cdot L^{-1}$ was used based on UF/IFAS recommended rate assuming 2-cm depth of incorporation. The soil:solution ratio used was 1:2, shaken for 24 h, centrifuged at 10000 rpm for 20 min, filtered, and analyzed for Zn using ICP-AES. The amount of Zn sorbed to the soil was calculated from the difference between the initial and equilibrium solution concentrations:

$$s_e = \frac{v_0}{m}(C_0 - C_e) \quad \text{[Eq. 1]}$$

where S_e is the adsorbed concentration at equilibrium ($mg \cdot kg^{-1}$), V_0 is the initial volume of solution (L), m is the soil mass (kg), C_0 is the initial concentration of the standard solution ($mg \cdot L^{-1}$), and C_e is the soil solution concentration at equilibrium ($mg \cdot L^{-1}$). The linear sorption isotherm was determined from the following model:

$$s_e = k_D C_e \quad \text{[Eq. 2}$$

where K_D is the sorption coefficient ($L \cdot kg^{-1}$).

Statistical analysis. A linear mixed effect model by Pinheiro and Bates (2000) was used to analyze the differences between treatments effect and Tukey honestly significant difference test was used to separate means. R software (R version 3.5.1, R core Team, 2013) was used for this analysis.

Result and Discussion

Soil analysis. Zinc concentration in the soil for the 280 $kg \cdot ha^{-1}$ N treatment increased significantly by 28% more than 168 $kg \cdot ha^{-1}$ N treatment (Fig. 1). The application treatments of N rates by soil, and foliar application for Zn shows a significant differences of Zn concentration but no consistent trend was established. Zn concentration in the soil in the irrigated zone (30 cm away from the tree) was about 150% significantly greater than in the non-irrigated zone (60 cm away from the tree) (Fig. 2). Zn deficiencies in HLB-affected trees are more pronounced during the cool, wet seasons and often disappear in warmer season. Comparing the season of soil samples analysis, the cool season have Zn concentration in the soil significantly higher than the warmer season. The low Zn concentration in the soil at this season could be caused by slow diffusion process that was responsible for transport of Zn to the plant roots (Havlin et al., 2013). Soil temperature, which could be affected by season, could increase/decrease Zn availability through its solubility and diffusion (Havlin et al., 2013). N appears to affect crop Zn status by promoting plant growth as well as by changing the root environment's pH (Alloway, 2008). N is the main factor limiting

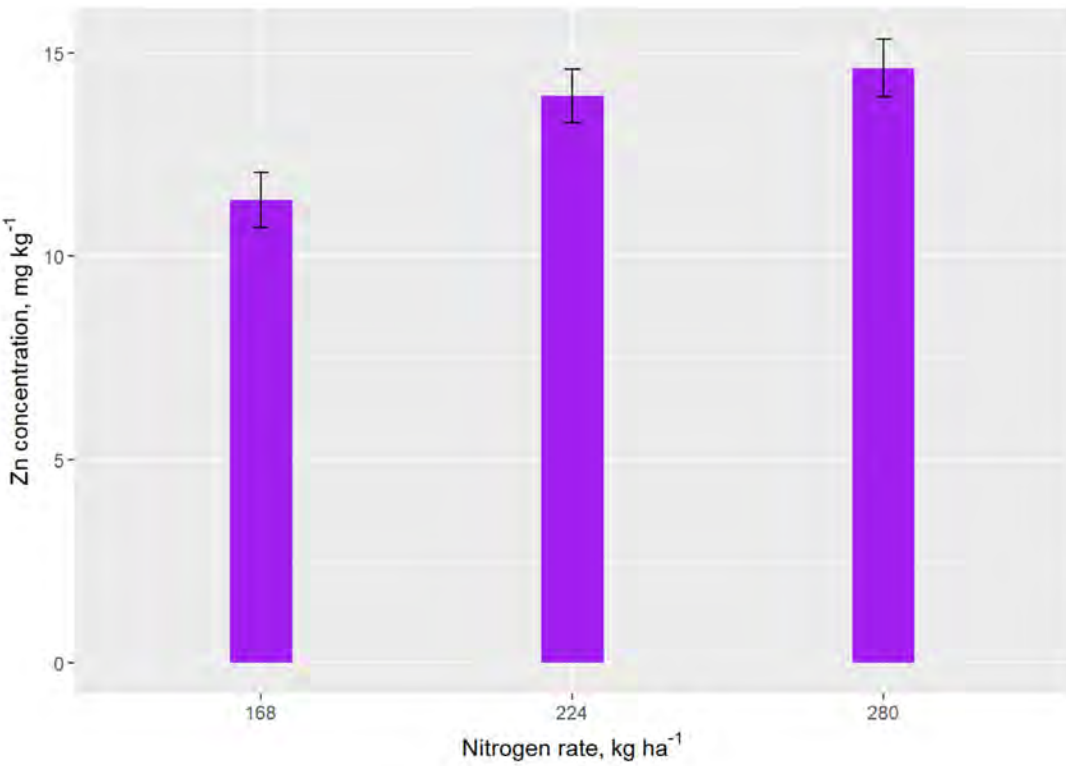

Fig. 1. Effect of zinc concentration in the soil subjected to varying nitrogen rates at a research plot near the Citrus Research and Education Center, Lake Alfred, FL. Error bars denote standard deviation of 3 replications.

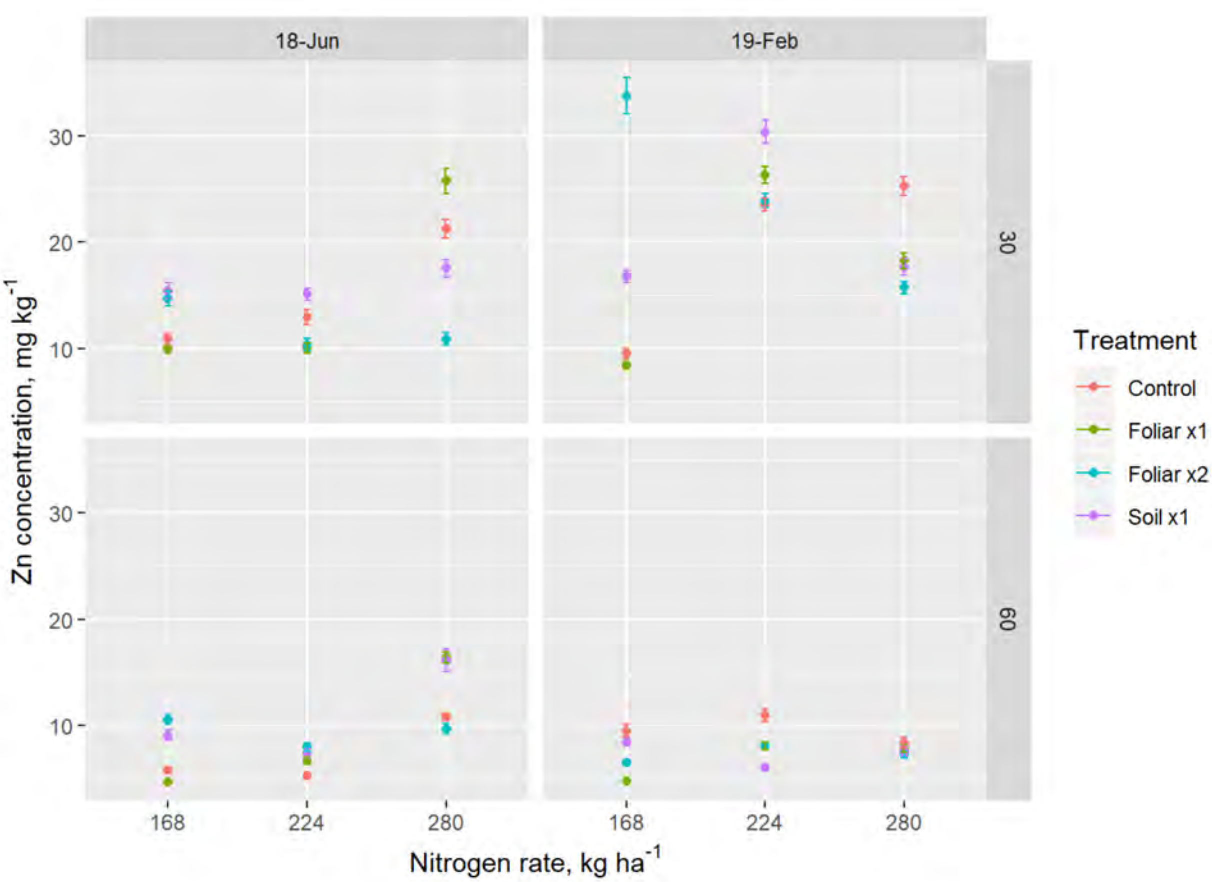

Fig. 2. Zinc response to sampling distance and nitrogen rates by soil and foliar application method and rates at a research plot near the Citrus Research and Education Center, Lake Alfred, FL.

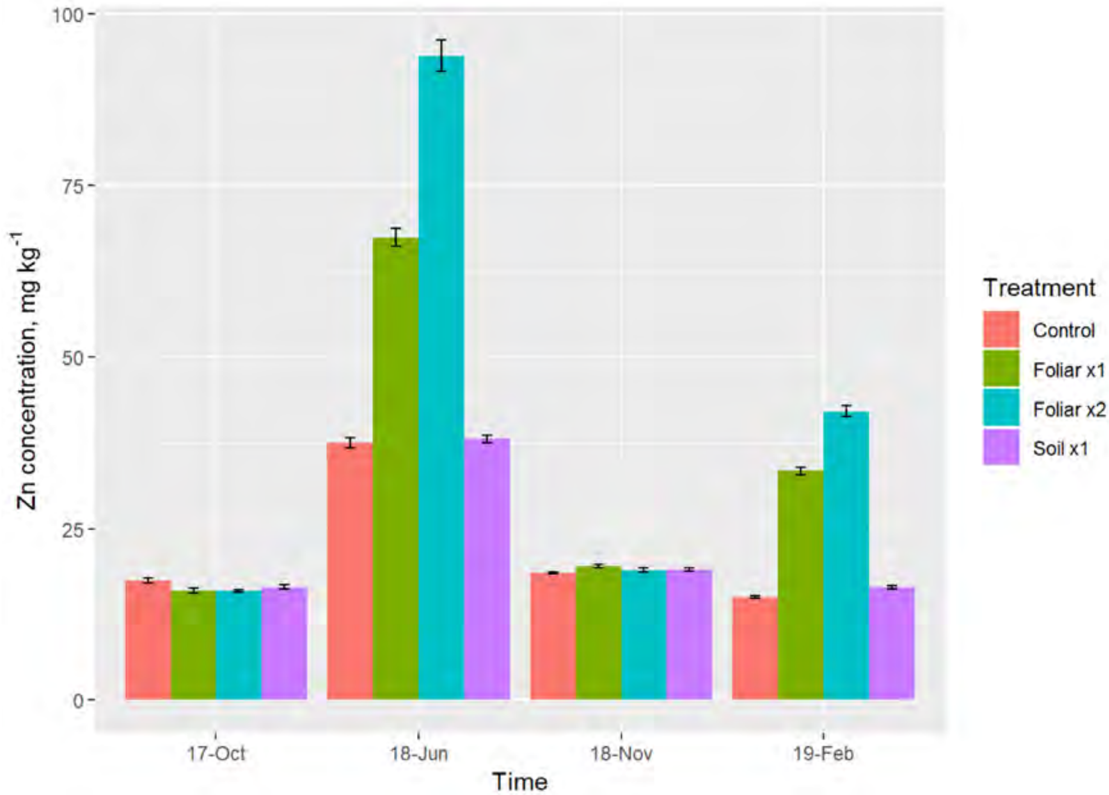

Fig. 3. Orange tree (*Citrus sinensis*) foliar zinc concentration with respect to season and application method and rate at a research plot near the Citrus Research and Education Center, Lake Alfred, FL. Error bars denote standard deviation of 3 replications.

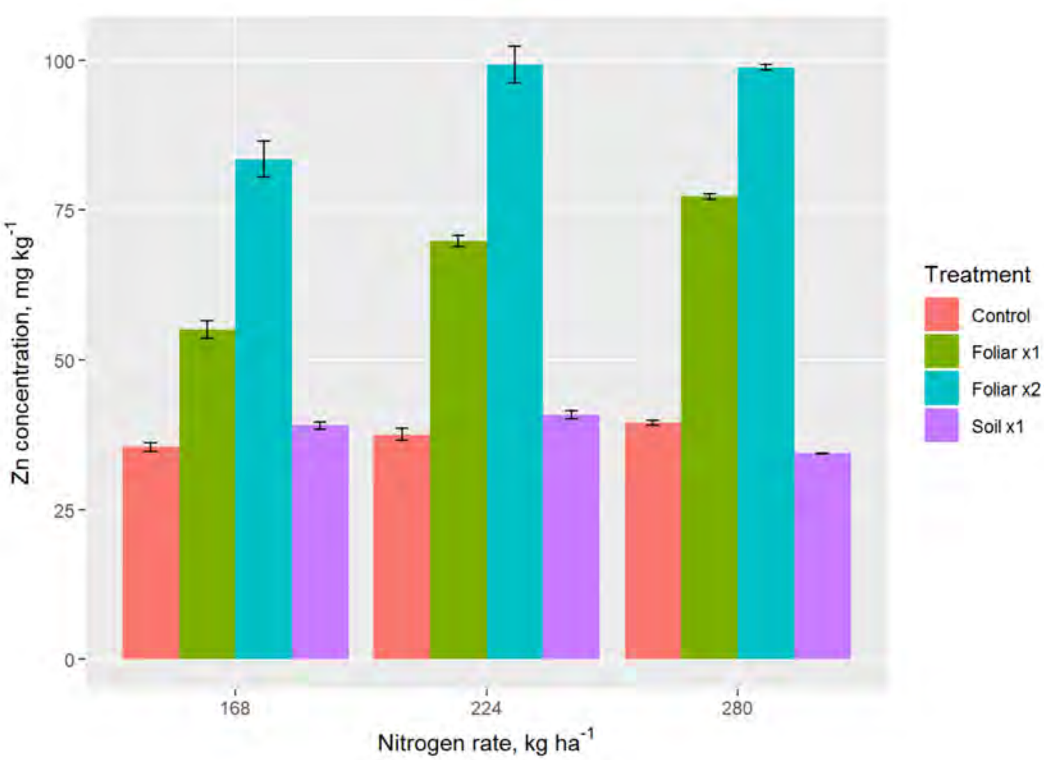

Fig. 4. Response of treatments to foliar zinc concentration in orange (*Citrus sinensis*) trees the leaf as a function of nitrogen rates at a research plot near the Citrus Research and Education Center, Lake Alfred, FL. Error bars denote standard deviation of 3 replications.

growth and yield in many soils and therefore, not surprisingly, yield improvements have been found by applying nitrogen and Zn fertilizers through positive interactions (Alloway, 2008). N application in the absence of Zn can lead to zinc deficiency due to a dilution effect caused by an increase in N growth (Kirk and Bajita, 1995). This can also lead to a negative interaction, however, if other micronutrients, such as copper, are also marginal in the soil (Kirk and Bajita, 1995). Growth promoted by nitrogen can cause a concentration of copper dilution that is then exacerbated by zinc applied (Kirk and Bajita, 1995).

Leaf analysis. Foliar treatment showed 110% increase in concentration of Zn in the leaf compared to soil treatment in the summer season (Fig. 3). There was 54% increase in Zn leaf concentration for the double rate with foliar Zn application compared to the single foliar Zn application rate for summer season (Fig.3). Zn concentration in the leaf tissue was significantly greater in early summer months compared to late fall and winter season (Fig. 3). Irrespective of N rate, double application rate of foliar treatment was significantly higher than standard application rate for foliar or soil. Leaf ageing might have been responsible for the fluctuation of Zn concentration through the sampling season. This agrees with the previous research by Obreza et al. (2014) on changes in concentration of N, P, K, Ca, and Mg in citrus leaves with age. They found that leaf nutrient concentration continuously changes with season and differs from one nutrient to another. The primary resistance to Zn absorption is through waxy cuticle layer of the leaves. The leaf cuticle thickness increases with plant age causing the foliar nutrient application to vary as the leaves age by season.

Sorption Study. The sorption study of Zn revealed high sorption coefficients at 0–15 cm and 15–30 cm soil depths while 30–45 cm and 45–60 cm depths showed no sorption (Fig. 5). Zn sorption increased significantly by 6400% at 0–15 cm compared to 30–45 cm and 45–60 cm soil depths (Fig. 5). Zinc sorption was 141% greater at 0–15 cm compared to 15–30 cm. Hippler et al. (2015) showed that Zn soil application can supply orange trees with nutrient requirements, but low fertilizer solubility and high soil sorption capacity limit fertilization efficiency. Since most of zinc applied was bounded to the 0–30 cm soil depth matrix, the soil solution concentration would be very low and thus also supply by mass flow to the roots (Marschner, 1993). Because of the low zinc concentration in the soil solution, supplying the roots with mass flow accounts for only a small fraction of plant demand (Marschner, 1993). The soil was predominantly sandy with a low percentage of organic matter (0.4%); this could have made the organic matter fraction responsible for Zn sorption in the 0–15 cm and 15–30 cm soil depth (Table 1). Zn might have formed stable complexes with high molecular-weight organic compounds that exist as insoluble complexes and might have made Zn applied to the soil unavailable for uptake (Havlin et al., 2013). Kiekens (1980) reported that the adsorption of zinc by clays and organic matter seemed to involve two different mechanisms: One mechanism operates mainly under acidic conditions and is closely linked to cation exchange, while the other mechanism operates under alkaline conditions and involves primarily chemisorption and organic ligand complexation.

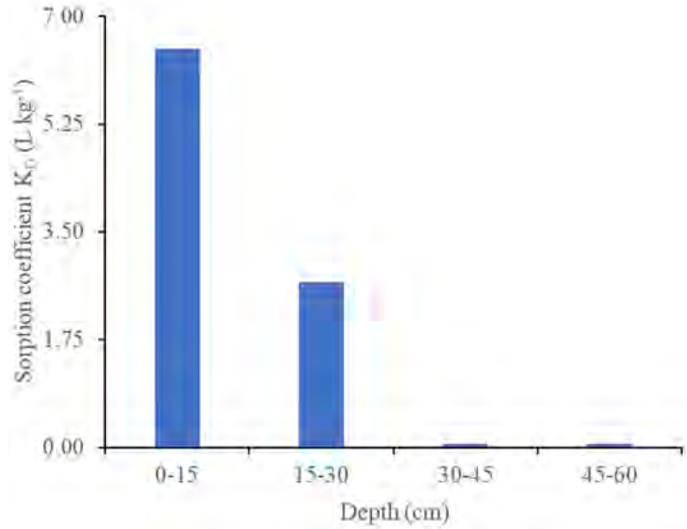

Fig. 5. Linear sorption coefficient of Zn as a function of soil depth at a research plot near the Citrus Research and Education Center, Lake Alfred, FL.

Conclusion

The soil is predominantly sandy which makes the organic matter fraction responsible for Zn sorption in the 0–15 cm and 15–30 cm soil depth. Zn might have formed stable complexes with high molecular-weight organic compounds that exist as insoluble complexes. Foliar treatment accounts for greater Zn concentration in the leaves. There was no interaction of between Zn and N in either of the leaf or soil. Increase in the application rate of foliar applied treatment improved the Zn concentration in the leaf above the critical level.

Literature Cited

Alloway, B.J. 2008. Zinc in soils and crop nutrition. International Zinc Association, Brussels, Belgium.

Alvarez, S., Rohrig, E., Solís, D., and M.H. Thomas. 2016. Citrus greening disease (Huanglongbing) in Florida: Economic impact, management and the potential for biological control. Agric. Res. 5(2):109–118.

Dordas, C. 2008. Role of nutrients in controlling plant diseases in sustainable agriculture. A review. Agron. Sustain. Dev. 28(1):33–46.

Gupta, U.C., K. Wu, and S. Liang. 2008. Micronutrients in soils, crops and livestock. Earth Science Frontiers 15(5):110–125.

Handique, U., R.C. Ebel, and K.T. Morgan. 2012. Influence of soil-applied fertilizer on greening development in new growth flushes of sweet orange. Proc. Fla. State Hort. Soc. 125:36–40.

Hanlon, E., J. Gonzalez, and J. Bartos. 1997. IFAS extension soil testing laboratory (ESTL) and analytical research laboratory (ARL) chemical procedures and training manual. Extension publication, University of Florida, Gainesville, FL.

Hänsch, R., and R.R. Mendel. 2009. Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). Current Opinion in Plant Biology 12(3):259–266.

Harbridge, J. 2007a. Determination of nitrate in 2M KCl soil extracts by flow injection analysis QuikChem Method 12-107-04-1-J. Lachat Instruments, Loveland, CO.

Harbridge, J. 2007b. Determination of ammonia in 2M KCl soil extracts by flow injection analysis QuikChem Method 12-107-04-1-J. Lachat Instruments, Loveland, CO.

Havlin, J.L., J.D. Beaton, S.L. Tisdale, and W.L. Nelson. 2013. Soil fertility and fertilizers: An introduction to nutrient management, 8th edition, Pearson Educational, Inc., Upper Saddle River, N.J.

Hippler, F.W.R., R.M. Boaretto, J.A. Quaggio, R.A. Azevedo, and D. Mattos, D. 2015. Towards soil management with Zn and Mn: Estimates of fertilization efficacy of Citrus trees. Annals of Applied Biology 166(3):484–495.

Hodges, A.W., M. Rahmani, T.H. Spreen, and T.J. Steven . 2014. Economic impact of the Florida's citrus industry, 2012-2013. Extension publication FE-633. University of Florida, Gainesville, FL.

Kadyampakeni, D. M., K.T. Morgan, A.W. Schumann, P. Nkedi-Kizza, and K. Mahmoud. 2014. Ammonium and nitrate distribution in soil using drip and microsprinkler irrigation for citrus production. Soil Sci. Soc. Am. J. 78(2):645–654.

Kiekens, L. 1980. Absorptieverschijnselen van zware metalen in gronden. Doctoral thesis, Ghent University, Ghent, Belgium.

Kirk, G.J.D. and J.B. Bajita. 1995. Root-induced iron oxidation, pH changes and zinc solubilisation in the rhizosphere of lowland rice. New Phytologist 131:129–137.

Marschner H. 1993. Zinc uptake from soils. In: Robson A.D. (eds) Zinc in soils and plants. Developments in plant and soil sciences. Springer, Dordrecht

Mehlich, A. 1984. Mehlich-3 soil test extractant—A modification of Mehlich-2 extractant. Commun. Soil Sci. Pl. Anal. 15:1409–1416.

Obreza, T.A. and K.T. Morgan. 2008. (eds.). Nutrition of Florida citrus trees. Extension publication SL-253. University of Florida, Gainesville, FL.

Obreza, T.A., and M.E. Collins. 2002. Common soils used for citrus production in Florida. Extension publication SL-193. University of Florida, Gainesville, FL.

Pinheiro, J.C. and D.M. Bates. 2000. Mixed-effects models in S and S-PLUS. Springer, New York, N.Y.

Pustika, A.B., B. Subandiyah, P. Holford, G.A.C. Beattie, T. Iwanami, and Y. Masaoka. 2008. Interactions between plant nutrition and symptom expression in mandarin trees infected with the disease huanglongbing. J. Australasian Plant Dis. Notes 3:112–115.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reed, S.T., and D.C. Martens. 1996. Copper and zinc. p. 703–722 In D.L. Sparks, A.L. Page, P.A. Helmke, and R.H. Loeppert (eds.), Methods of soil analysis Part 3—Chemical methods. Soil Science Society of America, American Society of Agronomy, Madison, WI.

Roistacher, C. 1996. The economics of living with citrus diseases: Huanglongbing (greening) in Thailand. International Organization of Citrus Virologists Conference Proceedings (1957–2010) 13(13):279–285

U.S. Department of Agriculture, National Agricultural Statistics Service (USDA-NASS). Citrus Fruits 2017 Summary, Aug. 2017.

Proc. Fla. State Hort. Soc. 132:68-71. 2019.

Translocation of Oxytetracycline in Citrus Plants after Root Drench and Stem Delivery

NABIL KILLINY*[1], FARAJ HIJAZ[1], FUAD AL-RIMAWI[1,2], YASSER NEHELA[1], AND OZGUR BATUMAN[3]

[1]*Department of Plant Pathology, Citrus Research and Education Center, IFAS, University of Florida, 700 Experiment Station Rd., Lake Alfred, FL 33850*

[2]*Chemistry Department, Faculty of Science and Technology, Al-Quds University, Jerusalem, Palestine*

[3]*Department of Plant Pathology, Southwest Florida Research & Education Center, IFAS, University of Florida, 2685 State Rd. 29 North, Immokalee, FL 34142*

ADITIONAL INDEX WORDS. antibiotic translocation, citrus, ELISA, HPLC, oxytetracycline

Huanglongbing (HLB) is the most destructive disease to the citrus industry. In North America, HLB is caused by *Candidatus* Liberibacter asiaticus (*C*Las) and is transmitted by the Asian citrus psyllid, *Diaphorina citri*. Recent studies showed that antibiotics such as oxytetracycline and streptomycin were effective against the *C*Las pathogen *in planta*. The objectives of this study were to investigate the uptake, translocation, and stability of oxytetracycline in citrus seedlings. Oxytetracycline was delivered via root or stem. The level of oxytetracycline in treated plants was monitored using high-performance liquid chromatography (HPLC) and/or enzyme-linked immunosorbent assay (ELISA). The HPLC and the ELISA methods showed similar results at high concentrations; however, the ELISA was more sensitive than the HPLC method. The highest level of oxytetracycline after root incubation was found in roots, followed by stem-xylem, stem-phloem, and in leaves. On the other hand, the level of oxytetracycline in the xylem and phloem was higher than that found in the root when delivered via stem. Oxytetracycline was still detectable in all tested tissues thirty-five days after treatment, indicating that oxytetracycline was relatively stable in citrus plants and could inhibit *C*Las growth for a few months in the field.

Citrus greening disease, which is also known as Huanglongbing (HLB), is currently threatening the citrus industry in Florida. HLB is caused by *Candidatus* Liberibacter asiaticus (*C*Las) and is transmitted by the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Grafton-Cardwell et al., 2013), while feeding on citrus phloem sap. Currently, HLB is widespread throughout the state of Florida and has significantly reduced citrus production.

Management of HLB mainly depends on the control of the *D. citri* using a wide range of insecticides. However, the heavy use of insecticides can negatively affect human health, beneficial insects, environment, and induce insecticide resistance in *D. citri* (Tiwari et al., 2013). Enhanced nutritional programs (ENPs) were also proposed to alleviate HLB symptoms and maintain the productivity of infected plants. However, ENPs were not effective in the field and can enhance the spread of HLB by increasing the lifespan of infected trees (Gottwald et al., 2012). Removal of infected trees was also suggested to reduce *C*Las inoculum; however, this strategy is difficult to implement in heavily infected regions such as Florida. Greenhouse studies showed that thermotherapy was effective against *C*Las, however this technology did not show any promising results in the field (Blaustein et al., 2017). Consequently, due to the difficulty of HLB management, the use of antibiotics is immediately needed for the control of this destructive disease.

The use of antibiotics for the control of HLB was initiated in the 1970s (Blaustein et al., 2017). Early studies showed that injection of tetracycline into the trunks of *C*Las-infected trees significantly reduced HLB symptoms (Martinez et al., 1970; Schwarz and van Vuuren, 1970). Early studies also showed that stem delivery of antibiotics was more efficient than foliar sprays and application under the bark (Capoor and Thirumal, 1973). Unfortunately, the use of antibiotics for the control of HLB was discontinued after a short period. However, in the last few years, the use of antibiotics for the control of HLB has regained a great interest by many scientists due to significant losses in the citrus industry. Recent studies showed that several antibiotics such as penicillin, streptomycin, ampicillin, and carbenicillin, were effective in eliminating the *C*Las titer and rescuing HLB-infected plants (Zhang et al., 2012; Zhang et al., 2015; Shin et al., 2016). In addition, several antimicrobials such as aluminum hydroxide, nicotine, D, L-buthionine sulfoximine, and surfactin were found to be effective against *C*Las (Doud et al., 2018).

Recently, oxytetracycline and streptomycin sulfate have been approved for the treatment of *C*Las-infected trees via foliar spray in Florida (Wang et al., 2017). In this study, we investigated the uptake, distribution, and stability of oxytetracycline in citrus plants.

Materials and Methods

PLANT MATERIALS. Healthy Mexican lime (*Citrus aurantifolia*) seedlings were used in this study. Plants were kept in a greenhouse (28 ± 1 ºC, 60 ± 5% relative humidity, L16:D8 h photoperiod) at the Citrus Research and Education Center (CREC), University of Florida, Lake Alfred, FL. All seedlings were about three months old and about 15 ± 5 cm tall.

*Corresponding author. Email: nabilkilliny@ufl.edu

Uptake of oxytetracycline by root drench. Oxytetracycline was purchased from Fisher Scientific (Pittsburgh, PA.). To study the uptake of oxytetracycline by citrus seedlings, the roots were cleaned from soil by washing under tap water, blot-dried on paper towels and immersed in 200 μg·mL^{-1} oxytetracycline solution. Control plants were incubated in distilled water. Five seedlings were used for each treatment. At the end of incubation time (16 h), the plants were washed with distilled water. The level of oxytetracycline in treated plants was determined by high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA).

Stem versus root delivery. To study the translocation of oxytetracycline through the stem application, several shallow cuts were made in the stem of each seedling using a scalpel and a large pipette tip cut lengthwise, placed around the cut area of the stem, and sealed with Parafilm. The space between the stem and the pipette tip was filled with 5 mL oxytetracycline solution (200 μg mL^{-1}) and incubated for 72 h with this antibiotic solution, while the roots were placed back into the soil. To compare stem delivery of antibiotics to root delivery, root delivery was conducted again, but for same time as stem delivery, 72 h. Control plants were incubated in distilled water without oxytetracycline. Five seedlings were used for each treatment in this experiment. At the end of incubation time, all seedlings were dissected and the antibiotic concentration was determined by HPLC.

Stability of oxytetracycline in plant tissues. To study the persistence of oxytetracycline in citrus seedlings, twenty-five citrus seedlings were incubated (via root delivery) in 200 μg mL^{-1} oxytetracycline solution for 72 h. At the end of incubation time, the roots were washed with distilled water to remove any adsorbed oxytetracycline, and the plants were returned to their original pots and left for different time intervals (0, 3, 7, 14, and 35 d). Control plants were incubated in distilled water. Oxytetracycline levels were measured using HPLC.

Extraction of oxytetracycline in plant tissues. Plant tissues were ground with liquid nitrogen using a mortar and pestle and 0.1 g of the homogenous sample was transferred to a 2-mL centrifuge tube. One mL aliquot of 0.1M HCl/0.01M EDTA solution was added to each tube and the sample was vortexed for 1 min followed by sonication for 30 min. The vortex and sonication procedures were repeated twice, and the samples were centrifuged at 12,000 rpm for 10 min at 20 ºC. The supernatant was transferred into a new tube and kept at –20 ºC until analysis using either ELISA or HPLC. To determine the efficiency of our extraction method, 100 mg of citrus leaves (control plant) was spiked with 100 μL of oxytetracycline solution of 1000 μg.mL^{-1}. Control samples were spiked with 100 μL distilled water. Oxytetracycline was extracted as described above and analyzed by HPLC and ELISA.

Analysis of oxytetracycline. *ELISA assay*. Oxytetracycline ACCEL ELISA kit was purchased from Plexense, Inc., (Davis, CA) and was used according to manufacturer recommendations.

HPLC assay. Oxytetracycline standard was purchased from Alfa Aesar (Ward Hill, MA). A stock solution of oxytetracycline (1000 μg·mL^{-1}) was prepared in methanol. A set of dilutions was made in water and were injected into the HPLC to construct the standard curve.

The detection of oxytetracycline was accomplished with an Agilent 1200 system coupled to a diode array detector (HPLC-DAD) and a Luna C18 column (150 × 4.6 mm, Phenomenex, Torrance, CA) with isocratic mobile phase of buffer solution and methanol (70:30, v:v). The pH of the phosphate buffer was adjusted to 3.0 using phosphoric acid. The flow rate of the mobile phase was 0.8 mL/min, injection volume was 20 mL, the column temperature was set at 28 °C, and the detector wavelength was set at 355 nm.

Statistical analysis. Data were analyzed using JMP 9.0 software (SAS, Cary, N.C.). The *t*-test ($P < 0.05$) was used to compare between the level of oxytetracycline obtained by HPLC and that measured by ELISA kit. The *t*-test ($P < 0.05$) was also used to compare between the levels of oxytetracycline measured after stem and root delivery. Analysis of variance (ANOVA) followed by post hoc pairwise comparisons using Tukey-Kramer honestly significant different test (Tukey HSD) were used to compare levels of oxytetracycline among 0, 3, 7, and 14 dpt ($P < 0.05$).

Results

Percentage recovery of oxytetracycline. The recovery of oxytetracycline from spiked leaf tissues using the ELISA kit and HPLC method was 85.6 ± 8.7% and 87.4 ± 9.4%, respectively. No oxytetracycline was detected in controls or blank samples. The high percentage recovery indicates that our extraction method was efficient for the detection of oxytetracycline. No significant

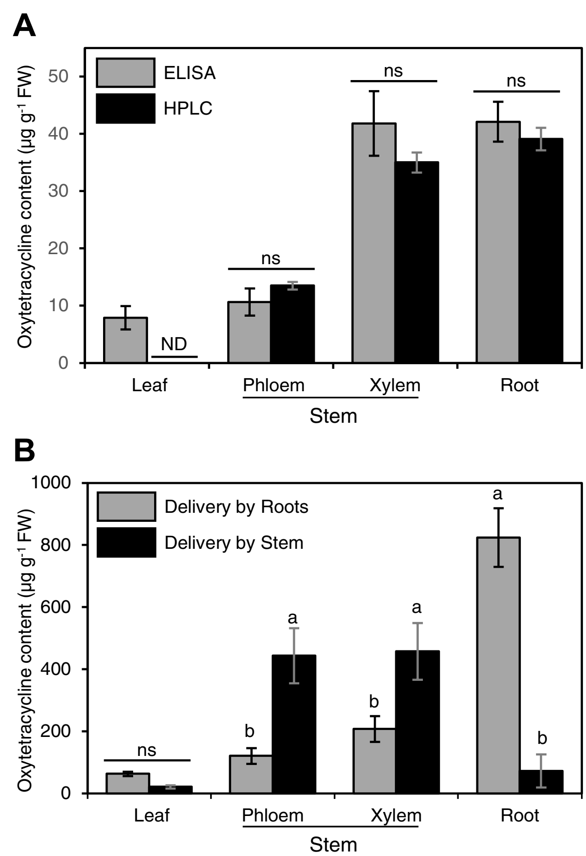

Fig. 1. Oxytetracycline content (μg g^{-1}) in fresh weight (FW) found in different Mexican lime (*Citrus aurantifolia*) seedling tissues after 16 h incubation of the roots in 200 μg·mL^{-1} oxytetracycline solution, and analyzed by HPLC and ELISA kit (A). (B) HPLC analysis of oxytetracycline content in various tissues in citrus plants treated with 200 μg·mL^{-1} oxytetracycline solution for 72 h via root or stem delivery methods. For these analyses, the stem bark was dissected into the outer bark tissue (representing the phloem) and the inner bark (representing the xylem). Data are the means ± SD of five biological replicates. Columns with different letters indicate statistically significant differences by Tukey HSD ($P < 0.05$). ND: not detected; ns: not a significant difference.

difference in detecting oxytetracycline levels was observed between HPLC and ELISA methods.

Translocation of oxytetracycline in citrus seedlings after root drenches (16 h). Oxytetracycline was detected in all parts of the plants (root, stem xylem, stem phloem, and leaves), indicating that oxytetracycline was translocated throughout the plant (Fig. 1A). The highest concentration of oxytetracycline was found in roots and xylem, followed by phloem and leaves (Fig. 1A). The concentration of oxytetracycline in leaves after 16 h of incubation was below the limit of quantification (LOQ) of the HPLC method, and therefore, only the ELISA results were reported for leaves (Fig. 1A). Nevertheless, no significant differences in levels of oxytetracycline detected in other tissues (root, xylem, and phloem) were observed between the HPLC and ELISA results (Fig. 1A).

Translocation of oxytetracycline in citrus seedlings after stem and root delivery (72 h). In this experiment, we assessed translocation dynamics of oxytetracycline by comparing between the stem and root delivery. The HPLC results showed that the levels of oxytetracycline in the xylem and phloem after stem delivery were significantly higher than those obtained after root delivery (Fig. 1B). On the other hand, the levels of oxytetracycline in the root after stem delivery were significantly lower than those obtained after root delivery (Fig. 1B). No significant differences were observed in the levels of oxytetracycline in the leaves via root or stem delivery (Fig. 1B).

The highest concentration of oxytetracycline was detected in the root followed by the xylem, phloem, and leaves via root delivery (Fig. 2A). The UV-visible spectra of oxytetracycline in oxytetracycline-treated treated plants were similar to that of oxytetracycline standard (Fig. 2A).

Stability of oxytetracycline in citrus plants. The level of oxytetracycline in the root and xylem showed a continuous decline with time (Fig. 2B). The level of oxytetracycline in the leaves and phloem followed a different trend than those that were observed in root and the xylem; it peaked after seven and fourteen days and declined thereafter (Fig. 2B).

Discussion

Evaluation of oxytetracycline extraction and analysis methods. The extraction solvent showed high recovery of oxytetracycline from plant tissues. We compared the HPLC

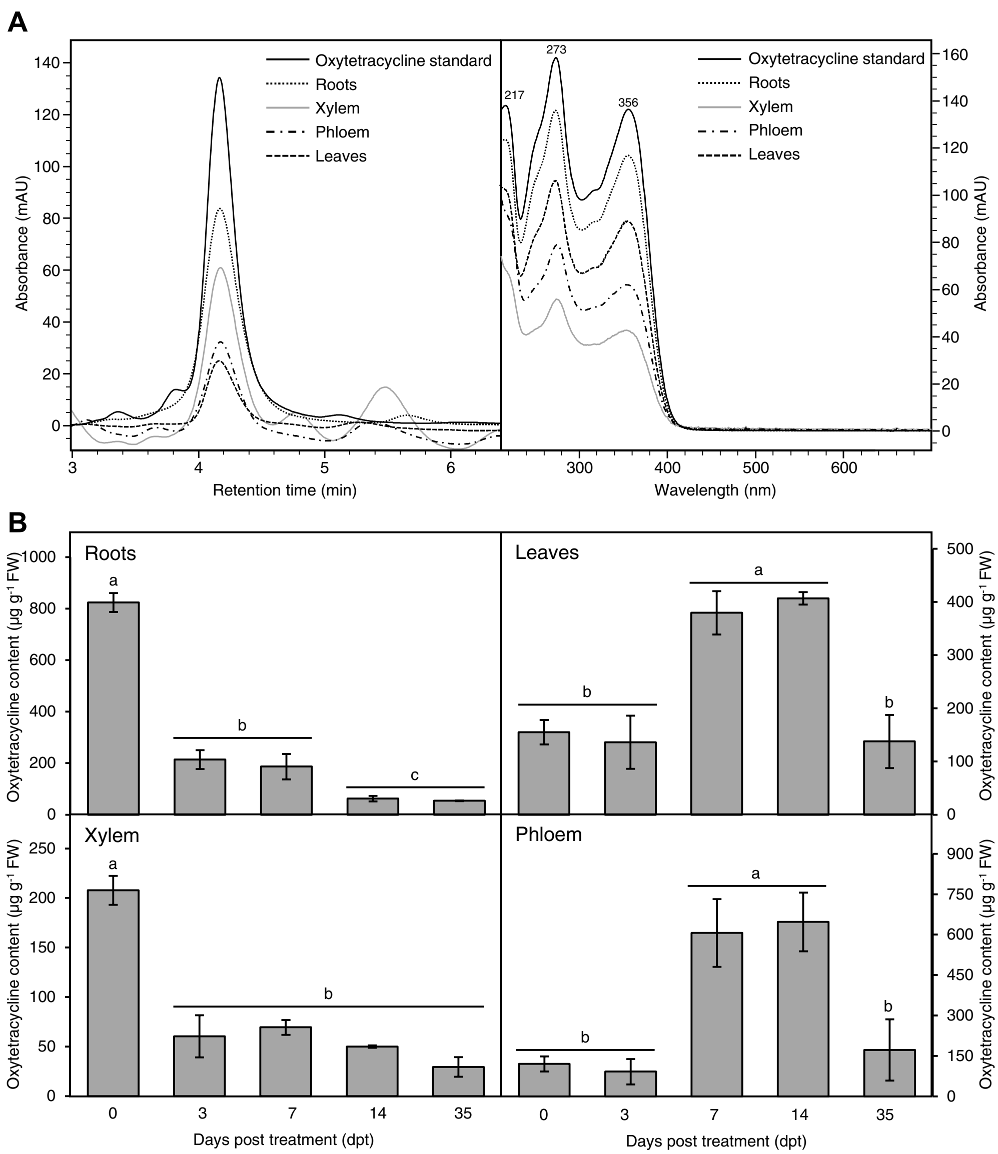

Fig. 2. HPLC results of oxytetracycline (μg g^{-1}) fresh weight (FW) detected in the root, xylem, phloem, and leaf of treated Mexican lime (*Citrus aurantifolia*) seedlings. (A) HPLC chromatogram (left) and UV-visible spectra (right) of standard and oxytetracycline in plants incubated in 200 μg·mL^{-1} oxytetracycline solution for 72 h via root drench method. (B) The level of oxytetracycline at 0, 3, 6, 14, and 35 d post-treatment in different plant tissues as measured by HPLC after incubation in 200 μg.mL^{-1} oxytetracycline solution for 72 h via root drench method. Plant roots were washed with distilled water after treatments and the plants returned to their original soil until analysis. For these analyses, the stem bark was dissected into the outer bark tissue (representing the phloem) and the inner bark (representing the xylem). Data are the means ± SD of five biological replicates. Columns with different letters indicate statistically significant differences by Tukey HSD ($P < 0.05$).

method to the ELISA kit, and our comparison indicated that both methods were suitable for the detection of oxytetracycline. However, each method has its own advantages. The HPLC has a high dynamic range from 5–200 μg·mL^{-1}, and it provides extra confirmation of oxytetracycline (UV-spectra and retention time). On the other hand, the ELISA was more sensitive (1.56 ng·mL^{-1}) than HPLC; however, it had a very narrow linear dynamic range (1.56-50 ng·mL^{-1}).

Translocation of oxytetracycline. Oxytetracycline was detected in the xylem, phloem, and leaves of citrus seedlings by ELISA after their roots were incubated in oxytetracycline solution for 16 h. These results indicated that oxytetracycline was taken up by the roots and translocated through the xylem. The highest concentration was found in the roots, followed by the xylem, phloem, and leaves. The level of oxytetracycline in the leaves was not detected by HPLC (below LOQ), and therefore, was measured using ELISA. The presence of oxytetracycline at high concentration in the phloem indicated that oxytetracycline could be effective against *C*Las, since it resides in the phloem. Previous research indicated that high efficiency of antibiotics against plant pathogens in vitro, does not guarantee high efficiency in planta. For example, several antibiotics were effective against spiroplasmas and phytoplasmas in vitro; however, only oxytetracycline was effective against these agents in planta, indicating that it has the potential for a high translocation rate in the phloem (Daniels, 1982).

Stem versus root delivery. Our results showed that the levels of oxytetracycline in the phloem and xylem of citrus seedlings after stem delivery were higher than those obtained after root delivery. On the other hand, the level of oxytetracycline in the root treated after root delivery was higher than that measured in the root after stem delivery. This result showed that low amounts of oxytetracycline were translocated from the stem to the root after stem delivery. In agreement with our results, no antibiotic activity was detected in the small roots of sweet orange citrus trees that were injected with 10–30 g of oxytetracycline/tree via trunk injection, indicating limited downward movement (Timmer et al., 1982). Twigs and leaves showed high antibiotic activity, indicating higher upward movement of oxytetracycline most probably via xylem vessels (Timmer et al., 1982). Lee et al. (1982) also showed that oxytetracycline activity was high in the canopy following trunk injection, whereas roots showed low or no activity. On the other hand, high antibiotic activity was observed in the roots after drench treatment (Lee et al., 1982). The combination of drench and trunk injection resulted in high activity in the root and canopy of treated plants (Lee et al., 1982).

Stability and persistence of oxytetracycline in citrus seedlings. Oxytetracycline was still detectable in all tissues tested including in the phloem, xylem, leaves, and roots up to thirty-five days after treatment, indicating that oxytetracycline was relatively stable in citrus plants. In agreement with our results, the activity of oxytetracycline was still detected (2-3 μg·g^{-1}) in leaves thirty-six days after injection of 6.0 g into the trunk of coconut trees (McCoy, 1976). The activity of oxytetracycline also persisted in the twigs and leaves of sweet oranges 3–8 months after trunk injection of 10–30 g/tree (Timmer et al., 1982). The observed half-life of oxytetracycline in coconut palm trees was estimated to be 2–3 weeks (Hunt et al., 1974; McCoy, 1976).

Conclusion

Our results showed that oxytetracycline was translocated in citrus plants after root and stem delivery methods. The presence of oxytetracycline at high concentrations in the phloem suggested that it could be effective against *C*Las. Oxytetracycline was detectable in all tested tissues 35-day posttreatment, indicating that it was relatively stable in citrus plants. Future studies should evaluate the efficacy of oxytetracycline against the *C*Las pathogen in the field.

Literature Cited

Blaustein, R.A., G.L. Lorca, and M. Teplitski. 2017. Challenges for managing *Candidatus* Liberibacter spp. (Huanglongbing disease pathogen): Current control measures and future directions. Phytopathology 108:424–435.

Capoor, S.P. and M.J. Thirumal.1973. Cure of greening affected citrus plants by chemotherapeutic agents. Plant Dis. Report 57:160–163

Daniels, M.J. 1982. Editorial: Possible adverse effects of antibiotic therapy in plants. Clin. Infect. Dis. 4:S167–S170.

Doud, M.S., C. Yang, Y. Huang, Y. Duan, C.A. Powell, Y. Zhong, and M. Zhang. 2018. Antimicrobial compounds effective against *Candidatus* Liberibacter asiaticus discovered via graft-based assay in citrus. Sci Rep. doi: 10.1038/s41598-018-35461-w

Gottwald, T.R., J.H. Graham, M.S. Irey, T.G. McCollum, and B.W. Wood. 2012. Inconsequential effect of nutritional treatments on huanglongbing control, fruit quality, bacterial titer and disease progress. Crop Prot. 36:73–82.

Grafton-Cardwell, E.E., L.L. Stelinski, and P.A. Stansly. 2013. Biology and management of Asian citrus psyllid, vector of the Huanglongbing pathogens. Annu Rev. Entomol. 58:413–432.

Hunt, P., A.J. Dabek, and M. Schuiling. 1974. Remission of symptoms following tetracycline treatment of lethal yellowing-infected coconut palms. Phytopathology 64:307–312.

Lee, R.F., L.W. Timmer, and L.G. Albrigo. 1982. Effect of oxytetracycline and benzimidazole treatments on blight-affected citrus trees. J Am. Soc. Hortic. Sci. 107:133–1138.

Martinez, A.L, D.M. Nor, and A.L. Armedil. 1970. Suppression of symptoms of citrus greening disease in the Philippines with tetracycline antibiotics. Plant Dis. Rptr. 54:1007–9.

McCoy, R.E. 1976. Uptake, translocation, and persistence of oxytetracycline in coconut palm. Phytopathology 66:1038–1042.

Schwarz, R.E. and S.P. van Vuuren. 1970. Decreases in fruit greening of sweet orange by trunk injections with tetracyclines. Plant Dis. Rptr. 5.5:747–50.

Shin, K., M.S. Ascunce, H.A. Narouei-Khandan, X. Sun, D. Jones, O.O. Kolawole, E.M. Goss, and A.H.C. van Bruggen. 2016. Effects and side effects of penicillin injection in huanglongbing affected grapefruit trees. Crop Prot. 90: 06–116.

Timmer, L.W., R.F., Lee, and L.G. Albrigo. 1982. Distribution and persistence of trunk-injected oxytetracycline in blight-affected and healthy citrus. J. Am. Soc. Hort. Sci. 107:428–432.

Tiwari, S., N. Killiny, and L.L. Stelinski. 2013. Dynamic insecticide susceptibility changes in Florida populations of *Diaphorina citri* (Hemiptera: Psyllidae). J. Econ. Entomol. 106:393–399.

Wang, N., E.A. Pierson, J.C. Setubal, J. Xu, J.G. Levy, Y. Zhang, J. Li, L.T. Rangel, and J. Martins. 2017. The *Candidatus* Liberibacter–host interface: Insights into pathogenesis mechanisms and disease control. Ann. Rev. Phytopathol. 55:451–482.

Zhang, M., C.A. Powell, Y. Guo, M.S. Doud, and Y. Duan. 2012. A graft-based chemotherapy method for screening effective molecules and rescuing Huanglongbing-affected citrus plants. Phytopathology 102:567–574.

Zhang, M., C. Yang, and C.A. Powell. 2015. Application of antibiotics for control of citrus huanglongbing. Adv. Antibiot. Antibodies 1: e101.

Proc. Fla. State Hort. Soc. 132:72–74. 2019.

Whole-plant Ontogenic Pattern of Flush Phenology and Plant Growth Regulator Responses

Sheng-Yang Li, Christopher Vincent*, and Kirsten Pelz-Stelinski

University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Rd., Lake Alfred, FL 33850

Additional index words. citrus, flushing, shoot initiation, auxin, gibberellin

Flushing phenology in citrus is important to integrated management of multiple insect pests, which thrive and reproduce on new flush, but it has been described as irregular. The pattern of new flush emergence in citrus is modulated by external factors and internal signals. We initiated trials of plant growth regulators (PGRs) in the greenhouse to manipulate plants to produce a more synchronized flushing pattern, with naphthalene acetic acid (NAA) to suppress and gibberellic acid (GA_3) to accelerate. In the greenhouse experiments, 21.6 ppm foliar GA_3 application induced more flushes on the 1-year-old potted plants, compared with other rates that are lower than 21.6 ppm. NAA application delayed first flush up to 20 days but the optimum rate depended on soil vs. foliar application rates were shown a varied suppressing effect in sprouting, depending on foliar vs. soil application. Furthermore, observation of the pattern of emergence regardless PGR treatment revealed clusters of first flush emergence. We hypothesized this was controlled by bud age. In the field trial, mature trees were pruned to induce a synchronized flush, then GA_3 was applied foliarly at various times after flush budbreak to test whether bud age affected the flushing response to GA_3. There was a significant response to application at 8 weeks after budbreak, where there was not to application at 4, 6, 8, or 10 weeks. However, we observed flushing induced on mature branches rather than on the most recent flush. Results suggest that the ontogenic response of flushing to exogenous GA_3 application is on the whole-tree level response rather than individual bud age.

Flush patterns establish the canopy structure and may influence the possible inflorescence shoot in the following year. Flush management may contribute to current citrus cultivation systems in the context of huanlongbing (HLB) disease. New flush is central to the spread of HLB, and manipulation of flush phenology could lead to more effective coordination of vector management. Citrus flush is likely controlled by internal factors, such as endogenous hormone level and bud age (Krajewski and Rabe, 1995). For example, auxins maintain apical dominance which inhibits axillary sprouting. A synthetic auxin was utilized as a shoot growth inhibitor to improve shoot management in pruned citrus trees (Lundberg and Smith, 1974; Phillips and Tucker, 1974).

In comparison, gibberellic acid (GA_3) enhances shoot growth and is associated with bud break (Altman and Goren, 1974). We hypothesize that these can be utilized to delay (auxin) and trigger new flush (GA_3). In this study, flush response to a synthetic auxin, naphthalene acetic acid (NAA) and GA_3 is evaluated to determine whether the flush response is affected by bud age or not.

Materials and Methods

We performed three experiments: In the first greenhouse experiment, NAA and GA_3 were applied at 5 rates in both soil and foliar application. All potted plants, 1-year-old 'Hamlin' (*Citrus sinensis*) grafted on 'Swingle' citrumelo (*C. paradisi* × *Poncirus trifoliata*) were tipped before treating with NAA and GA_3 to either soil or canopy in a randomized complete block arrangement. The dosage information is shown in Table 1. The second study was initiated to assess the effects of higher rates of NAA application, 0, 12, 60, 300, 1000 ppm to soil or canopy to the same variety and ages as Experiment 1. In both studies, the date of first flush emergence was observed. In the third experiment, conducted in the field, 60 sweet orange (*C. sinensis*) field-grown trees, 'B6-68' sweet orange grafted onto either 'C-35' citrange (*C. sinensis* × *C. trifoliata*) or 'Swingle' citrumelo rootstock, of similar size and vigor and with few HLB-symptoms were selected. The field study was conducted at the experimental grove (28°05'10.3"N 81°36'52.2"W), in Haines City, FL, managed by Citrus Research and Education Center.

A randomized complete block design was adopted with 5 trees per block and 12 blocks coupled with 4 treatment groups of trees with the application to accorded flush shoot age and the control group. In the beginning of the trial all trees were manually pruned to trigger a coordinated new flush. After the first flush was observed, we labelled 20 pruned shoots per tree around the canopy. GA_3 was applied to the canopy on the subsequent flush shoot at 1) 4 weeks after flush initiation (WAF), 2) 6 WAF, 3) 8 WAF, 4) 10 WAF (Table 1 and Fig. 1), or the control (CTL), which was

Table 1. The plant growth regulators (PGRs) and application methods assigned to the citrus plants in greenhouse experiments.

	Experiment 1		Experiment 2
Condition	Greenhouse, potted		
Plant age	1-year-old		
Scion/Rootstock	Hamlin/Swingle citrumelo		
PGR	NAA & GA_3		NAA
Application method	Foliar & soil application		
Application rate (ppm)	0.04	0.02	0
	0.17	0.09	12
	0.87	0.47	60
	4.33	2.33	300
	21.6	11.7	1000

*Corresponding author. Email: civince@ufl.edu

pruned and did not receive any GA_3 application. New sprouting on the first 5 nodes of each labeled shoot was observed at 4 weeks after GA_3 application for each treatment, as well as in the control. We count that the flush shoot has the response to gibberellin application if any one of 5 nodes sprouted. Most of the sprouting buds were observed to be the terminal ones. Some field trees lost a few labelled flush shoots due to the psyllid nymph feeding. On these trees only 10–15 shoots were valid for evaluation.

All data were analyzed by using linear mixed effects models (package: nlme) in R (version 3.6.0), in which rootstock cultivars and grove management practices and their interaction were considered as fixed affect, while the experimental repeats and the interaction effect between experimental repeat and rootstock variety were considered as random effects. The flush shoot count is expressed as new shoots per node on the five most distal nodes of labelled shoots. These were calculated as a mean per plant. Flush count data collected from a few plants were discarded and regarded as missing data due to the sever psyllid damage or off-type scion-rootstock combination. Tukey least significant differences comparisons were performed using the agricolae package in R, and *P*-values < 0.05 were considered significant.

Results

PGRs serve a role as flush suppressor and inducer. In the first greenhouse experiment, 11.7 ppm, the highest dosage, of NAA showed a delaying effect on subsequent flush. No significant differences were observed in inducing flush among the rates of GA_3 in soil application (data not shown). The highest foliar rate, 21.6 ppm, induced higher proportion of plants to flush. This result indicated that 21.6 ppm is sufficient to induce the flush in young citrus plants.

Comparison of NAA application methods in delaying effect. In the both soil and foliar applications, all rates delayed the subsequent flush. The effectiveness of each application method with the specific rate indicates that foliar application results in delaying effect with lower rates, 60 to 300 ppm (Fig. 1). The delaying effect can last up to 40 days after tipping. Comparing to soil application method, the same delaying effect requires higher rates, 300 to 1000 ppm to achieve the similar delaying effect.

Bud age and foliar GA_3 application effect on flushing response of HLB-affected plant. In the greenhouse experiment, GA_3 at 21.6 ppm was preliminarily confirmed to induce flushes in young citrus plants. A similar rate, 20 ppm of GA_3 was foliar-applied to field plants which are affected by HLB. The sprouting nodes were recorded at 4 weeks after GA_3 application to the trees with corresponded flush shoot age. Most sprouting nodes were the terminal ones from the flush shoot and shown the longer flush growth (data not shown). No sprouting node on the recent flush shoot was shown in the CTL plants (Fig. 2). A few sprouting nodes were observed on flush shoots in the plants with 4 WAF, 6 WAF, 8 WAF, and 10 WAF separately. However, only the plants with 8 WAF had the significantly higher sprouting rate, 10.7% than the control plants. Meanwhile, more sprouting nodes were shown on the previous branches and shoots than the most recent ones which were induced by pruning.

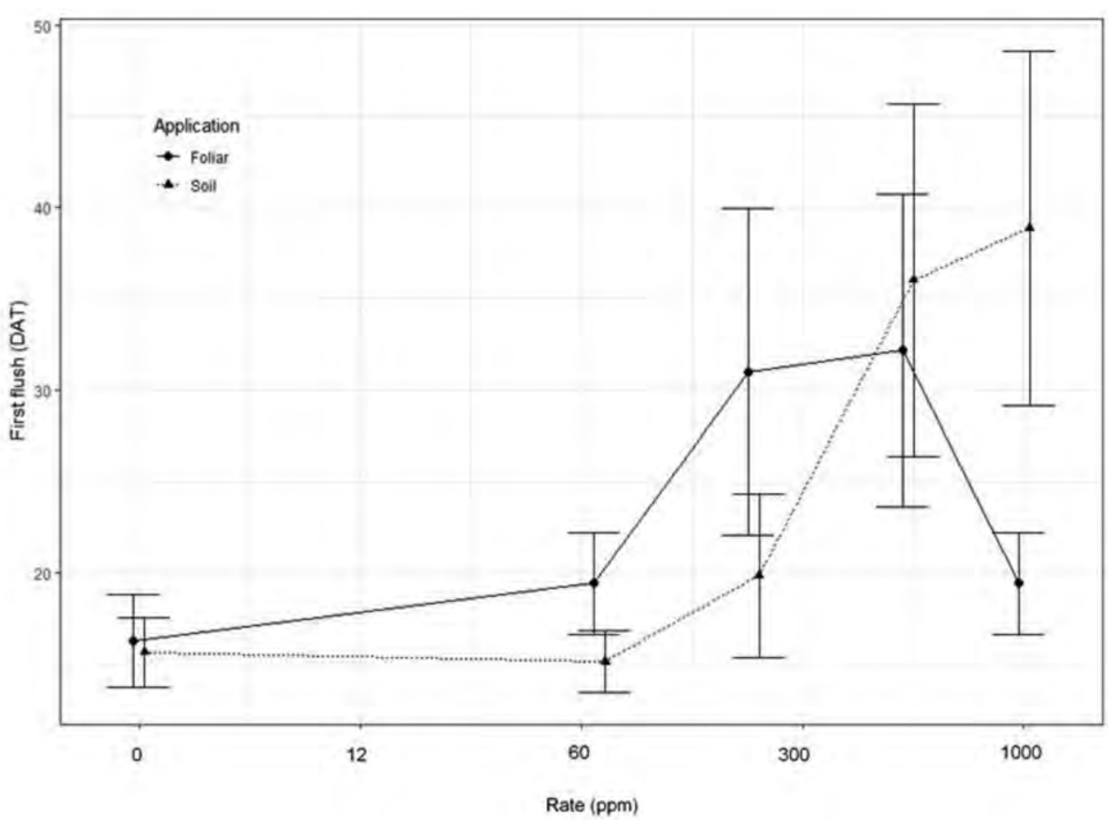

Fig. 1. Day of first flush of sweet orange, *Citrus sinensis*, after tipping (DAT) affected by different rates and application methods of naphthalene-acetic acid (NAA). The vertical bar represents standard error.

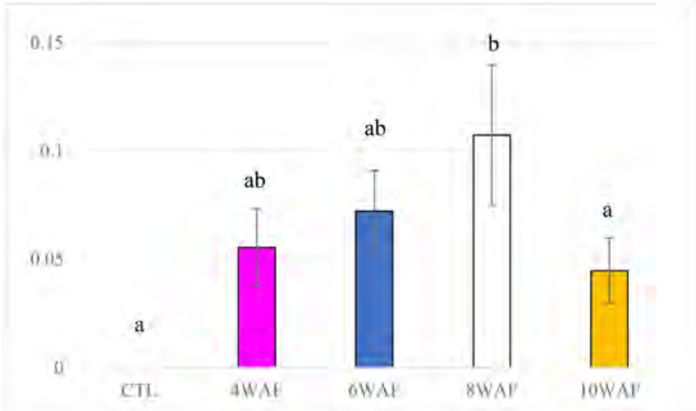

Fig. 2. The average flush shoot count of 20 labelled sweet orange (*Citrus sinensis*) shoots in the tree canopy in response to GA_3 application timing. GA_3 was applied at different weeks after flush initiation (WAF). The bars represent means and vertical bars represent standard error (n = 12). Different letters indicate the significant difference at $P \leq 0.05$ via Tukey test.

Discussion

In greenhouse experiments, the inhibitor role of NAA was shown to delay flush initiation. The efficacy of application method is different at comparable rates. Foliar application of NAA delays the flush up to 40 d at the lower rate but a similar delaying effect requires higher rate in soil application. Unlike the application in direct foliar contact, the efficiency of roots absorbing NAA may be lower, which would explain the difference.

Shoots with older nodes can have higher flush rates in the coming spring than recent flush shoots with younger nodes from the pruned stub (Krajewski and Rabe, 1995). No new flush on most current flush shoot in control plants in our study were shown as late as 12 weeks after flush initiation induced by pruning. In comparison, the plants with at least 4-week-old nodes showed the sprouting tendency in response to GA_3 application. The plants with 8-week-old nodes showed a significantly higher sprouting rate than the control plants. This fact indicates that GA_3 can induce new sprouting. The concept of GA_3 economy in citrus may give another hint to explain this field observation. Comparing to the antisense and sense overexpression of GA_{20}-oxidase gene, the shoot growth and endogenous GA_1 content are not different once they are grafted onto the non-transgenic 'Carrizo' citrange rootstock (Fagoaga et al., 2007). This study supports that the root plays the role in GA economy, which contributes to the bioactive GA formation or distribution. Then, the higher sprouting nodes on older branches and shoots can be attributed to the GA economy. Exogenous GA_3 may not immediately affect the sprouting on the applied young flush shoots but may travel to the root then distribute to the canopy in another bioactive GA form. If the root really plays a role in GA economy which affects flush phenology, the root should be considered as a factor in flush phenology.

Conclusions

NAA can effectively delay flush either applied to soil or canopy, but effective rates can vary with different application methods. Foliar application delays new flush at lower rates than soil application. GA_3 foliar application at 20 ppm can induce new flush but was most effective in the plants with 8-week-old flush shoots. Most sprouting nodes are the terminal nodes. Larger amounts of sprouting nodes were observed on the previous shoots and branches located in the inner canopy. Flush induction by GA_3 application likely results from whole plant signals, rather than individual bud maturity.

Literature Cited

Altman, A., and R. Goren. 1974. Growth and dormancy cycles in citrus bud cultures and their hormonal control. Physiol. Plant 30:240–245.

Fagoaga, C., F.R. Tadeo, D.J. Iglesia, L. Huerta, I. Lliso, A.M. Vidal, M. Talon, L. Navarro, J.L. García-Martínez, and L. Peña. 2007. Engineering of gibberellin levels in citrus by sense and antisense overexpression of a GA 20-oxidase gene modifies plant architecture. J. Exp. Bot. 58:1407–1420.

Krajewski, A.J. and E. Rabe. 1995. Bud age affects sprouting and flowering in Clementine mandarin (*Citrus reticulata* Blanco). HortScience 30:1366–1368.

Lundberg, E.C. and T.S. Smith. 1974. A possible sprout inhibitor for Florida citrus. Proc Fla. State Hort. Soc. 87:20–23.

Phillips, R.L. and D.P.H. Tucker. 1974. Chemical inhibition of sprouting of pruned lemon trees. HortScience 9:199–200.

Proc. Fla. State Hort. Soc. 132:75–79. 2019.

Genetic and Chemical Validation of *Citrus grandis* × *Citrus latipes* Hybrids

Nabil Killiny*[1], Manjul Dutt[2], Qibin Yu[2], Fred G. Gmitter[2], and Jude W. Grosser[2]

[1]*Department of Plant Pathology, University of Florida, Citrus Research and Education Center/IFAS, 700 Experiment Station Rd., Lake Alfred, FL 33850*

[2]*Department of Horticultural Sciences, University of Florida, Citrus Research and Education Center/IFAS, 700 Experiment Station Rd., Lake Alfred, FL 33850*

Additional index words. *Citrus latipes*, hybrids, Pummelo, SSR, volatile organic compounds

Three Pummelo × *Citrus latipes* hybrids were selected from a population produced by crossing the huanglongbing (HLB) susceptible C2-5-12 Pummelo as the female parent with pollen from *C. latipes* (HLB tolerant) obtained from the U.S. Department of Agriculture in Riverside, CA. Seeds were germinated in a high pH (8) clay soil supplemented with Phytophthora (*P. nicotianae* and *P. palmivora* mixed). Three vigorously growing putative hybrid seedlings were selected from this population initially based on leaf morphology and seedling vigor. The selected hybrid seedlings also exhibited the compact tree architecture as seen in the pollen parent. Hybrids were verified by simple sequence repeat (SSR) analyses. Volatile metabolite profiles distinguished the hybrids from the parents; however, they were closer to the father–parent *C. latipes*. Interestingly, one hybrid (hybrid 2) was more different than the others. The chemical analysis confirmed the SSR results; however it gave more insight about the differences and the similarities between the hybrids and their ancestors.

Currently, there is no cure for huanglongbing (HLB) disease and its management mainly depends on the control of its insect vector using insecticides. With the continuous loss in citrus yield caused by HLB, development of citrus cultivars that tolerate HLB is urgently needed. The new tolerant cultivars could supplant the established susceptible cultivars and improve profitability by reducing the costs associated with pest management. In addition, understanding of the mechanisms behind citrus tolerance to HLB could help citrus breeders to develop commercially tolerant citrus cultivars.

Resistance of citrus cultivars to *Candidatus* Liberibacter asiaticus (*C*Las), the causal agent of HLB or its vector in the field, *Diaphorina citri* Kuwayama, could save citrus trees from HLB. Field and greenhouse observations have indicated that *D. citri* has specific host plant preferences (Halbert and Manjunath, 2004; Richardson and Hall, 2014). In the same manner, field observations revealed that some citrus cultivars were more tolerant to *C*Las than others (Halbert and Manjunath, 2004). Greenhouse-controlled studies also showed 'Eureka' lemon (*Citrus limonia* Osbeck) and *C. latipes* (Swingle) Tanaka to be more tolerant to the *C*Las pathogen than others (Folimonova et al., 2009).

Unfortunately, the mechanism behind the tolerance of these species is not well understood. Tolerance of these species to HLB could arise from their resistance to the HLB pathogen or to *D. citri*. Recently, we investigated the volatile and polar metabolite profiles of many commercial citrus cultivars to explore the biochemical differences between them (Killiny et al., 2016; Hijaz et al., 2016). Higher levels of metabolites involved in plant defense or those that possess strong antimicrobial activities were found in the tolerant cultivars (Killiny et al., 2016; Hijaz et al., 2016). In another study, we analyzed many of the metabolites of 'Sugar Belle' mandarin hybrid (*C. reticulata* 'Clementine' × *C. reticulata* 'Minneola') and four of its close relatives (Killiny et al., 2017). Remarkably, we found that 'Sugar Belle' mandarin was high in thymol, which has strong antimicrobial and anti-fungal activity (Killiny et al., 2017). 'Sugar Belle' was also high in many other non-volatile compounds including synephrine, benzoic acid, ferulic acid, caffeic acid, *chiro*-inositol, *myo*-inositol, which could act as antimicrobial agents and/or protect 'Sugar Belle' mandarin from stress (Killiny et al., 2017). We also investigated the Australian finger lime (*C. australasica*) and 'Sugar Belle' (HLB tolerant) with a newly released mandarin hybrid 'Bingo' (*C. reticulata* hybrid × *C. kinokuni* 'Mukakukishu') (Killiny et al., 2018). Comparison of their metabolomic profiles revealed that the Australian finger lime was very different from 'Sugar Belle', indicating that they could have different tolerance mechanisms against *C*Las.

Although the spread of HLB disease throughout the citrus groves in Florida has been a curse to the citrus industry, it gave the citrus breeding program a chance to screen the entire field germplasm collection against HLB, providing an opportunity to identify selections with tolerance to this disease. Some resistance selections have a direct cultivar potential, but these selections should be cross-bred with other commercial cultivars to achieve commercialization. The advances in citrus tissue culture, molecular genetics and biotechnology could enhance the development and availability of commercial tolerant citrus cultivars, thereby

We thank Luke Thompson, and Shelley E. Jones for the technical assistance and we also thank Lorraine Jones and Floyd Butz for maintaining the trees in greenhouses

*Corresponding author. Email: nabilkilliny@ufl.edu

reducing the cost of citrus production and minimizing the reliance on insecticide. In this study, we evaluated three new citrus hybrids that were developed by crossing the HLB susceptible C2-5-12 Pummelo [*C. grandis* (L.) Osbeck] with the HLB-tolerant *C. latipes* in order to produce new commercial citrus cultivars, with potential tolerance toward HLB.

Materials and Methods

Hybridization and seedling selection. Crosses were made in the spring of 2013 using *C. grandis* C2-5-12 as the female parent. C2-5-12 is an open-pollinated seedling of the DPI Pummelo cultivar 'Ling Ping Yau', originally selected as a parent for citrus rootstock improvement research. *C. latipes* pollen was obtained from the U.S. Department of Agriculture's National Clonal Germplasm Repository for Citrus at Riverside, CAf. Seeds were sown in the late fall in bins of calcareous soil (pH = 8), inoculated with *Phytophthora nicotianae* and *P. palmivora* cultures obtained from Jim Graham, at the Citrus Research and Education Center, Lake Alfred, FL. Robust seedlings were selected based on growth rate, health, and color and transferred to 4 × 4 pots in commercial potting soil (Metromix 500, Sun Gro Horticulture, Agawam, MA). These seedlings were propagated as cuttings in the mistbed. Well-rooted cuttings were used for all subsequent experiments.

Genomic DNA extraction. Genomic DNA was isolated from 4-year-old greenhouse grown hybrids of *Citrus grandis* × *Citrus latipes*. Fully expanded leaf tissues (100 mg) were frozen in liquid nitrogen and disrupted using a TissueLyser II (Qiagen®, Valencia, CA) for quick pulverization. Tissue lysis was performed at 30 Hz for 1 min and total DNA was extracted with GeneJET plant genomic DNA extraction kit (Thermo-Fisher Scientific, Waltham, MA) according to the manufacturer's protocol. The concentration of DNA was determined using NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific®). The DNA samples were diluted to the concentration of 25 $ng \cdot \mu L^{-1}$ in distilled water and used for simple sequence repeat (SSR) analysis.

Plant genotyping using Simple Sequence Repeat (SSR) markers. SSR analysis was used to verify the hybridity status of the three progeny. SSR analysis was performed according to Chen et al. (2008), using Genemarker v2.6.3 to generate allele tables and graphs. To identify zygotic seedlings of *C. grandis* × *C. latipes*, eight primers, each with two expected alleles were utilized as described previously by Chen et al. (2008). Four fluorescently labeled universal M13 primers, using 6FAM, VIC, NED, and PET, were synthesized by Applied Biosystems Inc. and used for ABI G5 high throughput genotyping analysis on a Genetic Analyzer (3130 XL, Applied Biosystems®, Foster City, CA). Fluorescently labeled EST-SSR PCR products were fractionated, and chromatographic peaks were analyzed and exported into an Excel file using GeneMarker® analysis software v2.6.3 (SoftGenetics®, State College, PA).

Chemical validation using Hexane leaf extract and GC-MS. Leaf volatiles were extracted using hexane as described by (Hijaz et al., 2016) with slight modifications. Briefly, three leaves were collected from each tree and were pooled together for analysis. Five different biological samples (each sample from a different tree) were collected and each sample was analyzed twice (two technical replicates) and averaged. Leaf samples (~0.1 g) were diced from leaf blades using a razor blade and placed into 2-mL screw cap tubes with 5 mm stainless steel balls for maceration. Sample tubes were placed into liquid nitrogen for at least 5 min and then macerated using a TissueLyser II (Qiagen, Valencia, CA). An aliquot of 0.5 mL of hexane was added to each tube. The tubes were placed on ice and sonicated for 5 min, then placed on a tube rotator for 1 h at 8 °C. Following extraction, the samples were centrifuged for 3 min at 10,000 rpm and 0.2 mL of supernatant was transferred to an amber vial with 350 μL fused inserts (C4000-LV2, National Scientific, Rockwood, TN). *Trans*-2-nonenal was added at a final concentration of 1$\mu L\ mL^{-1}$ as an internal standard. Hexane extracts (0.5 μL) were injected splitlessly into the injector heated to 250 °C.

The GC-MS system consisted of a Clarus 680 GC fitted with Elite-5MS column (30 m × 0.25 mm I.D. × 0.25 μm film thickness) was used for separation of analytes (Perkin Elmer, Shelton, CT). The carrier gas was ultrapure helium at a flow rate of 1 $mL\ min^{-1}$. The oven program was 40 °C for 2 min, and then increased to 200 °C at a rate of 7 $°C\ min^{-1}$ for a total run time of 25 min. The mass spectrometer (Clarus SQ8) was operated in electron impact mode, 70 eV, 180 °C ion source temperature, and scanning from 40 to 400 *m/z*.

Detected volatile peaks were integrated using TurboMass software v. 5.4.2 (Perkin Elmer) and mass spectra were compared to those of authentic standards and/or identified by at least two of three mass spectral libraries (Wiley Flavor and Fragrances of Synthetic and Natural Compounds, Wiley 9th ed. Mass Spectral Database, and NIST 2011 MSD) with a matching score of at least 700. Unidentified metabolites were reported by *m/z* and retention time or linear retention index. Reference standard compounds were purchased from Sigma-Aldrich (St. Louis, MO) at the highest available purity. Compound peak areas were normalized to the internal standards used for each type of compound and then to 1 g of fresh leaf weight.

Quantification of leaf volatiles was based on the peak areas obtained from a series of reference standards diluted in hexane and injected under the same chromatographic conditions as samples. Calibration curves were constructed from the linear regressions obtained by plotting the concentration vs. peak area for each standard at each concentration. Similarly, reference standards from the major classes of metabolites were derivatized and used for the construction of calibration curves.

Results and Discussion

Putative C2-5-12 Pummelo × *Citrus latipes* hybrids were selected from a large population based on their growth in a calcareous phytophthora infested soil. Subsequently, leaf morphology and seedling vigor were also used to narrow our selection to the three utilized in this study (Fig. 1). The narrow tree architecture of the new hybrids and the obvious morphology of the leaves indicated to the hybrid status of the three progenies (Fig. 1). The morphology of the leaves of the new hybrids was a mix of their parents (Fig 1). The leaves of the new hybrids showed a medium wing, whereas the Pummelo parent has a small wing and the *C. latipes* has a large wing (Fig. 1). These results indicated that the crossbreeding between Pummelo and *C. latipes* was successful.

In SSR, all eight primers (CX6F02B, CX6F04G, CX6F06Y, CX6F10R, CX6F17B, CX6F18G, CX6F29Y, and CX0035R) revealed alleles in the offspring that matched those of the parents, showing that they were likely related to the parents but not confirming them as hybrids (Table 1). Four of the primers (CX6F04G, CX6F06Y, CX6F10R, and CX6F18G) produced allele combinations in all three progeny that would only be possible

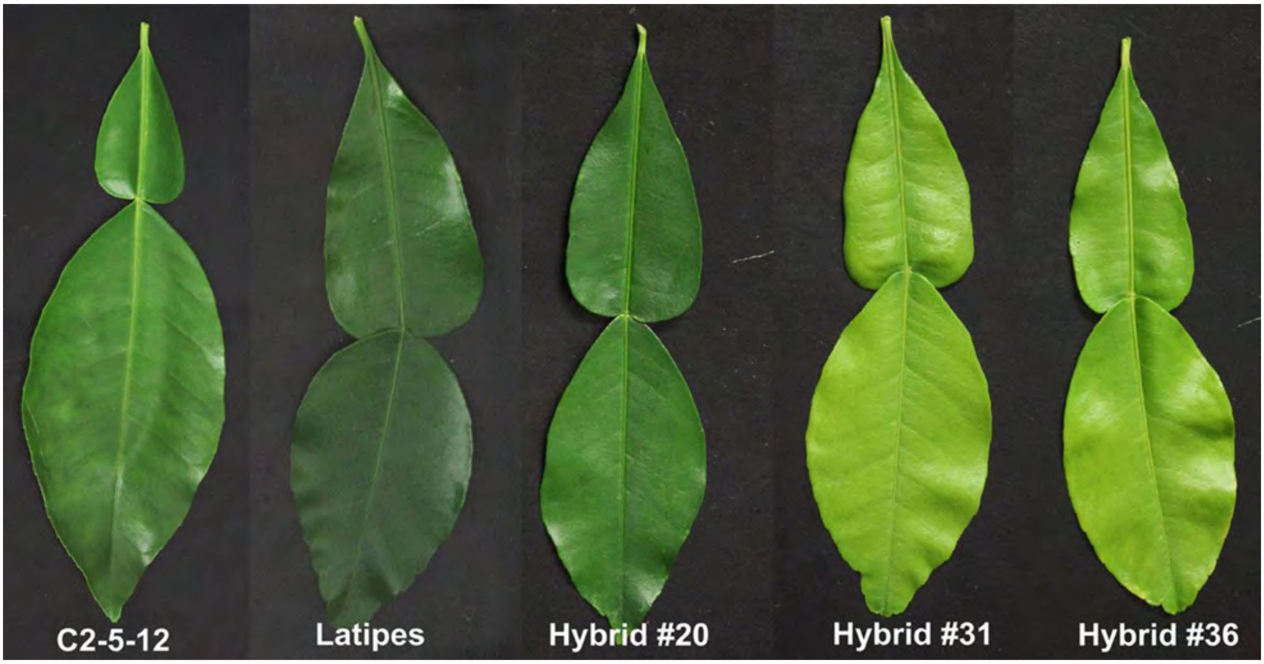

Fig. 1. Leaf morphology of the three hybrids evaluated in this study and their parents (C2-5-12 Pummelo *and Citrus latipes*).

Table 1. Detected alleles [represented by different-sized amplified fragments measured in basepairs (bps)] using eight SSR primers in pummelo and latipes and their corresponding sexual hybrids.

	Primers[z]															
	CX6F02B		CX6F04G		CX6F06Y		CX6F10R		CX6F17B		CX6F18G		CX6F29Y		CX0035R	
C. grandis	168	168	163	169	157	157	176	176	132	132	160	161	152	152	183	183
C. latipes	168	168	157	169	166	166	170	170	126	132	148	148	152	152	183	183
X1_20	168	168	169	169	157	166	170	176	126	132	148	160	152	152	183	183
X1_31	168	168	169	169	157	166	170	176	126	132	148	161	152	152	183	183
X1_36	168	168	169	169	157	166	170	176	126	132	148	160	152	152	183	183

[z]Note that four of the primers (CX6F04G, CX6F06Y, CX6F10R, and CX6F18G) produced allele combinations in all three progeny that would only be possible in a hybrid.

in a hybrid, confirming their hybrid status. The results of SSR analysis show that all three selections (Hybrid #20, Hybrid #31, and Hybrid #36) are hybrids of C2-5-12 *C. grandis* × *C. latipes*.

The volatile compounds data yielded a scatter plot showing that the volatile profile of each donor parent was distinctive from each other (Fig. 2A). The loading plot showed that *C. latipes* contained higher levels of VOCs than Pummelo (Fig. 2B). In addition, the scatter plot showed that the volatile profiles of the new hybrids were also different from their parents (Fig. 2A). The loading plot also showed that the new hybrid contained higher amounts of VOCs compared to its parents (Fig. 2B). Furthermore, the scatter plot showed that the volatile profile of hybrid 1 (# 20) and 3 (# 36) were closer to each other than hybrid 2 (hybrid # 36). The loading plot showed that hybrid 2 (#31) contained higher amounts of volatiles compared with hybrid 1 (#20) and 3 (#36) (Fig. 2B). The cluster analysis showed that hybrid 1 (# 20) and 3 (# 36) were similar (>80% similarity) to each other, whereas hybrid 2 (#31) was more similar (>80% similarity) to *C. latipes* than Pummelo (Fig. 2C).

The heat map showed that the Pummelo was low in most of the detected volatiles (Fig. 2C). *C. latipes* was high in several volatiles including α- and β-phellandrene, linalool, indole, nerol, neryl acetate, γ-carene, methyl-anthranilate (Fig. 2C). The heat map also showed that the VOC content in the new hybrids was higher than that of their parents (Fig. 2C). In addition, the heat map showed that hybrid II (#31) contains high amounts of VOCs compared to other hybrids (Fig. 2C). The heat map also showed that many of the volatile compounds that were absent in Pummelo parent were detected in at least one of the new hybrids. This result confirmed the crossbreeding between Pummelo and *C. latipes*. These results showed that the metabolomic analysis reveals more details about the new hybrids and their biochemical relationship to their parents.

In fact, citrus leaf volatiles have been utilized extensively to study the similarities and variations between donor parents, their hybrids, and between hybrids themselves. For example, Gancel et al. (2005) studied the leaf volatile compounds of six citrus somatic allotetraploid hybrids resulting from various combina-

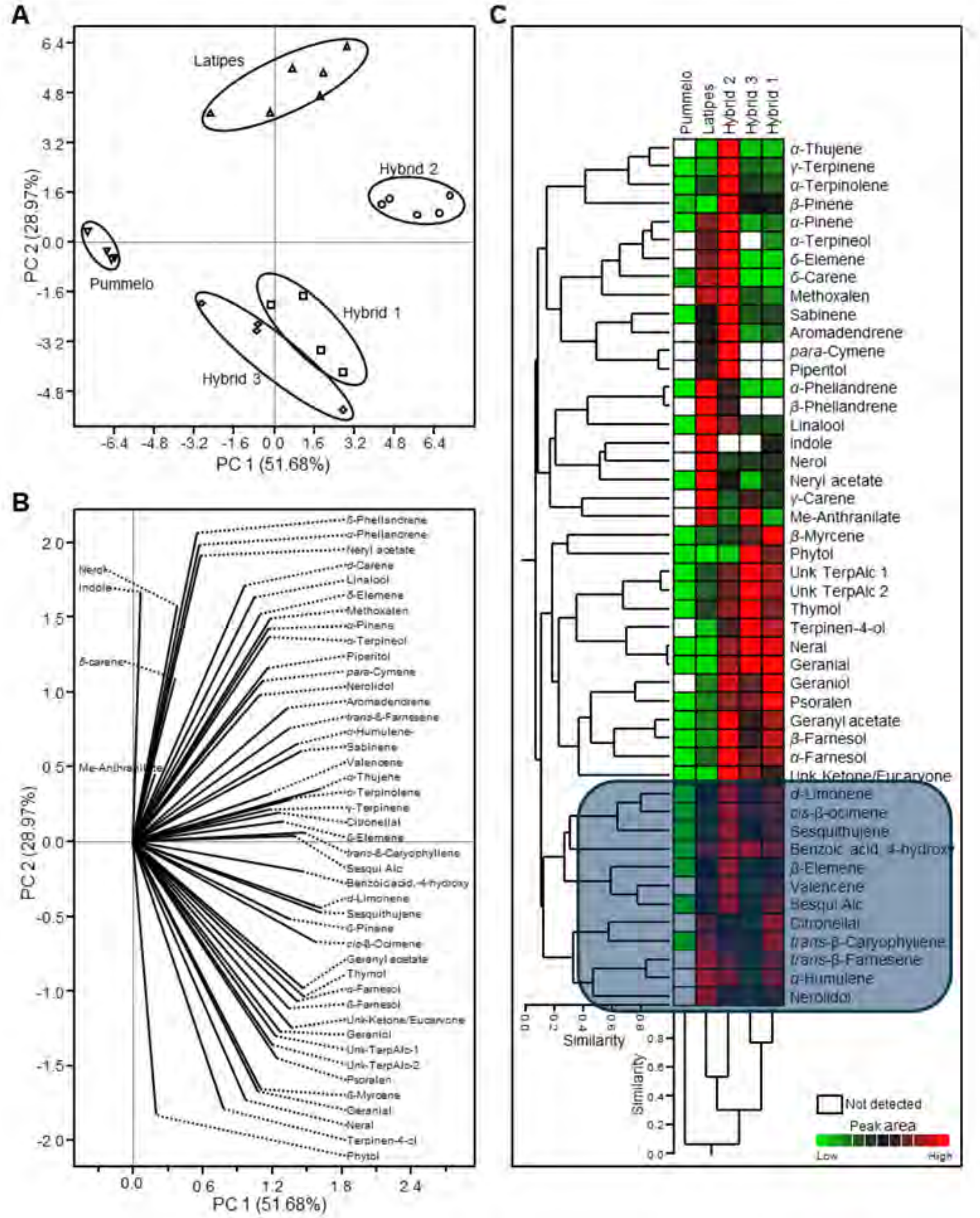

Fig. 2. Principal component analysis (PCA) of all of the volatile compounds detected in the parent donor parents (C2-5-12 Pummelo and *Citrus latipes*) and their hybrids (1: # 20, 2: # 31, and 3: # 36). The scatter plot (**A**), the loading plot (**B**), and cluster analysis and heat map (**C**) for the volatile compounds detected in donor parents and their hybrids.

tions of lime, lemon, citron, sweet orange, and grapefruit. The volatile compounds were extracted using a pentane/ether (1:1) mixture, analyzed by GC-MS, and compared with those of their respective parents. The chemical analysis showed that hybrids produced from citrus parent exhibited the same relative contents in hydrocarbons and oxygenated compounds as the acid citrus, while the (grapefruit + orange) hybrid behaved similarly to its two parents (Gancel et al., 2005). In agreement with Gancel et al. (2005), our results showed that most compounds detected in *C. latipes* were detected in its hybrids except for three compounds, which were absent in hybrid #20 and #31.

In addition, the metabolic analysis could reveal rich information about the biosynthetic pathways in the new hybrids and the possible roles of the detected metabolites in plant tolerance (Hijaz et al., 2016; Killiny and Hijaz, 2016). For example, the high level of thymol, neral, and geranial in hybrid # 36 could make it tolerant to *C*Las since they possess strong antimicrobial activity (Hijaz et al., 2016, Killiny et al., 2017). In our future work, we will evaluate the tolerance of these hybrids to the *C*Las pathogen and *D. citri*, and we will determine the mechanisms underpinning the tolerance of the tolerant hybrids.

Literature Cited

Chen C., J.W. Grosser, M. Calovic, P. Serrano, G. Pasquali, J. Gmitter, and F.J. Gmitter, Jr. 2008. Verification of mandarin and Pummelo somatic hybrids by expressed sequence tag–simple sequence repeat marker analysis. J. Amer. Soc. Hort. Sci. 133(6):794–800.

Folimonova, S.Y., C.J. Robertson, S.M. Garnsey, S. Gowda, and W.O.

Dawson. 2009. Examination of the responses of different genotypes of citrus to huanglongbing (citrus greening) under different conditions. Phytopathology 99:1346–1354.

Gancel, A. L., P. Ollitrault, Y. Froelicher, T. Tomi, G. Jacquemond, F. Luro, and J.M. Brillouet. 2005. Leaf volatile compounds of six citrus somatic allotetraploid hybrids originating from various combinations of lime, lemon, citron, sweet orange, and grapefruit. J. Agric. Food Chem. 53(6):2224–2230.

Halbert, S.E. and K.L. Manjunath. 2004. Asian citrus psyllids (Sternorrhyncha: psyllidae) and greening disease of citrus: A literature review and assessment of risk in Florida. Fla. Entomol. 87:330–353.

Hijaz, F., Y. Nehela, and N. Killiny. 2016. Possible role of plant volatiles in tolerance against huanglongbing in citrus. Plant Signal Behavior 11:e1138193.

Killiny, N. and F. Hijaz. 2016. Amino acids implicated in plant defense are higher in *Candidatus* Liberibacter asiaticus-tolerant citrus varieties. Plant Signal Behavior 11:e1171449.

Killiny, N., M.F. Valim, S.E. Jones, A.A. Omar, F. Hijaz, F.G. Gmitter, and J.W. Grosser. 2017. Metabolically speaking: Possible reasons behind the tolerance of 'Sugar Belle' mandarin hybrid to huanglongbing. Plant Physiol. Biochem. 116:36–47.

Killiny, N., S.E. Jones, Y. Nehela, F. Hijaz, M. Dutt, F.G. Gmitter, and J.W. Grosser. 2018. All roads lead to Rome: Towards understanding different avenues of tolerance to huanglongbing in citrus cultivars. Plant Physiol. Biochem. 129:1–10.

Richardson, M.L. and D.G. Hall. 2014. Resistance of *Poncirus and citrus × Poncirus* germplasm to the Asian citrus psyllid. J. Citrus Pathol. 1(1):266.

Proc. Fla. State Hort. Soc. 132:80–81. 2019.

—Scientific Note—

Improving Root Hair Growth on a Citrus Rootstock—A Possible Fertilizer Strategy to Increase Tolerance to HLB

Laura Waldo*, Arnold Schumann, and Tim Ebert

University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Rd., Lake Alfred, FL 33850

Additional index words. phosphate, phosphorus, root hair, triple calcium phosphate, tricalcium phosphate

Plant root hairs are responsible for a large percentage of nutrient uptake from the growing medium (soil or water). Typically citrus roots fail to produce root hairs naturally due to environmental conditions present in the growth media. Research published by Liu, et al. (2006), demonstrated that root hair induction was possible on 8 different monocotyledonous and dicotyledonous plant species when available phosphorus levels were kept at low, but not deficient concentrations in solution. When bioavailable phosphorus is kept at high or near absent concentrations, root hairs failed to develop. Carrizo citrange (*Citrus sinensis* Osb. × *Poncirus trifoliata* L. Raf.) seedlings were germinated into peat media plugs using a biodome style chamber. Three months post germination, 27 uniformly sized seedlings were selected and placed into three solution culture tanks (9 seedlings each). Three different cultures were developed to maintain bioavailable phosphorus levels at high (10 ppm), low (0.7 ppm), and nearly absent (0.2 ppm). Tank solutions (refreshed fortnightly) were: Tank 1—control fertilizer solution, a complete nutrient solution with soluble potassium phosphate as only source of phosphorus (P) and pH adjusted to 6.8 using sodium bicarbonate; Tank 2—equivalent ratio of nutrients as control fertilizer, with tricalcium phosphate (TCP) as sole source of phosphorus in solution (pH approximately 6.8); and Tank 3—same nutrient solution as Tank 2, with TCP as sole source of P in solution plus calcium carbonate added to buffer pH above neutral (approximately 7.2). Root sampling for microscopy (scanning electron microscopy, SEM) and leaf sampling for nutrient analysis was done six months post transplanting into solution culture. Two weeks before sampling, the chemistries of each tank were tracked and daily solutions were analyzed for available phosphate and pH. Phosphate analysis was measured in the lab using a SmartChem 170, Discrete Analyzer (Unity Scientific, Milford, MA). Several roots per tank were sampled, and processed for scanning electron microscopy at the Citrus Research and Education Center Microscopy Laboratory, Lake Alfred, FL.). Four images were taken at 300× magnification from each treatment and counted by four different individuals (no significant difference between counters). The area of each root counted, was calculated using ImageJ (an open source Java image processing program). These counts were then analyzed using analysis of variance with multiple comparisons using the Tukey test. The release of phosphate from TCP is pH dependent, and slow, maintaining an equilibrium of phosphate at very low concentrations. At pH 6.8, TCP buffers the available phosphate at concentrations between 0.54 and 0.92 ppm (Tank 2). At a slightly higher pH, 7.2, TCP buffers the available phosphate at concentrations between 0.05 and 0.49 ppm (Tank 3). While the concentration of phosphate in solution was low, the leaf P concentration for Tank 2 was 0.24%, above the optimum nutrient status (0.12 to 0.16%) based on University of Florida/IFAS recommendations for healthy citrus trees (Obreza and Morgan, 2008). Tank 3 leaf P was significantly lower (0.08%), which is below the critical deficient threshold of 0.09%. Tank 2 had a significantly higher amount of root hairs than Tanks 1 and 3 (Fig. 1); greater than 1000 more root hairs/mm² than Tank 1, and almost 2000 more root hairs/mm² than Tank 3 were counted. Based on these preliminary results, we conclude that TCP is capable of maintaining an equilibrium that is favorable to the development

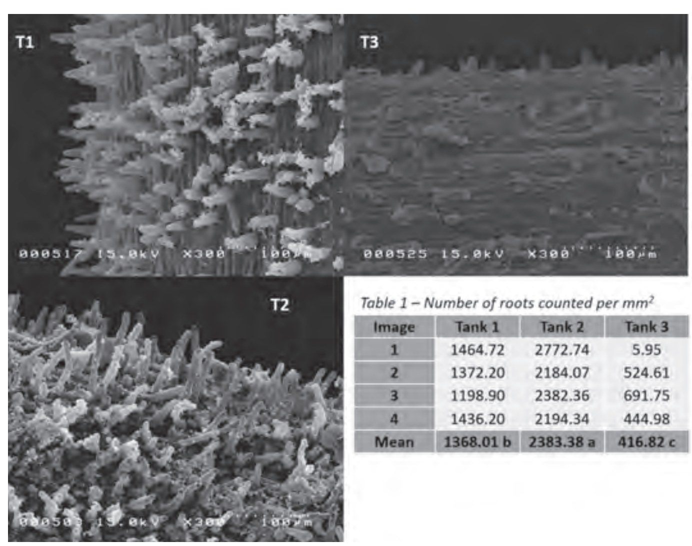

Table 1 – Number of roots counted per mm²

Image	Tank 1	Tank 2	Tank 3
1	1464.72	2772.74	5.95
2	1372.20	2184.07	524.61
3	1198.90	2382.36	691.75
4	1436.20	2194.34	444.98
Mean	1368.01 b	2383.38 a	416.82 c

Fig. 1. Number of root hairs /mm² on Carrizo citrange (*Citrus sinensis* Osb. × *Poncirus trifoliata* L. Raf.) seedlings, as influenced by phosphorus source in the growing medium. T1 = Tank 1, T2 = Tank 2, and T3 = Tank 3.

The funding for this research came from the Citrus Research and Education Foundation (CRDF). We would like to thank Daniel Stanton and the CREC microscopy laboratory for help with the microscopy.

*Corresponding author. Email: ljwaldo@ufl.edu

of root hairs. These root hairs are able to seek out and scavenge nutrients in solution with their increased surface area in order to take in enough phosphorus to keep the plants healthy. Using TCP and available calcium to reduce bioavailable P to lower concentrations may be a novel approach for field applications in HLB endemic citrus groves, improving nutrient uptake and possibly increasing citrus tree health.

Literature Cited

Liu, G-D., J. Dunlop, and P. Thai. 2006. Induction of root hair growth in a phosphorus-buffered culture solution. Agricultural Sciences in China 5(5): 370-376.

Obreza, T.A. and K.T. Morgan. 2008. Nutrition of Florida citrus, 2nd edition. Extension publication SL-253. University of Florida, Gainesville, FL.

Citrus Section

Proc. Fla. State Hort. Soc. 132:82. 2019.

—Scientific Note—

Phenology of 'Valencia' and 'Hamlin' Sweet Oranges on Two Different Rootstocks

Daniel Adu Boakye and Fernando Alferez*

University of Florida, IFAS, Southwest Florida Research and Education Center, 2685 State Rd. 29 North, Immokalee, FL 34142

Additional index words. citrus greening, citrus yield, fruit drop

Fruit growth and maturation of 'Hamlin' and 'Valencia' sweet oranges (*Citrus sinensis*) was evaluated on two different rootstocks (US-897 and US-942). This study was initiated at the June drop stage at Southwest Florida Research and Education Center demonstration grove, Immokalee, FL. The experiment was 2 × 2 factorial randomized complete block design with 10 replications. For this, fruit typology was divided into single terminal fruit (one fruit per branch) and clustered fruit (several fruit per branch). One-hundred-twenty (120) single fruits and 240 clustered fruits were flagged. Additionally, we assessed fruit organization and distribution in the trees to determine if there were differences in the number of clustered vs. single terminal fruit per rootstock. Monthly, until harvest, we monitored stem diameter, fruit diameter, albedo and flavedo thickness, peel maturation, and fruit drop rate. Irrespective of the variety we found more proportion of single terminal fruit vs. clustered fruit in trees on US-942 than in trees on US-897.

In all cases, the stem diameter of clustered fruits was significantly smaller from that of single orange fruits at June drop stage, but later in the fruit maturation process, stem diameter was not significantly different. Regarding fruit diameter, US-942 rootstock significantly influenced the fruit diameter of both single and clustered fruits of 'Hamlin' as compared to rootstock US-897. Rootstock US-942 interaction with 'Hamlin' and 'Valencia' induced an increase in fruit size from June to October. By December, no differences in fruit size were observed. Diameter of single and clustered fruits of 'Valencia' grown on US-942 was significantly higher than those of 'Hamlin' on both rootstocks.

In general, 'Hamlin' showed higher fruit drop rate than 'Valencia'. Interestingly, and irrespective of the variety, fruit organized in clusters dropped more than single fruits; the drop rate for clustered fruits was around 30% and 60% higher for 'Hamlin' and 'Valencia' respectively as compared to single fruits. The fact that varieties grown on US-942 tended to produce significantly more single terminal fruit than clustered fruit as compared to US-897, may explain previous observations of more productivity and less fruit drop of trees grown on US-942.

*Corresponding author. Email: alferez@ufl.edu

Proc. Fla. State Hort. Soc. 132:83. 2019.

—Scientific Note—

Effect of Individual Protective Cover on Physiology and Growth of Citrus Trees

Susmita Gaire, Ozgur Batuman, Ute Albrecht, and Fernando Alferez*

University of Florida/IFAS, Southwest Florida Research and Education Center, 2685 State Rd. 29 North, Immokalee, FL 34142

Additional index words. citrus greening, psyllid exclusion, tree health

For Florida citrus industry survival, it is of prominent importance to prevent psyllid insects, the vector of huanglongbing (HLB) disease, from being in contact with newly planted disease-free citrus trees from the very beginning after transplantation, to avoid the risk of HLB infection. Individual protective covers (IPCs) aim to protect individual young trees from psyllids during at least the first two years of the critical growth phase. However, a study on the efficacy of individual protective covers (IPCs) to prevent psyllid occurrence and HLB has not been performed and little is known on the effect of IPCs on physiology and growth of citrus trees. A study was initiated in Jan. 2018, to compare the performance of 'Valencia' oranges (*Citrus sinensis*) on 'Cleopatra' (*C. reshni*) rootstock with and without IPCs at Southwest Florida Research and Education Center. Our goal was to understand how IPCs influence tree growth and health.

The experiment was a completely randomized design with a two-by-three factorial arrangement with five replications. The factorial treatment structure included two levels of covering (with and without IPC) and three doses of insecticides. We assessed and compared several horticultural and physiological measurements periodically. These included: trunk diameter measurements (rootstock/scion), spring flushing and blooming patterns, canopy/leaf area index, leaf drop rate, canopy volume, chlorophyll content, and foliar starch and soluble sugar content. In addition, we assessed for the presence or absence of psyllids and other pests and diseases, including canker and greasy spot incidence. After two years, our results show that all the trees inside IPC are *Candidatus* Liberibacter asiaticus (*C*Las)-negative, as compared to two-thirds of infected trees without the IPC. Although no psyllids were found inside IPCs, other pests of concern were identified, including armyworms and mites. This indicates that scouting the trees and performing additional treatments may still be necessary. IPCs seemed to protect against canker and greasy spot incidence, although a seasonal component cannot be ruled out. Seasonality seems to play a role also in modulating horticultural and physiological parameters in IPC-covered trees.

Our next objective will be to investigate how seasonality may influence physiology of the trees inside the IPCs.

*Corresponding author. Email: alferez@ufl.edu

Proc. Fla. State Hort. Soc. 132:84. 2019.

—Scientific Note—

Deep Learning for Diagnosis of Citrus Leaf Symptoms

ARNOLD SCHUMANN*, PERSEVERANÇA MUNGOFA, AND LAURA WALDO

Citrus Research and Education Center, University of Florida, 700 Experiment Station Rd., Lake Alfred, FL

ADDITIONAL INDEX WORDS. artificial intelligence, smartphone app, machine vision, citrus nutrition, pests, diseases

The task of visually identifying nutrient deficiencies with leaf symptoms can be challenging, especially in the presence of confounding symptoms from diseases and pests. We hypothesized that deep learning machine vision developed as a smartphone app could substitute for expert human diagnosis of in-field leaf symptom expression. The overall goal of this study is to develop an app for enabling farmers, home-owners, and extension agents, without previous experience, to accurately identify nutrient deficiencies and common pest and disease symptoms in citrus. Nine symptom classes were developed for this prototype application. Four are nutrient deficiency symptoms (magnesium, manganese, zinc, and iron; Mg, Mn, Zn, and Fe respectively), three represent disease symptoms (HLB, citrus canker, greasy spot), and one shows pest damage (two-spotted spider mites). The ninth class represents asymptomatic healthy leaves. Citrus leaves representing each symptom (~130 per class) were collected in the field and photographed with a smartphone camera using at least eight megapixel resolution. Leaves with no symptoms, greasy spot, canker, and mite damage were photographed on both adaxial and abaxial sides. The images were tagged with their respective identities using the YoloV3 text labeling convention (Redmon and Farhadi, 2018) and used to train a YoloV3-spp convolutional artificial neural network on the Darknet framework <https://github.com/AlexeyAB/darknet>. A separate validation set of images was created from additional leaves, using three replications of 15 leaves for each class. For the nutrient deficiency classes, each batch of 15 leaves was analyzed for their nutrient concentrations in order to confirm the diagnosed deficiencies. Percentage diagnostic accuracy for each class was estimated from each 15-leaf replicate by comparing machine vision results with those determined by human experts. The average accuracy of the trained YoloV3-spp network to correctly identify all leaf symptoms was 89%, ranging from 63% for Zn to 100% for greasy spot, canker, and asymptomatic healthy leaves. After further investigating the validation images, we discovered that most of the incorrectly diagnosed Zn symptoms were due to the similar appearance of Mn deficiency, and that many of the images used for training did not exhibit classic pure Zn deficiency symptoms. A revised image set will be collected for retraining the Zn and other symptoms to improve detection accuracy. The trained neural network file was copied to an Apple Mac computer, merged into an app, and compiled with the Xcode compiler for the iPhone smartphone (Fig. 1, Fig. 2). Preliminary tests with the iPhone app show that machine vision can diagnose leaf symptoms in less than one second. The smartphone app will be thoroughly field-tested and additional symptom classes will be added before deploying it to the App Store as a new scouting tool for diagnosing citrus leaf symptoms in Florida.

Fig. 1 (left). Screen capture of the iPhone app identifying a citrus leaf symptom.

Fig. 2 (below). The prototype iPhone app being used to diagnose citrus greasy spot disease in less than one second.

This research was funded by the HLB Multi-Agency Coordination (MAC) System.

*Corresponding author. Email: schumaw@ufl.edu.

Literature Cited

Redmon J. and A. Farhadi. 2018. Yolov3: An incremental improvement. arXiv preprint arXiv:1804.02767

Proc. Fla. State Hort. Soc. 132:85. 2019.

—Scientific Note—

Phytotoxicity Threshold for Neutral Electrolyzed Water in Citrus

Timothy Ebert*, Laura Waldo, William Holmes, Napoleon Mariner Jr. and Arnold Schumann

University of Florida/IFAS, Citrus Research and Education Center, 700 Experiment Station Rd., Lake Alfred, FL 33850

Additional index words. pest management, electrolyzed water, greasy spot, citrus scab

This project investigated a method to control pests and diseases in a citrus under a protective screen (CUPS) environment to enable sustainable and economically viable citrus production. The screen prevents Huanglongbing disease by excluding the vector, the Asian citrus psyllid. However, the screen alters the growing environment and greasy spot (*Mycosphaerella citri* Whiteside) and citrus scab (*Elsinoe fawcettii* Bitancourt and Jenk) are problems. Neutral electrolyzed water (NEW) is a surface sterilant and therefore may be an effective tool for managing these diseases. NEW water has been used effectively in strawberry, tomato and gerbera production for disease management (Abbasi and Lazarovits, 2006; Hirayama et al., 2016; Mueller et al., 2003).

The specific objective of this study was to develop guidelines regarding concentration and frequency of application for using electrolyzed water in CUPS to minimize the phytotoxic damage this product may cause to citrus trees.

NEW was made by passing an electric current through a potassium chloride salt solution to generate hypochlorous acid and potassium hypochlorite. A total of five concentrations from 0 to 500 mg/L chlorine were tested against seedling non-grafted *Citrus sinensis* 'Valencia' plants with a mean height of 34 cm. growing in a greenhouse. Application intervals were once per week, twice, and five times per week for a total of 81 d. There were four replicates per treatment. Chlorophyll was measured weekly using an Apogee MC100 SPAD meter. Other measurements were taken at the end of the experiment: trunk diameter, number of leaves, tree height, leaf area and dry mass, and 11 essential nutrients nitrogen, phosphorus, potassium, magnesium, calcium, sulfur, boron, zinc, manganese, iron, and copper. The data were analyzed using the GLM procedure in SAS with dose, application frequency and their interaction as the independent variables.

Plants treated with 500 mg/L chlorine five times per week dropped most of their leaves by the end of the experiment. Plants treated with 250 mg/L chlorine five times per week were also damaged, showing reduced chlorophyll content, number of leaves, dry mass, and leaf area. Plants treated with 250 mg/L chlorine twice per week did not appear to show significant phytotoxic damage. The potassium in NEW water resulted in elevated concentrations of potassium in the leaves and a reduction in calcium concentrations.

Future work may include the application of electrolyzed water to mature trees in a CUPS environment and monitor disease incidence and yield. A trial is currently underway with both tangerine (*Citrus reticulata* 'Murcott') and grapefruit (*C.* × *paradisi* 'Ray Ruby') to assess phytotoxic damage and to determine if electrolyzed water has any effect on incidence of citrus scab and greasy spot diseases.

Literature Cited

Abbasi, P.A. and G. Lazarovits. 2006. Effect of acidic electrolyzed water on the viability of bacterial and fungal plant pathogens and on bacterial spot disease of tomato. Canadian J. Microbiol. 52:915–923.doi:10.1139/w06-048

Hirayama, Y. S. Asano, K. Watanabe, Y. Sakamoto, M. Ozaki, K. Okayama, S.T. Ohki and M. Tojo. 2016. Control of Colletotrichum fructicola on strawberry with a foliar spray of neutral electrolyzed water through an overhead irrigation system. J. Gen. Plant Pathol. 82:186–189. doi:10.1007/s10327-016-0667-6

Mueller, D.S., Y.C. Hung, R.D. Oetting, M.W. van Iersel, J.W. Buck. 2003. Evaluation of electrolyzed oxidizing water for management of powdery mildew on gerbera daisy. Plant Dis. 87:965–969. doi:10.1094/PDIS.2003.87.8.965

This work was supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2018-70016-27387

*Corresponding author. Email: tebert@ufl.edu

Proc. Fla. State Hort. Soc. 132:86. 2019.

—Scientific Note—

Highlands Youth Citrus Project: Mimicking the Florida Citrus Industry and Finding Success

Laurie Ann Hurner*

University of Florida/IFAS, Highlands County Extension, 4509 George Blvd., Sebring, FL 33875

Additional index words. citrus, Extension, life skills

The Highlands County Extension developed a new program to enable young people to become knowledgeable about Florida citrus production. The objectives of this program were to a) develop a youth program for the future of the citrus industry and b) develop life skills for youth including recordkeeping, responsibility, public speaking and communication skills.

The program's findings were:

- Previous project years the project provided insecticide (imidacloprid) sprays for protection against Huanglongbing (HLB) citrus greening disease, mimicking the current application schedule of the commercial citrus industry.
- Prior to the 2017 fair, all trees were tested and 10% of the trees were infected with HLB disease.
- In 2018, all trees were tested and 25% were found to be infected with HLB disease.
- For the 2019 program, the decision was made to discontinue insecticide sprays and provide individual protective screen structures for the trees.
- Evaluation after the installation of protective screen structures in 2019, all trees were tested and found to be 100% HLB disease free.
- This is very important as it proves that a tree cover system on young trees will work in the fight against HLB.

Recommendations for future efforts:

- The project should continue to use the individual screen structures for the project and teach participants how to grow trees successfully within these structures.
- Annual testing for HLB disease presence should be done to determine the success or failure of the screen structure system.

*Corresponding author. Email: lhurner@ufl.edu

Proc. Fla. State Hort. Soc. 132:87. 2019.

—Scientific Note—

Residual Toxicological Effects of Afidopyropen on Adult Asian Citrus Psyllid

Monica Triana, Barry Kostyk, and Jawwad Qureshi*[1]

University of Florida, IFAS, Southwest Florida Research and Education Center, 2685 State Rd. 29 North Immokalee, FL 34142

ADDITIONAL INDEX WORDS. chemical control, residual effects, psyllid management

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, (Homoptera: Liviidae), is an invasive insect pest of citrus in Florida. It is an efficient vector of the bacterium, *Candidatus* Liberobacter asiaticus, causal organism of citrus greening or huanglongbing (HLB) disease. The pest was first detected in Florida in 1998 and now occurs on all citrus throughout the state. HLB was first detected in Florida in 2005 and is spreading rapidly. Effective means of control are required to manage pest and disease while maintaining ecological and economic sustainability.

Biological control plays an important role in reducing the eggs, nymphs, and adults of *D. citri* in Florida citrus. Predators such as ladybeetles (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae), spiders (Aranae), and hoverflies or syrphids (Diptera: Syrphidae) (Michaud, 2004) are important biological control agents. Among lady-beetles species in Florida, *Curinus coeruleus* Mulsant, *Olla v-nigrum* Mulsant, *Harmonia axyridis* Pallas, *Cycloneda sanguinea* (L.), and *Exochomus childreni* Mulsant are the most common (Michaud, 2004; Stansly and Qureshi 2009). These predators were observed to respond positively to psyllid infestations and inflict 80 to 100% mortality of psyllid eggs and nymphs (Qureshi and Stansly 2009). However, more than one method of control is needed to enhance psyllid reduction and incidence of HLB.

Chemical control using soil and spray application of insecticides is presently a critical component of *D. citri* management. The systemic neonicotinoid insecticides, thiamethoxam, imidacloprid, and cloth–ianidin and a new insecticide (cyantraniliprole) are allowed in Florida citrus but their use as soil applications is limited by rate restrictions to young trees. Suppression of ACP by sprays of broad-spectrum insecticides prior to flushing has proved to be an effective strategy for reducing populations (Qureshi and Stansly 2010). Nevertheless, it is also necessary to control *D. citri* during the growing season which in Florida commences with spring flush and lasts through mid-fall. Although several insecticides have been evaluated and recommended for use, there is a continuous need of new insecticides to add to the tool box to diversify the insecticides available to control *D. citri* (Qureshi et al. 2014).

Sefina® is a new product manufactured by BASF (Florham Park, NJ)that contain active ingredient afidopyropen, responsible to disorient psyllids which finally starve to death. Sefina® was evaluated in foliar sprays to test the residual effects on adult *D. citri*. For this study a colony of *D. citri* was maintained on orange jasmine *Murraya paniculata* at the University of Florida Southwest Florida Research and Education Center, Immokalee, FL. Two-year-old potted *Citrus sinensis* 'Valencia' orange trees were sprayed with treatment solutions using a 1-gal hand held sprayer to reach a level for the solution to runoff and allowed to dry before moving plants to the greenhouse. Four treatments were evaluated: water (as a control); Sefina® (14 oz/100 gal) alone; Sefina® with 0.25% vol/vol Dyne-amic (Helena Agri-Enterprises, LLC, Collierville, TN) surfactant; and Sefina® with 1% v/v JMS Stylet Oil (JMS Flower Farms, Vero Beach, FL). Adult *D. citri* were immobilized with a low dose of CO_2 and transferred to the orange trees. Adult insects were caged on lower surfaces of the leaves inside a ventilated "clip-cage" (transparent plastic cup, 3.6 cm × 2.6 cm diameter; 4 cm high) with a thin polypropylene mesh (30 holes/inch) attached to the bottom by silicone glue. Insects were placed on the leaves at 2, 24, and 72 h, and 7 and 14 d after spraying and allowed to feed. Mortality was assessed at 24, 48, and 72 h and 7 and 14 d after caging. Significant psyllid mortality was observed in all Sefina® treatments compared to the water treatment at 2, 24, and 72 h. Mortality was lowest in the Sefina® plus Dyne-amic treatment at 72 h. and 7 d after application.

Literature Cited

Michaud, J.P. 2004. Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida. Biological Control 29: 260-269.

Qureshi, J.A. and P.A. Stansly. 2009. Exclusion techniques reveal significant biotic mortality suffered by Asian citrus psyllid *Diaphorina citri* (Hemiptera: Psyllidae) populations in Florida citrus. Biological Control 50:129–136.

Qureshi, J.A. and P.A. Stansly. 2010. Dormant season foliar sprays of broad-spectrum insecticides: An effective component of integrated management for *Diaphorina citri* (Hemiptera: Psyllidae) in citrus orchards. Crop Protection 29:860–866.

Qureshi, J.A., B. Kostyk, and P.A. Stansly. 2014. Insecticidal suppression of Asian citrus psyllid *Diaphorina citri* (Hemiptera: Liviidae) vector of Huanglongbing pathogens. PLoS ONE 9(12):e112331. doi:10.1371/journal.pone.0112331

*Corresponding author. Email: jawwadq@ufl.edu

Proc. Fla. State Hort. Soc. 132:88–92. 2019.

Seed and Seedling Nursery Characteristics for 10 USDA Citrus Rootstocks

Rayane Barcelos Bisi
U.S. Horticultural Research Laboratory, USDA, ARS, Fort Pierce, FL 34945; and Southwest Florida Research and Education Center, University of Florida/IFAS, Immokalee, FL 34142

Ute Albrecht
Southwest Florida Research and Education Center, University of Florida/IFAS, Immokalee, FL 34142

Kim D. Bowman
U.S. Horticultural Research Laboratory, USDA, ARS, Fort Pierce, FL 34945

Additional index words. huanglongbing, nucellar seedling, off-type, SSR, Swingle, true-to-type, zygotic seedling

***Abstract*. Six new hybrid rootstocks, 'US-1279', 'US-1281', 'US-1282', 'US-1283', 'US-1284', and 'US-1516', were released from the U.S. Department of Agriculture (USDA) citrus breeding program to provide improved tree tolerance to huanglongbing (HLB), the most destructive disease facing the citrus industry in the United States and many other parts of the world. Five of these new rootstocks were released based on field performance in trials with the rootstocks propagated by stem cuttings, rather than the traditional propagation using nucellar seedlings. In this study, we evaluated the fruit, seed, and seedling characteristics of these new rootstocks, along with four other USDA rootstocks of commercial importance. The study included a determination of the percentage of true-to-type and off-type seedlings by both plant morphology and simple sequence repeat (SSR) markers. All 10 rootstocks produced an acceptable number of seeds and good seedling emergence from those seeds. The rootstocks 'Swingle', 'US-802', 'US-812', 'US-1283', 'US-1284', and 'US-1516' had a high percentage of true-to-type seedlings and correspondingly good potential to be propagated by seeds. However, no true-to-type plants were observed among seedlings from the rootstocks 'US-1279', 'US-1281', and 'US-1282', indicating that economical seed propagation will be impossible for these cultivars. The 10 SSR marker sets used in this study were observed to easily differentiate the 10 rootstocks studied, and readily distinguished true-to-type and off-type seedlings among progeny from all 10 rootstock clones. This study presents information of significant value for commercial nurseries involved in propagation of citrus rootstocks, and those involved in citrus rootstock breeding and development around the world. We propose the use of these 10 SSR marker sets as readily applicable for accurate identification of most citrus rootstock cultivars and their true-to-type seedlings.**

The rootstock is an important component of a healthy and productive citrus tree, influencing the fruit yield, fruit quality, tree size, and tolerance of diseases (Bowman and Joubert, 2020; Castle et al., 2011; McCollum and Bowman, 2017; Wutscher and Bowman, 1999). HLB disease (also known as citrus greening) is arguably the most important and most destructive disease in much of the world's citrus industry. Some hybrids of trifoliate orange (*Poncirus trifoliata*) with *Citrus* spp. have been identified as tolerant to HLB when used as a scion (Albrecht and Bowman, 2019; Folimonova et al., 2009) or as a rootstock (Albrecht and Bowman, 2011, 2012; Bowman et al., 2016a, 2016b). Hybrids of this parentage are known to possess many other outstanding rootstock characteristics. As a consequence, use of HLB-tolerant rootstocks is considered one of the most effective tools currently available to combat the disease. After new rootstocks are tested and released, one of the first challenges is to obtain enough plants of the new rootstock clones in nursery propagation.

Although propagation of citrus rootstocks can be accomplished effectively by stem cuttings or micropropagation (Albrecht et al., 2017; Bowman and Albrecht, 2017), commercial propagation of citrus rootstocks usually depends on the production of genetically uniform clonal plants from seed. Within the genus *Citrus*, many species show the phenomenon of nucellar polyembryony, which means that seeds contain multiple embryos produced by ordinary mitotic division of cells of the nucellus (nucellar embryos) and no male gamete contributes to their formation (Garcia et al., 1999). For these species, some or a large portion of the seedlings will therefore be genetically identical to the seed parent. Historically, clones have been used as citrus rootstocks only when they provide a relatively high proportion of genetically uniform nucellar seedlings (Bowman and Joubert, 2020). Eliminating the zygotic plants among primarily nucellar rootstock seedling populations in the citrus nursery is important to maintain genetic homogeneity, thereby assuring growers of uniform rootstock performance in the field (Ruiz et al., 2000). In the citrus nursery, zygotic seedlings of rootstocks are eliminated, or rogued, based on visual appearance. However, separating the seedlings only by leaf morphology, size, and growth habit is not always reliable, because some seedlings of zygotic origin for particular rootstocks are difficult to visually identify (Anderson et al., 1991). If these zygotic seedlings are mistakenly used for propagation, the result can be unpredictable or reduced tree performance, including a high level of variability in tree size and health.

Several methods can be used to identify true-to-type and off-type seedlings, including isozyme analysis, random amplified polymorphic DNA (RAPD) analysis, amplified fragment length polymorphisms (AFLP) analysis, or SSR analysis (Rao et al., 2008). Isozyme analysis has limitations because of the small number of available loci in the genome and the scarce variability at those loci. RAPD and AFLP markers have limitations because of their dominant nature (heterozygous and homozygous individuals are not easily distinguished), which reduces in half the ability to detect zygotic plants in some progenies (Ruiz et al., 2000). SSR markers typically have a high number of polymorphic loci with numerous alleles (Karhu et al., 1996; Raybould et al., 1998; White and Powell, 1997), and have proven a useful tool to identify zygotic and nucellar seedlings (Russell et al., 1997).

Six new hybrid rootstocks 'US-1279', 'US-1281', 'US-1282', 'US-1283', 'US-1284', and 'US-1516' were released from the USDA citrus breeding program during 2014 and 2015 to provide improved rootstock tolerance to HLB (Bowman and McCollum, 2015; Bowman et al., 2016b). Sweet orange scions on these rootstocks demonstrated tree health and fruit productivity that was superior to the most widely used rootstocks under conditions severely challenged by HLB. All the new hybrids originated from crosses of mandarin (*Citrus reticulata*) or pummelo (*Citrus maxima*), and trifoliate orange (*Poncirus trifoliata*). At the time of release, fruits

Received for publication 26 Sept. 2019. Accepted for publication 17 Dec. 2019.
Published online 5 March 2020.
This research was presented as a poster and paper proceeding at the Florida State Horticultural Society Annual Meeting, 9–11 June 2019, Orlando, FL.
U.A. and K.D.B. are the corresponding authors. E-mail: ualbrecht@ufl.edu or kim.bowman@usda.gov.

and seeds were not available for five of these hybrids, and little was known about their potential for seed propagation. During the 2018 season, fruiting trees were available from these new rootstocks to evaluate fruit, seed, and seedling characteristics, and determine the percentage of true-to-type and off-type seedlings by visual and SSR markers of these new rootstocks, along with four other USDA rootstocks of commercial importance. The information provided in this study is of substantial value to commercial nurseries and others interested in propagation of these rootstocks. In addition, it provides a readily applicable methodology for evaluating nursery characteristics that can be applied to other new rootstocks in the future.

Materials and Methods

Plant material. Grafted seed trees of six citrus hybrid rootstocks released by the USDA in 2014–15, and four previously released rootstocks of commercial value, were used as a source of fruit and seed. Hybrids and their parentages are listed in Table 1. In January 2019, open-pollinated mature fruits from 'US-802', 'US-812', 'US-852', 'US-1279', 'US-1281', 'US-1282', 'US-1283', 'US-1284', and 'US-1516' were collected from 4-year-old grafted seed trees at the USDA, A.H. Whitmore Foundation Farm (Leesburg, FL) in a mixed planting of many genotypes, where outcrossing is likely. Fruit from the rootstock 'Swingle' were unavailable at that time. Seeds of 'Swingle' used in subsequent parts of this study were purchased from a commercial source (Lyn Citrus Seed, Arvin, CA).

Fruit and seed characteristics. For most of the rootstocks, four groups of eight fruits per rootstock were used to determine fruit length (mm), fruit diameter (mm), and the length/diameter ratio. The number of fruits per Florida standard 70-L field box was calculated by using an estimation of ratio of sphere volume to packing volume, which has been reported in the literature as ≈0.634 (Jaeger and Nagel, 1992; Torquato et al., 2000).

$$\text{number of fruits per field box} = \frac{(\text{volume of field box}) \times (0.634)}{(\text{fruit volume})}$$

Seeds were extracted from each fruit, and the number of seeds per fruit, seed weight (mg), and number of seeds per 50 mL were determined, and the number of seeds per liter and number of seeds per field box were calculated.

Seedling growth. After extraction from the fruit, seeds from each rootstock were washed, treated with a solution of 8-quinolinol sulfate (10 $g \cdot L^{-1}$; Sigma Chemical Co., St. Louis, MO), air dried, and stored at 4 °C until they were sown. For most rootstocks, six groups of 49 seeds were planted in the last week of Mar. 2019. Before planting, seedcoats were removed individually by peeling, and seeds disinfected for 20 min with 1% (v/v) sodium hypochlorite (Clorox Co., Oakland, CA) and containing 0.01% (v/v) Tween 20 (Sigma-Aldrich, St. Louis, MO), and then rinsed with water. For some rootstocks, fewer than 294 seeds were available, and replications were adjusted accordingly (see Table 1). Seeds were planted into racks of 3.8 × 21-cm cone cells (Cone-tainers; Stuewe and Sons, Tangent, OR) containing steam-sterilized soilless potting medium (Pro Mix BX; Premier Horticulture, Inc., Quakertown, PA), with one seed per cell. Seedlings in cone cells were maintained in a temperature-controlled greenhouse at the U.S. Horticultural Research Laboratory (Ft. Pierce, FL) and irrigated as needed, alternating between water and water-soluble fertilizer mix of 20N–10P–20K (Peters Professional; The Scotts Company, Marysville, OH), applied with a proportioner at a rate of 400 $mg \cdot L^{-1}$ N.

Seedling assessment. After 80 d, seedling emergence above the soil and number of multiple seedlings were determined, and seedlings were identified as true-to-type (morphologically identical to the clonal source plants) or off-type by visual assessment from two individuals with combined experience of 30 years in citrus nursery work. Visual assessment was based on leaf morphological traits and plant growth rate. Only seedlings that were at least 30% the height of typical seedlings for that cultivar were considered as possible true-to-type seedlings, to avoid the uncertainty with visually assessing very small and stunted plants.

Plant tissue collection. Eighty days after seeding, six leaves were collected from each of 24 seedlings of each rootstock, to confirm the accuracy of the visual method, using SSR markers. For most rootstocks, 14 true-to-type and 10 off-type seedlings, identified by visual assessment, were evaluated. For the rootstocks 'US-1279', 'US-1281', and 'US-1282', no obvious true-to-type seedlings were observed, so all seedlings examined with SSR analysis were visually off-type, but represented the closest to true-to-type characteristics found in each replicate set of plants. For the rootstocks 'US-802' and 'Swingle', only seven and nine visually off-type seedlings were found and evaluated by SSR, respectively. Thus, for 'US-802' and 'Swingle', 17 and 15 true-to-type seedlings were evaluated by SSR, respectively.

DNA extraction. Leaves were ground in liquid nitrogen with a mortar and pestle, and stored at –80 °C. A total of 100 mg of ground tissue was used for DNA extraction using the Plant DNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

Table 1. Citrus rootstock cultivar names, parentage, release date, and number of seeds and replications used in the seedling study.

Rootstock	Parentage	USDA release date	Number of seeds (reps)[z]
Swingle	*Citrus paradisi* × *Poncirus trifoliata*	1974	294 (6)
US-802	*Citrus maxima* 'Siamese' × *P. trifoliata* 'Gotha Road'	2007	294 (6)
US-812	*Citrus reticulata* 'Sunki' × *P. trifoliata* 'Benecke'	2001	293 (6)
US-852	*C. reticulata* 'Changsha' × *P. trifoliata* 'English Large'	1999	57 (3)
US-1279	*C. reticulata* 'Changsha' × *P. trifoliata* 'Gotha Road'	2014	343 (7)
US-1281	*C. reticulata* 'Cleopatra' × *P. trifoliata* 'Gotha Road'	2014	9 (1)
US-1282	*C. reticulata* 'Cleopatra' × *P. trifoliata* 'Gotha Road'	2014	107 (5)
US-1283	*C. reticulata* 'Ninkat' × *P. trifoliata* 'Gotha Road'	2014	294 (6)
US-1284	*C. reticulata* 'Ninkat' × *P. trifoliata* 'Gotha Road'	2014	294 (6)
US-1516	*C. maxima* 'African' × *P. trifoliata* 'Flying Dragon'	2015	294 (6)

[z]Total number of seeds planted and () number of replications.

Table 2. Primer sequences and annealing temperatures of SSR markers used in this study.

SSR marker	Forward sequence (5′-3′)	Reverse sequence (5′-3′)	Annealing temp (°C)
M165	CATCAAGGCATTGGTCTAGCTC	TTGGGTGGCAGAATTAGCTG	63
M172	TGTAAGGCCGTTACCCCTCCA	TACCATCTCCCCATGTAACGCT	63
M13	CCCTTGTTTTACGCCACTAG	CTGATCCAGATCCAACTTACG	63
M156	CCAAGAGAATATCCGGTGGAC	AAAGTACCCTTCATGATCACCC	63
M21	TTCTTCAGGGTGTAATCCAG	AGCAAGAGTTCTAGTGTTAGC	60
M50	GCGGTCGCTTAGTGAACTGT	TTGAATCCCGACCTTCTACC	60
M112	GCAAACCACACAGTTATATCCG	CTTCGATACCGACATCAGCA	60
M126	TACGGACATCTTCTAAACCGACC	GTCTGGACTCATTTGACTTGCAC	60
M157	GGGTTCTTTCATCTGCCGAATG	CGAGGAATCCCCAAAGCTGAAG	61
M163	TCACGACTCTATCCCATGTC	ACAATCCGCACTACTAATCC	61

SSR marker analysis. Based on preliminary studies, 10 SSR markers were selected for this study. The nucleotide sequences of the primers used to detect these markers are listed in Table 2. Marker analysis was performed using the Type-It Microsatellite polymerase chain reaction (PCR) kit (Qiagen) according to the manufacturer's instructions. Each reaction contained 5 ng DNA template and 2 μM each of reverse and forward primers in a total reaction volume of 25 μL. Forward primers were labeled with 6-FAM (fluorescein) or HEX (hexachloro-fluorescein) and purchased from Life Technologies Corporation (Carlsbad, CA). PCRs were performed using a Bio-Rad T100 Thermal Cycler (Bio-Rad, Hercules, CA). A first cycle of denaturation at 95 °C for 5 min was followed by 28 cycles at 72 °C for 30 s (denaturation), 60 to 63 °C for 90 s (annealing), and 72 °C for 30 s (extension), followed by a final extension step at 60 °C for 30 min. Annealing temperatures varied by primer set and are listed in Table 2.

One microliter of PCR product was mixed with 14 μL Hi-Dye formamide solution (Amresco, Solon, OH) premixed with the GeneScan Rox 500 Size Standard (Applied Biosystems, Inc., Foster City, CA). This mixture was denatured at 95 °C for 3 min, then immediately cooled to 4 °C and subjected to automated fragment analysis by an ABI 3730xl DNA sequencer (Applied Biosystems, Inc.) following manufacturer's instructions. Analyses were performed using GeneMapper 5.0 software (Thermo Fisher Scientific, Waltham, MA). Alleles were manually assigned to clear and consistent fluorescence peaks. Inconsistent fluorescence peaks, such as stutters, pull-ups, or dinosaur tails were excluded (Pan, 2006).

SSR marker comparison of hybrids and parental species. All 10 primer pairs were examined with known clonal source plants of the 10 rootstock cultivars and eight representatives of the parental species, 'Cleopatra' mandarin (*Citrus reticulata*), 'Sunki' mandarin (*C. reticulata*), 'Ninkat' mandarin (*C. reticulata*), 'Pandan Wangi' pummelo (*Citrus maxima*), 'Sha Tian You' pummelo (*C. maxima*), 'US-145' pummelo (*C. maxima*), 'Rich 16-6' trifoliate orange (*Poncirus trifoliata*), and 'Flying Dragon' trifoliate orange (*P. trifoliata*). For some of the rootstocks in the study, the precise parental clones are either uncertain or were unavailable for the SSR analysis.

The Polymorphism Information Content (PIC) was calculated for each marker by applying the formula

$$\mathrm{PIC} = 1 - \sum_{j=1}^{n} P_{ij}^2,$$

where Pij is the frequency of the jth allele for marker I, and summation extends over n alleles (Qi et al., 2012). The fragment sizes of each seedling were compared with the fragment sizes of the clonal source plant for each individual, to identify the off-type and true-to-type seedlings. Seedlings were confirmed true-to-type when all fragments were identical to those of the clonal source plant for that cultivar.

Statistical analysis. Data were analyzed using Statistica v.13.3 (TIBCO Software Inc., Palo Alto, CA). Comparison of the means was performed by Tukey's honestly significant difference test when P was smaller than 0.01.

Results

Fruit and seed characteristics. There were significant differences for all fruit and seed characteristics among the rootstocks studied (Table 3). The rootstocks 'US-802' and 'US-1516' had the largest fruit and the highest number of seeds per fruit, with 44 and 36 seeds, respectively. US-1283 produced the smallest fruit (1154 fruit per field box), and there were an average of 21 seeds per fruit, resulting in the largest number of seeds (23,858) per field box of fruit for any of the rootstocks. The average number of seeds per fruit for a cultivar was generally associated with the average fruit size, with larger fruit having more seeds. The number of stored seeds per liter ranged from 2250 for 'US-1281' to 6477 for 'US-1284'.

Visual assessment of seedlings. Most of the rootstocks had a large percentage of emerged seedlings, with more than 95% emergence observed for the rootstocks 'US-802', 'US-812', 'US-1516', 'US-1283', 'US-1284', and 'Swingle' (Table 4). The rootstock with the lowest percentage of emerged seedlings (68.24%) was 'US-1282'.

None of the seeds from rootstocks 'US-1281' and 'US-1282' produced multiple seedlings and 100% of seedlings were identified as off-type by visual assessment. 'US-1279' seeds also produced 100% of off-type seedlings, with only 0.3% multiple seedlings per seed. The percentage of strong true-to-type seedlings ranged from 96% for 'US-802', to 0% for 'US-1279', 'US-1281', and 'US-1282'. The rootstocks 'Swingle', 'US-802', 'US-812', and the new hybrids 'US-1283' and 'US-1284' had the highest percentage of emerged seedlings (96% to 99%) and the highest percentage of strong true-to-type seedlings based on visual assessment.

Although there was a general association of the percentage of multiple seedlings and

Table 3. Fruit and seed characteristics of different citrus hybrid rootstocks.

Rootstock	Fruit length (mm)	Fruit diam (mm)	Length/diam ratio	Number of fruit per field box	Number of seeds per fruit	Seed wt (mg)	Seeds per liter	Seeds per field box
US-802	78 a	87 a	0.90 c	164 d	44 a	170 a	3,070 de	7,171 c
US-812	48 c	53 cd	0.90 c	677 bc	12 b	158 ab	3,308 de	8,212 c
US-852	53 b	58 c	0.91 c	502 c	17 b	137 ac	2,784 e	8,307 c
US-1279	46 cd	47 ef	0.96 b	898 b	15 b	100 d	5,371 b	13,309 b
US-1281	44 cd	48 df	0.93 bc	872 ab	9 b	112 bd	2,250 e	7,848 c
US-1282	46 c	51 de	0.90 c	793 b	11 b	107 cd	4,034 c	8,276 c
US-1283	41 d	44 f	0.93 bc	1,154 a	21 b	105 cd	5,501 b	23,858 a
US-1284	43 cd	53 cd	0.81 d	786 b	19 b	84 d	6,477 a	14,971 b
US-1516	75 a	74 b	1.01 a	226 d	36 a	138 ac	3,526 cd	8,187 c

Mean separations for significant analysis of variance within columns were by Tukey's honestly significant difference test at $P \leq 0.01$.

Table 4. Seedling characteristics of different citrus hybrid rootstocks.

Rootstocks	Emerged seedling (%)	Multiple seedling (%)	True-to-type seedling (%)	Off-type seedling (%)
Swingle	96 a	24 cd	94 a	4 c
US-802	99 a	45 ab	96 a	2 c
US-812	98 a	58 a	92 a	4 c
US-852	89 ab	12 de	60 b	40 b
US-1279	75 bc	0.3 de	0 c	100 a
US-1281	89 a-c	0 de	0 c	100 a
US-1282	68 c	0 e	0 c	100 a
US-1283	98 a	57 a	91 a	4 c
US-1284	97 a	37 bc	86 a	5 c
US-1516	96 a	12 de	63 b	27 b

Mean separations for significant analysis of variance within columns were by Tukey's honestly significant difference test at $P \leq 0.01$.

Table 5. Amplified alleles of simple sequence repeat markers for 10 citrus hybrid rootstocks and eight parental species.

	Markers									
Rootstocks	M165	M172	M13	M156	M21	M50	M112	M126	M157	M163
Parent clones										
Cleopatra	214	263, 272	133, 142	191	373	149, 155	251	177, 185	242	250
Ninkat	214	260, 272	136, 140	176, 185	373	149	251	185	243, 245	250
Flying Dragon	226, 234	249, 253	128	170, 179	365, 367	143	248	181	236, 242	232
Rich 16-6	226, 234	249, 253	128	170, 179	365, 367	143, 155	248	181	236, 242	232
Pandan Wangi	206, 220	246, 252	134	182	361, 364	155	248	170	233	247, 250
Sha Tian You	206	252	130, 134, 142	179, 182	361	143	248	170	233	247
US-145	220	247, 252	130, 142	182	361	143, 155	248	170	233	241, 250
Sunki	214	255, 263	133, 145	185	373	149	251	185	242	250
Hybrids										
Swingle	214, 234	249, 252	128, 142	179, 182	361, 365	143, 161	248, 257	170, 185	233, 236	232, 250
US-802	229, 234	247, 253	128, 134	179, 182	361, 365	143	248	170, 181	233, 236	232, 241
US-812	214, 226	253, 263	128, 133	179, 185	367, 373	143, 149	248, 251	181, 185	242	232, 250
US-852	214, 226	243, 253	128, 136	179, 191	367, 373	143, 149	248, 251	181, 185	242	232, 247
US-1279	214, 226	249, 260	128, 136	179, 191	371	143, 149	248, 251	185	242	232, 250
US-1281	214, 234	249, 272	128, 133	179, 191	373	143, 155	248, 251	185	242	232, 250
US-1282	214, 226	249, 272	128, 133	179, 191	367, 373	143, 155	248, 251	185	242	232, 250
US-1283	214, 226	249, 272	128, 136	176, 179	367, 373	143, 149	248, 251	185	242, 243	232, 250
US-1284	214, 226	253, 260	128, 140	176, 179	365, 373	143, 149	248, 251	181, 185	242, 243	232, 250
US-1516	206, 234	249, 252	130, 142	179, 182	362, 367	143, 155	248	170, 181	233, 236	232, 250

Table 6. Fragment size, number of total alleles (NTA) and polymorphism information content (PIC) of 10 simple sequence repeat (SSR) markers.

SSR markers	Fragment size	NTA	PIC
M165	206–234	6	0.75
M172	243–272	10	0.86
M13	128–145	8	0.82
M156	170–191	6	0.75
M21	361–373	7	0.81
M50	143–161	4	0.61
M112	248–257	3	0.50
M126	170–185	4	0.67
M157	233–245	5	0.73
M163	232–250	4	0.60

the percentage of true-to-type seedlings, there were cases in which multiple zygotic seedlings emerged from a single seed. Because of the size heterogeneity of seedlings, some seedlings did not grow large enough during the evaluation period to be defined as either true-to-type or off-type. Consequently, in Table 4, the sum of true-to-type and off-type seedlings is lower than 100% for some cultivars.

SSR marker comparison of rootstock hybrids and parental clones. All 10 primer pairs successfully amplified multiple alleles per marker in the hybrid rootstocks and the parental species (Table 5). The number of alleles ranged from 3 (M112) to 10 (M172) with an average of 5.7, and fragment size ranging from 128 and 373 (Table 6). In total, 57 alleles were detected, and all of the markers were polymorphic. The PIC values varied among the SSR markers from 0.50 (M112) to 0.86 (M172).

SSR analysis for true-to-type seedlings. The SSR analyses confirmed the results of the visual ratings to differentiate between true-to-type and off-type seedlings (Table 7). For 7 of the 10 rootstocks, seedling types were identified visually with 100% accuracy. For the other three rootstocks ('Swingle', 'US-802', and 'US-852'), the accuracy was 92% to 96%, with all of the errors involving true-to-type plants, which were wrongly classified by visual assessment as off-type.

Discussion

This study presents information of significant value for commercial nurseries involved in propagation of the new citrus rootstocks, growers interested in planting trees on those rootstocks, and researchers involved in citrus rootstock breeding and development. Citrus rootstocks that produce fruit with a large number of seeds and good seedling emergence are preferred by nurseries. All six of the new rootstocks, along with the commercial standard rootstocks, showed good results for these traits.

Probably of even greater importance for a rootstock cultivar to be effectively propagated by seed, a high proportion of the seedlings must be strong growing and genetically identical to the parent cultivar. Nucellar polyembryony, which produces uniform and clonal (true-to-type) seedlings from a single seed, has traditionally been a major factor for selecting citrus rootstocks in breeding programs (Bowman and Joubert, 2020). This trait continues to be valuable for a new rootstock to have commercial success, even though alternative methods of vegetative propagation allow for potential commercialization where nucellar seedlings are never produced. The proportion of true-to-type seedlings of the six new rootstocks ranged from 0% to 91%, with the rootstocks 'US-1283', 'US-1284', and 'US-1516' having a high percentage of true-to-type seedlings and good potential to be propagated by seeds. In contrast, no true-to-type seedlings were recovered from the rootstocks 'US-1279', 'US-1281', and 'US-1282', indicating that economical seed propagation will be impossible for these cultivars. Alternative propagation by stem cuttings and micropropagation (Albrecht et al., 2017; Bowman and Albrecht, 2017) will be necessary for any commercial use of these three rootstocks.

The SSR markers used in this study amplified a large number of alleles. The value of the PIC indicates the effective number of alleles that can be detected per marker in a set of individuals (Chandra et al., 2014). The average PIC value in this study was 0.71, suggesting that the SSR markers used were very effective in detecting alleles and should be effective in distinguishing between the rootstock clones (Botstein et al., 1980).

In addition, the SSR markers used in this study were demonstrated to also be effective for distinguishing between true-to-type and off-type seedlings. Although SSR marker analysis is not economically feasible for use as routine practice in a commercial nursery, it is a valuable tool for verifying the proportion of true-to-type seedlings emerging from seeds of new rootstocks, and to help define the morphological traits to be used for visual roguing in the nursery. Understanding the genetic uniformity among seedlings of each rootstock is essential to choose the best method for propagation, avoid large economic losses in the nursery, and minimize the catastrophic damage to field plantings that can result from planting trees that have been propagated on off-type and inferior zygotic seedlings.

The SSR markers described here also appear to have value to verify rootstock cultivar identity in which there may be confused or mislabeled clonal lines, seed source trees, or batches of seed. The cost of using SSR evaluation for seed trees or groups of seedlings would be substantial and prohibitively expensive for routine use. However, when mistaken identity is suspected, using SSR markers to validate rootstock identity could be used to eliminate the risk of catastrophic tree loss in the field and limit legal liability.

It has been previously noted that the dominant trifoliate leaf trait of *Poncirus* over the monofoliate trait of *Citrus* (Soost and Cameron, 1975) allows for easy visual identification of zygotic hybrid seedlings (Chen

Table 7. Percentage of true-to-type (TTT) and off-type (OT) citrus seedlings identified by visual assessment and by simple sequence repeat (SSR) marker analysis, and correspondence of the two methods.

		Visual		SSR		
Rootstocks	Number of seedlings	TTT	OT	TTT	OT	Correspondence (%)
Swingle	24	15	9	17	7	92
US-802	24	17	7	19	5	92
US-812	24	14	10	14	10	100
US-852	24	14	10	15	9	96
US-1279	24	0	24	0	24	100
US-1281	8	0	8	0	8	100
US-1282	24	0	24	0	24	100
US-1283	24	14	10	14	10	100
US-1284	24	14	10	14	10	100
US-1516	24	14	10	14	10	100

et al., 2008). In our study, with trifoliate-leaved first-generation hybrids of *Poncirus trifoliata* with *Citrus* spp., most zygotic seedlings had a distinctly different leaf shape from the true-to-type nucellar seedlings, and therefore allowed for relatively reliable visual identification of true-to-type seedlings. It should be noted that true-to-type seedlings of rootstocks that are not F1 hybrids of *Citrus* × *Poncirus* are often much more difficult to identify by morphology. In either case, SSR marker analysis can provide good validation of the proportion of rootstock seedlings that are true-to-type. SSR marker analysis also can help distinguish among rootstocks of similar parentage (and morphology) when correct identity is in question.

Literature Cited

Albrecht, U., M. Bordas, B. Lamb, B. Meyering, and K.D. Bowman. 2017. Influence of propagation method on root architecture and other traits of young citrus rootstock plants. HortScience 52:1569–1576.

Albrecht, U. and K.D. Bowman. 2011. Tolerance of the trifoliate citrus hybrid US-897 (*Citrus reticulata* Blanco × *Poncirus trifoliata* L. Raf.) to Huanglongbing. HortScience 46:16–22.

Albrecht, U. and K.D. Bowman. 2012. Tolerance of trifoliate citrus rootstock hybrids to *Candidatus* Liberibacter asiaticus. Scientia Hort. 147:71–80.

Albrecht, U. and K.D. Bowman. 2019. Reciprocal influences of rootstock and scion citrus cultivars challenged with *Ca.* Liberibacter asiaticus. Scientia Hort. 254:133–142.

Anderson, C.M., W.S. Castle, and G.A. Moore. 1991. Isozymic identification of zygotic seedlings in Swingle citrumelo (*Citrus paradisi* × *Poncirus trifoliata*) nursery and field populations. J. Amer. Soc. Hort. Sci. 116:322–326.

Botstein, D., R.L. White, M. Skolnick, and R.W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Amer. J. Hum. Genet. 32(3):314–331.

Bowman, K.D. and U. Albrecht. 2017. Efficient propagation of citrus rootstocks by stem cuttings. Scientia Hort. 225:681–688.

Bowman, K.D. and J. Joubert. 2020. Citrus rootstocks, p. 105–127. In: M. Talon, M. Caruso, and F.G. Gmitter (eds.). The genus citrus. Woodhead Publishing, Cambridge, MA.

Bowman, K.D. and G. McCollum. 2015. Five new citrus rootstocks with improved tolerance to huanglongbing. HortScience 50:1731–1734.

Bowman, K.D., L. Faulkner, and M. Kesinger. 2016a. New citrus rootstocks released by USDA 2001–2010: Field performance and nursery characteristics. HortScience 51:1208–1214.

Bowman, K.D., G. McCollum, and U. Albrecht. 2016b. Performance of ‘Valencia’ orange [*Citrus sinensis* (L.) Osbeck] on 17 rootstocks in a trial severely affected by huanglongbing. Scientia Hort. 201:355–361.

Castle, W.S., K.D. Bowman, J.C. Baldwin, J.W. Grosser, and F.G. Gmitter. 2011. Rootstocks affect tree growth, yield, and juice quality of ‘Marsh’ grapefruit. HortScience 46:841–848.

Chandra, A., M.P. Grisham, and Y.B. Pan. 2014. Allelic divergence and cultivar specific SSR alleles revealed by capillary electrophoresis using fluorescence-labeled SSR markers in sugarcane. Genome 57(6):363–372.

Chen, C., K.D. Bowman, Y.A. Choi, P.M. Dang, M.N. Rao, S. Huang, and F.G. Gmitter. 2008. EST-SSR genetic maps for *Citrus sinensis* and *Poncirus trifoliata*. Tree Genet. Genomes 4(1):1–10.

Folimonova, S.Y., C.J. Robertson, S.M. Garnsey, S. Gowda, and W.O. Dawson. 2009. Examination of the responses of different genotypes of citrus to huanglongbing (citrus greening) under different conditions. Phytopathology 99:1346–1354.

Garcia, R., M.J. Asins, J. Forner, and E.A. Carbonell. 1999. Genetic analysis of apomixis in Citrus and Poncirus by molecular markers. Theor. Appl. Genet. 99(3-4):511–518.

Jaeger, H.M. and S.R. Nagel. 1992. Physics of the granular state. Science 255(5051):1523–1531.

Karhu, A., P. Hulme, M. Karjalainen, P. Karvonen, K. Karkkainen, D. Neale, and O. Savolainen. 1996. Do molecular markers reflect patterns of differentiation in adaptive traits of conifers? Theor. Appl. Genet. 93:215–221.

McCollum, G. and K.D. Bowman. 2017. Rootstock effects on fruit quality among ‘Ray Ruby’ grapefruit trees grown in the Indian River District of Florida. HortScience 52:541–546.

Pan, Y.B. 2006. Highly polymorphic microsatellite DNA markers for sugarcane germplasm evaluation and variety identity testing. Sugar Tech 8(4):246–256.

Qi, Y.W., Y.B. Pan, F.Y. Lao, C.M. Zhang, L.N. Fan, H.Y. He, and Q.W. Li. 2012. Genetic structure and diversity of parental cultivars involved in China mainland sugarcane breeding programs as inferred from DNA microsatellites. J. Integr. Agr. 11(11):1794–1803.

Rao, M.N., J.R. Soneji, C. Chen, S. Huang, and F.G. Gmitter. 2008. Characterization of zygotic and nucellar seedlings from sour orange-like citrus rootstock candidates using RAPD and EST-SSR markers. Tree Genet. Genomes 4(1):113–124.

Raybould, A.F., R.J. Mogg, C. Aldam, C.J. Gilddon, R.S. Thorpe, and R.T. Clarke. 1998. The genetic structure of sea beat (*Beta vulgaris* ssp. maritima) populations. III. Detection of isolation by distance at microsatellite loci. Heredity 80:127–132.

Ruiz, C., M.P. Breto, and M.J. Asins. 2000. A quick methodology to identify sexual seedlings in citrus breeding programs using SSR markers. Euphytica 112(1):89–94.

Russell, J.R., J.D. Fuller, M. Macaulay, B.G. Hatz, A. Jahoor, W. Powell, and R. Waugh. 1997. Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. Theor. Appl. Genet. 95:714–722.

Soost, R.K. and J.W. Cameron. 1975. Citrus, p. 507–540. In: J. Janick and J.N. Moore (eds.). Advances in fruit breeding. Purdue University, West Lafayette, IN.

Torquato, S., T.M. Truskett, and P.G. Debenedetti. 2000. Is random close packing of spheres well defined? Phys. Rev. Lett. 84(10):2064–2067.

White, G. and W. Powell. 1997. Isolation and characterisation of microsatellite loci in *Swietenia humilis* (Meliaceae): An endangered tropical hardwood species. Mol. Ecol. 6:851–860.

Wutscher, H.K. and K.D. Bowman. 1999. Performance of ‘Valencia’ orange on 21 rootstocks. HortScience 34:622–624.

This paper was presented during the 2019 FSHS Annual Meeting and originally published in *HortScience* [HortScience 55(4):528–532. 2020. https://doi.org/10.21273/HORTSCI14598-19]. The paper is included in this Proceedings as a reprint (with permission).

Proc. Fla. State Hort. Soc. 132:93–101. 2019.

Influence of Rootstock Propagation Method on Traits of Grafted Sweet Orange Trees

Ute Albrecht, Shahrzad Bodaghi, and Bo Meyering
Southwest Florida Research and Education Center, University of Florida, Institute of Food and Agricultural Sciences, Immokalee, FL 34142

Kim D. Bowman
U.S. Horticultural Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Fort Pierce, FL 34945

Additional index words. cuttings, grafting, micropropagation, seedlings, tissue culture

***Abstract*. The rootstock plays a large role in modern citrus production because of its influence on tolerance to adverse abiotic and biotic soil-borne stresses, and on the general horticultural characteristics of the grafted scion. In recent years, rootstock has received increased attention as a management strategy to alleviate the devastating effects of the bacterial disease huanglongbing (HLB), also known as "citrus greening." In commercial citrus nursery production, rootstocks are typically propagated by seed. Because of the increased demand for HLB-tolerant rootstocks, seed supply is often inadequate for the most popular cultivars. Cuttings and tissue culture (TC) propagation are alternative methods to supply adequate quantities of genetically identical rootstocks to be used as liners for grafting. However, there are concerns among nursery owners and citrus growers regarding the possible inferiority of rootstocks that are not propagated by seed. This study investigates the influence of rootstock propagation method on traits of sweet orange trees grafted on four commercially important rootstock cultivars during the nursery stage and during the first year of growth in a commercial citrus orchard. Several of the measured traits during the nursery stage, including rootstock sprouting, grafted tree growth, and root mass distribution were significantly influenced by the rootstock propagation method, but traits were also influenced by the rootstock cultivar. Our results also suggest that for tissue culture-propagated plants, differences in the starting material and the culturing method can affect the grafted tree behavior. Except for canopy spread and scion to rootstock trunk diameter ratio, tree growth during the orchard stage was determined by the combination of propagation method and rootstock, rather than by propagation method alone.**

Rootstock selection has played an important role in the history of citrus production (Castle, 2010). The rootstock has a significant impact on tolerance to biotic and abiotic stress factors as well as on fruit quality, yield, and other horticultural parameters of a grafted citrus tree (Bowman and Joubert, 2020; Bowman et al., 2016a; Castle, 1995; Wutscher and Hill, 1995; Wutscher and Bowman, 1999). Since the arrival of the bacterial disease huanglongbing (HLB, a.k.a. citrus greening) in Florida, citrus production has declined steadily from 242 million boxes of citrus before 2005 to 74 million boxes in the 2018–19 production year (www.nass.usda.gov/fl). Most commercial citrus cultivars are susceptible to HLB, and disease management in Florida is focused on eliminating the disease vector and improving tree health and productivity using different horticultural strategies (Morgan et al., 2016; Stansly et al., 2014).

Rootstocks have received increased attention as a management strategy to alleviate the devastating effects of HLB. In contrast to most scion cultivars, several rootstock cultivars are tolerant to HLB and enable a grafted tree to remain productive for a longer time (Albrecht and Bowman, 2011, 2012; Bowman et al., 2016a). The physiological mechanism for rootstock effects on the scion under HLB endemic conditions have not yet been elucidated, but it may be associated with their ability to influence the scion metabolically (Albrecht et al., 2019; Killiny et al., 2018) or indirectly by affecting absorption of water and nutrients from the soil. The ability to influence uptake of water and nutrients is related to the root system architecture, which can vary considerably among rootstocks (Castle and Youtsey, 1977).

The phenomenon of nucellar embryony found in citrus and other fruit tree crops allows the clonal production of most rootstock cultivars from seed (Koltunow et al., 1995; Kumar and Rani, 2013), and seed propagation remains the preferred method of rootstock production in commercial citrus nurseries. In recent years, the increased demand for HLB-tolerant rootstocks has led to an inadequate seed supply for the most popular cultivars. Consequently, alternative propagation methods, namely cuttings and tissue culture propagation, are required to produce the desired quantities of trees.

Propagation by cuttings and tissue culture will result in genetically identical rootstocks that can be used as liners for grafting (Albrecht et al., 2017a; Bowman and Albrecht, 2017). For new cultivars, both propagation methods are indispensable to produce trees for field testing until seed source trees have reached sexual maturity (Bowman and Albrecht, 2017). In addition, for those rootstocks that produce few or no nucellar embryos but have other desirable traits (Barcelos Bisi et al., 2020), vegetative propagation is the only option to produce genetically identical plants.

Seed-grown rootstocks usually develop a single taproot during initial growth in the nursery. In contrast, rootstocks propagated by cuttings and tissue culture produce multiple adventitious roots (Albrecht et al., 2017b). Based on traditional views, there is concern among citrus growers and citrus nurseries that the different root architectures arising from vegetative propagation will result in trees of inferior quality. The use of rooting hormones and the maturity of the source plant (Ferguson et al., 1985) are additional sources of concern as they may affect growth behavior of rootstock liners prior and during the grafting stage.

In citrus, early studies regarding growth and root architectures of trees produced other than by seed were mostly conducted with the intent to compare scion cuttings with scion seedlings or grafted trees rather than rootstock propagation methods. These studies reported shallower and sparser root systems of cuttings compared with seedlings (Halma, 1931; Savage et al., 1945). However, Palma et al. (1997) suggested that the multiple adventitious roots produced by rooted microcuttings of *Citrus macrophylla* would be more efficient for nutrient uptake than the single perpendicular root of the seedlings. Castle (1977) found that despite differences in some root traits, the root distribution and root density of sweet orange trees on cuttings compared favorably with that of trees on seedlings. In addition to differences among propagation methods, considerable

Received for publication 12 Feb. 2020. Accepted for publication 19 Mar. 2020.
Published online 17 April 2020.
The manuscript is associated with a presentation given during the 2019 Annual Meeting of the Florida State Horticultural Society, which was held 9–11 June in Orlando, FL.
We would like to express our thanks to Beth Lamb (Phillip Rucks Nursery, Frostproof, FL) and Mireia Bordas (Agromillora, Wildwood, FL) for providing plant material; Anna and Nate Jameson (Brite Leaf Citrus Nursery, Lake Panasoffkee, FL) for providing space and tree care in their nursery; and Joby Sherrod (Duda & Sons) for providing space and tree care in their grove. This study was supported with funds from the UF/IFAS Citrus Research Initiative and the Citrus Research and Development Foundation (CRDF).
U.A. is the corresponding author. E-mail: ualbrecht@ufl.edu.

differences among rootstock genotypes were reported (Castle and Youtsey, 1977).

Studies on temperate fruit tree crops showed varying results depending on the propagation method, culturing conditions, and cultivar used (Webster, 1997 and references therein). Results also vary in other tree crops. For example, African plum trees propagated by vegetative methods had a lower root density than seed-grown trees and were suggested to be less competitive in intercropping systems (Asaah et al., 2012). A study on different Eucalyptus species found that micropropagation yielded an inferior root system compared with macropropagation during the first 16 months of field growth (Mokotedi et al., 2010). In contrast, a comparison of tissue culture cultivars and seedlings of *Eucalyptus camaldulensis* discovered no above- or belowground architectural differences that were associated with the propagation method (Bell et al., 1993).

A recent study conducted in our laboratory (Albrecht et al., 2017b) compared root architectures of rootstock liners produced by seed, cuttings, and tissue culture during the early weeks of growth and before grafting. In the present study, we are expanding our research to investigate root architectures, tree growth, and other traits of grafted sweet orange (*Citrus sinensis*) trees in the nursery and in a commercial citrus orchard. Our objectives were to investigate if the propagation method affects 1) growth traits of trees during the early weeks after grafting in the nursery, 2) root architectures and other traits of grafted field-ready nursery trees, and 3) grafted tree growth during the first year of establishment in a commercial citrus orchard. Four commercial citrus rootstock cultivars were included in the study to investigate the influence of rootstock and the combination rootstock and propagation methods.

Materials and Methods

Rootstock production

Rootstock cultivars. Four commercially important citrus rootstocks were used: 'US-802' ('Siamese' pummelo [*Citrus maxima*] × 'Gotha Road' trifoliate orange [*Poncirus trifoliata*]), 'US-812' ('Sunki' mandarin [*C. reticulata*] × 'Benecke' trifoliate orange), 'US-897' ('Cleopatra' mandarin × 'Flying Dragon' trifoliate orange), and 'US-942' ('Sunki' mandarin × 'Flying Dragon' trifoliate orange), four hybrid rootstocks that have gained major commercial importance in Florida since their release by the U.S. Department of Agriculture (USDA) (Bowman and Joubert, 2020; Bowman et al., 2016b).

Seed propagation. Seeds of the rootstock cultivars were extracted from fruits as described in Albrecht et al. (2017b). Plants were grown in the USDA Horticultural Research Laboratory (USHRL) greenhouses in Fort Pierce, FL beginning with sowing seeds into premoistened, soilless potting mix (Pro Mix BX; Premier Horticulture, Inc., Quakertown, PA) using racks of 3.8-cm × 21-cm cone cells (Cone-tainers; Stuewe and Sons, Tangent, OR). After germination, plants were irrigated as needed and fertilized biweekly using a water-soluble fertilizer with micronutrients (20N–10P–20K; Peters Professional, The Scotts Company, Marysville, OH) at a rate of 400 mg N/L. Insecticides were applied as needed. Any off-types arising from zygotic embryos were identified based on leaf morphological traits and discarded.

Cuttings propagation. Plants were grown in the USHRL greenhouses in Fort Pierce, FL. Single node cuttings of ≈2.5 cm in length were excised from woody sections of 1- to 2-year-old greenhouse-grown nucellar rootstock plants. Leaves remained attached to each node but were trimmed to reduce the leaf size to 20% to 30%. The basal end of each cutting was dipped in a commercial rooting powder (Hormodin 2, E.C. Geiger, Inc., Harleysville, PA) containing 0.3% indole-3-butyric acid (IBA), and cuttings were inserted into 3.8-cm × 21-cm cone cells (Cone-tainers; Stuewe and Sons) containing the same potting medium described above. Cones were placed on a mist bench, and misting was applied for a duration of 6 weeks as described in Bowman and Albrecht (2017); during this time period, the shadecloth on the greenhouse was closed from 9:00 AM to 6:00 PM daily. After 4 weeks, plants received a liquid fertilizer application as described above. Two weeks later, plants received another liquid fertilizer application at the same rate but including chelated iron (Sequestrene 138 Fe; Ciba-Geigy Corp., Greensboro, NC). Starting in week 7, the shadecloth remained open, and plants were maintained in the same manner described for seedlings.

Tissue culture propagation. Plants were grown in two different commercial citrus nurseries and produced using two different methodologies described in Albrecht et al. (2017b). The source of explants for tissue culture method 1 (TC1) were nucellar embryos from seeds obtained from fruit collected from foundation trees at the Bureau of Citrus Budwood Registration, Florida Department of Agriculture and Consumer Services, Chiefland, FL. Disinfected embryos were placed into clear polypropylene 473-ml deli containers containing Murashige and Skoog (MS) agar-nutrient medium (Murashige and Skoog, 1962) without added growth regulators. Embryos were dissected after pregermination of seeds and identified as nucellar based on leaf morphology and uniformity of growth of the regenerated plant. Multiple shoot clusters were produced by alternating between media containing MS medium with 1.0 mg/L benzyladenine (BA), 0.5 mg/L kinetin, and 0.5 mg/L naphthalene acetic acid (NAA) (Bowman et al., 1997), and MS medium or EXS-III basal medium (PhytoTechnology Laboratories, Lenexa, KS) with no added growth regulators. Clusters were divided and placed in new media on a cycle of about 5 weeks. Elongated shoots suitable for rooting were produced by serial transfers on hormone-free medium. Single shoots were obtained by removing sections with at least four nodes and placing them on a MS basal medium containing 2.0 mg/L NAA and 1 g/L active charcoal. After rooting for a period of about 6 weeks, plantlets were removed from the medium, roots were trimmed to a 3–6 cm length, and placed into 3.8-cm × 21-cm cone cells containing the same potting medium described for seedlings. Plants were kept in high humidity in a plant growth chamber (EGC Model M36, Environmental Growth Chambers, Chagrin Falls, OH) with a 16-h light (200 $\mu mol \cdot m^{-2} \cdot s^{-1}$)/8-h darkness photoperiod. Humidity was gradually reduced over 3–4 weeks to allow plants to acclimate to ambient greenhouse conditions. Plants were then transferred to the greenhouse and maintained as described for seedlings.

Explants for tissue culture method 2 (TC2) were buds from young apical shoots from certified disease-free budwood from the Bureau of Citrus Budwood Registration citrus germplasm collection in Chiefland, FL. Disinfected buds were cultured in test tubes containing a MS-based agar nutrient medium (Agromillora, composition proprietary). Cultures were maintained in a growth room under controlled conditions at a temperature of 24 to 26 °C and a 16-h light/8-h darkness photoperiod. Buds were transferred to jars and subcultured every 2 weeks to fresh nutrient medium. After several cycles, depending on the number of plants to be produced, explants were transferred to new cultures in which the shoot elongation was promoted. In these cultures, plantlets were maintained in the growth room under the same conditions as described above for 8–10 d until they reached a height of 6–8 cm. Elongated plants were individualized by cutting off the stem at the base, planted in 3.8- × 4.4-cm paper pots (Ellepots) containing a mix of peat (Pelemix, Las Salinas, Spain) and coconut fiber (Klasmann-Deilmann, Geeste, Germany), and transferred to misting tunnels inside the greenhouse for acclimatization. After 2–3 weeks, rooted plants were moved to growth benches, grown until 18–20 cm in height (8–10 weeks), and then moved to the USHRL greenhouses in Fort Pierce, FL, where they were transplanted into cone cells as described above.

Grafted nursery plants

Rootstock liners were divided into two sets. One set remained in the USHRL greenhouse and the other set was transported to a commercial citrus nursery. Liners that remained at the USHRL greenhouse were transplanted into 15.2-cm × 15.2-cm × 30.5-cm plastic tree pots (Stuewe & Sons) containing Pro Mix BX potting medium. Plants were arranged in a randomized design with rootstock and propagation method as fixed effects and each replication consisting of 9 plants. When stem diameters were of a suitable size (5–6 mm), liners were budded with certified disease-free 'Valencia' (*C. sinensis* L.) budwood using the inverted T method (Albrecht et al., 2017b). Plants were irrigated as needed using a handheld sprayer and fertilized biweekly using a water-soluble fertilizer (20N–10P–20K; Peters Professional, The Scotts Company) at a rate of 400 mg N/L, and insecticides were applied as needed.

In the commercial citrus nursery, rootstock liners were transplanted into 10.2-cm × 10.2-cm × 34.3-cm plastic tree pots (Stuewe & Sons) containing a coir-based potting medium (Pelemix, Kiryat Gat, Israel). When liners reached a suitable size (5–6 mm stem diameter) they were budded with certified disease-free 'Valencia' budwood using the inverted T method. Plants were arranged in the nursery in a design favored by the nursery owner that is optimized for rapid nursery production. Arrangement was by rootstock cultivar, with each block of rootstock containing all propagation methods. Plants were maintained in the greenhouse under conditions typical for the commercial nursery using an automated drip irrigation system (Netafim, Tel Aviv, Israel). Plants were fertigated daily using 20N–10P–20K with micronutrients (Totalgro, SDT Industries, Winnsboro, LA) at a rate of 150 ppm N. Plants were grown until ready for field planting, which was ≈6 months after budding.

Field-grown trees

Field-ready grafted trees were planted in a commercial citrus orchard near Felda, Hendry County, FL (lat. 26°36′33.3″ N, long. 81°26′42.1″ W. The soil type at this location is a sandy spodosol with little organic matter, low cation exchange capacity, poor drainage, and low water holding capacity (Mylavarapu et al., 2016). Trees were planted in six single rows on raised 6.5-m wide beds, separated by 6.5-m wide furrows, with a spacing of 2.4 m between trees and 1–2 border trees at the end of each row. Trees were planted in a split plot design with rootstock as the main plot and propagation method as the subplot and six replications (rows). Each subplot consisted of three trees. Tree management followed grower standards and included regular root drenches with neonicotinoids and foliar sprays of other insecticides as needed. Irrigation was by under-tree microjets. Trees were assessed 1 year after planting.

Plant assessments

Young grafted nursery trees. Plants maintained in the USHRL greenhouses were evaluated for sprout formation and bud survival during the first 12 weeks after budding (WAB). Sprout formation was expressed as the number of rootstock sprouts per plant and bud survival was expressed in percent. In addition, scion height and stem diameters were measured at 12 WAB and at 22 WAB, respectively.

Field-ready nursery trees. A subset of field-ready grafted trees from the commercial nursery was dissected to assess the plant biomass distribution and root architectures. Six trees of each scion/rootstock combination were used. Plants were separated into leaves, stems, and roots. Leaves were counted, scanned with a Cannon MG3620 scanner, and leaf area was determined using Assess 2.0 (Lakhdar Lamari, American Phytopathological Society) image analysis software. Leaves were dried in an oven at 53 °C to a constant weight to determine leaf dry weight and calculate the specific leaf area (SLA). SLA was calculated as the ratio of leaf area (cm^2) to leaf dry weight (g). Stem diameters were measured 2.5 cm above and below the graft union, and trees were separated at the graft union. Roots were removed from the potting medium and washed with tap water. The number of primary roots, defined as roots directly arising from the root crown (including tap root and adventitious roots), were counted, and root diameters were measured at their point of origin on the root trunk. Roots were separated into large-diameter roots (>0.5 cm in diameter), medium-size diameter roots (0.2–0.5 cm), and fibrous roots (<0.2 cm in diameter). A subset of fibrous roots was scanned, and root length was determined using Assess 2.0 software. Roots and stems were dried as described for leaves to determine stem and root dry weights and calculate the specific root length (SRL). Leaf, stem, and root biomasses were expressed as percentage of the total plant biomass. Shoot-to-root ratio was determined by dividing combined the dry weights of stems and leaves by the dry weight of roots. Biomasses of the different root classes (large, medium, and fibrous) were expressed as percentage of the total root biomass. The SRL was calculated as the ratio of length (m) to dry weight of fibrous roots (g).

Field-grown trees. Trees were assessed 1 year after field planting. Trunk diameters were measured at 5 cm above and below the graft union using a digital caliper. Tree height was determined from the soil level to the top of the canopy using a digital measuring pole (Sokkia, Kanagawa, Japan), excluding any erratic shoots. Canopy spread was determined as the average of canopy width across and parallel to the row. HLB foliar disease symptoms were assessed by visual ratings on a scale of 1 to 5, with 1 = no foliar disease symptoms, 2 = foliar symptoms on less than 25% of leaves, 3 = 25% to 50% of leaves with symptoms, 4 = 50% to 75% of leaves with symptoms, 5 = more than 75% of leaves with symptoms. Canopy thickness and canopy color were also rated on a scale of 1 to 5, with a rating of 1 representing the thinnest and most unhealthy (yellow) canopy and a rating of 5 representing a very thick and healthy/green canopy. Two ratings per tree were conducted on two opposite sides parallel to the row, and ratings were expressed as the average.

Statistical analysis

Analysis of variance (ANOVA) was conducted using TIBCO Statistica v13.3 (TIBCO Software Inc., Palo Alto, CA), and main effect means were separated using Tukey's honestly significant difference post hoc test. For young grafted nursery-grown trees, factorial ANOVA was employed with rootstock and propagation method as fixed effects. For field-ready nursery plants, factorial ANOVA was employed for all variables expressed as relative values (biomass ratios, specific leaf area, specific root length, etc.). For absolute measures (total biomass, leaf area, scion and rootstock trunk diameters, and primary root diameter) analysis was by one-way ANOVA across all rootstock cultivars. For field trials, factorial ANOVA was employed with rootstock and propagation method as fixed effects, and block included as a random factor. Means were separated by Tukey's honestly significant difference test. Differences were defined as statistically significant when the P value was <0.05.

Results

Young grafted nursery trees. The percentage of live buds measured 12 weeks after budding was similar among rootstocks propagated by seed, cutting, and tissue culture (85.4% to 94.4%), and there was a significant interaction among propagation methods and rootstock (Table 1). 'US-942' propagated by TC1 had the lowest percentage (52.8%) of live buds compared with most of the other rootstocks.

The number of rootstock sprouts measured during the first 12 weeks after budding varied significantly among propagation methods and rootstocks. Rootstocks propagated by TC2 had the largest number of sprouts (4.53) compared with the other propagation methods. Among the different rootstock cultivars, the largest number of sprouts were produced on 'US-812' and 'US-942' (3.69 and 3.82) and the lowest on 'US-802' (2.65).

A significant interaction was found among propagation methods and rootstock cultivar for the scion height. Scions grafted on 'US-942' propagated by seed were largest (19.5 cm), whereas scions grafted on 'US-802' produced by TC2 were smallest (6.4 cm). A significant interaction among propagation methods and rootstock cultivar was also found for scion diameters. Scion diameters were largest in scions grafted on 'US-942' and 'US-812' (5.36 mm and 4.95 mm) produced by seed, and on 'US-942' produced by TC1 (5.07 mm), and smallest in scions on 'US-802' produced by TC2 (2.48 mm).

Field-ready nursery trees. Because of the commercial nursery requirements, plants were arranged by rootstock cultivar on the greenhouse benches; absolute measures (total plant biomass, leaf area, scion and rootstock trunk diameters, and primary root diameter) were therefore only compared among propagation methods, and not among rootstock cultivars. The total biomass of plants was 52.3 g on average and did not vary significantly (P = 0.1950) among propagation methods (data not shown). The same was observed for the leaf area, which was 2179 cm^2 on average, with no significant differences (P = 0.1234) among propagation methods (data not shown). No significant differences among propagation methods were observed for scion trunk diameters

Table 1. Bud survival, rootstock sprouts, and other traits of young nursery-grown 'Valencia' trees grafted on different rootstocks propagated by seed (SD), cuttings (CT), and tissue culture (TC).

Factor	% Buds alive 12 WAB	No. of RS sprouts 0–12 WAB	SC ht 12 WAB (cm)	SC stem diam 22 WAB (mm)
Propagation method				
SD	92.4	2.94 b	17.1 a	4.83 a
CT	87.4	3.12 b	13.5 b	3.88 bc
TC1	85.4	2.60 b	13.1 b	4.25 b
TC2	94.4	4.53 a	13.0 b	3.79 c
	$P = 0.2002$	$P < 0.0001$	$P = 0.0021$	$P < 0.0001$
Rootstock				
US-802	97.9 a	2.65 b	10.8 b	3.26 c
US-812	89.6 ab	3.82 a	13.0 b	4.15 b
US-897	93.7 a	3.08 ab	16.3 a	4.48 b
US-942	77.8 b	3.69 a	16.8 a	4.93 a
	$P = 0.0012$	$P = 0.0013$	$P < 0.0001$	$P < 0.0001$
Propagation method × rootstock				
SD × US-802	100.0 a	1.5	18.0 ab	4.40 abc
SD × US-812	80.6 ab	3.28	15.7 abc	4.95 a
SD × US-897	100.0 a	3.03	15.3 abc	4.58 abc
SD × US-942	88.9 a	3.94	19.5 a	5.36 a
CT × US-802	100.0 a	2.53	9.8 bcd	3.17 def
CT × US-812	86.1 ab	3.64	14.0 abcd	3.70 bcde
CT × US-897	80.6 ab	3.03	16.1 abc	4.44 abc
CT × US-942	81.5 ab	3.33	14.3 abcd	4.34 abcd
TC1 × US-802	97.2 a	2.47	9.1 cd	2.99 ef
TC1 × US-812	94.4 a	3.28	12.0 abcd	4.38 abc
TC1 × US-897	97.2 a	2.36	15.6 abc	4.58 abc
TC1 × US-942	52.8 b	2.31	15.6 abc	5.07 a
TC2 × US-802	94.4 a	4.08	6.4 d	2.48 f
TC2 × US-812	97.2 a	5.08	10.4 bcd	3.57 cdef
TC2 × US-897	97.2 a	3.89	18.1 ab	4.31 abcd
TC2 × US-942	88.9 a	5.08	17.0 abc	4.82 ab
	$P = 0.0133$	$P = 0.2744$	$P = 0.0168$	$P = 0.0096$

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. WAB = weeks after budding; RS = rootstock; SC = scion.

($P = 0.9152$), and rootstock trunk diameters ($P = 0.8166$), which were 8.6 mm and 12.0 mm, respectively, on average (data not shown).

The scion-to-rootstock trunk diameter ratio of the field-ready grafted trees was not influenced by the propagation method, but by the combination of rootstock cultivar and propagation method (Table 2). Among the plants with the highest scion-to-rootstock trunk diameter ratio (0.76–0.78) were plants grafted on 'US-942'—regardless of the propagation method. The lowest ratios (0.63–0.65) were measured for plants grafted on 'US-802'.

The scion-to-rootstock biomass ratio varied significantly among propagation methods. Plants on rootstocks propagated by cuttings and TC2 had the highest ratio (3.23 and 3.03), and plants on seed-propagated rootstocks had the lowest ratio (2.60). Significant differences were also measured among rootstock cultivars, with plants on 'US-942' having the highest (3.24) and plants on 'US-802' having the lowest (2.65) ratio.

The specific leaf area of the grafted plants ranged from 150.7 to 169.8 cm^2/g, but no significant differences were measured among rootstock propagation methods and rootstock cultivars. The percentage of leaf biomass did not vary among rootstock propagation methods. However, significant differences were found among rootstock cultivars. The highest percentage of leaves was produced by trees on 'US-942' and 'US-897' (27.2% and 26.5%), and the lowest on 'US-802' (23.1%).

The percentage of stem biomass and root biomass varied significantly among rootstock propagation methods. Trees on rootstocks propagated by cuttings and TC2 had a higher proportion (51.8% and 50.6%) of stem tissue than trees on seed- and TC1-propagated rootstocks (46.3 and 48% respectively). Trees on seed-propagated rootstocks had a larger proportion of roots (27.7%) than trees on cuttings- and TC2-propagated rootstocks (23.5% and 24.6%). The root mass fraction also differed among rootstock cultivars, with the largest fraction found for 'US-802' (27.2%) and the lowest for 'US-942' and 'US-897' (23.5% and 24.5%).

Rootstock propagation method significantly influenced the number of primary roots and ranged from 1.4 for seed-rootstock to 4.9 for cuttings-propagated rootstocks (Table 3). No significant differences were observed among rootstock cultivars, although there was a trend ($P = 0.0651$) for 'US-942' producing the largest number (3.6) of roots. Rootstock propagation method also significantly ($P = 0.0001$) affected the diameter of the primary roots. Primary roots of seed- and TC1-propagated rootstock had the largest diameter (12.3 mm and 10.9 mm), and cuttings-propagated rootstocks had the lowest diameter (6.3 mm) (data not shown). Typical root systems of field-ready trees produced by seed, cuttings, and tissue culture are presented in Fig. 1.

Root size distribution was significantly affected by the propagation method but not by the rootstock cultivar. Seed and TC1-propagated rootstocks had a larger proportion (55.8% and 54.4%) of large roots than cuttings- and TC2-propagated rootstocks (32.4% and 42.8%). The reverse was observed for the proportion of medium-size roots, which was largest in cuttings- and TC2-propagated rootstocks (40.3% and 34.2%) and lowest in seed- and TC2-propagated rootstocks (20.6% and 21.9%). A significant interaction between propagation method and rootstock cultivar was found for the proportion of fibrous roots. Most of the cuttings-propagated rootstocks were among those with the largest proportion (26.6% to 29.1%) of fibrous roots. The lowest proportion (14.2%) was found for 'US-802' when propagated by TC2.

The specific root length (SRL) of fibrous roots was not significantly influenced by the rootstock propagation method but by the rootstock cultivar. SRL was higher for 'US-897' and 'US-942' (26.1 and 24.0 m/g) than for 'US-802' and 'US-812' (20.1 and 20.9 m/g).

Field-grown trees. Tree survival was 100% for trees grafted on seed-, cuttings-, and TC1-propagated rootstocks and 94.4% for trees grafted on TC2-propagated rootstocks. The average tree height of trees after 1 year of growth in a commercial citrus orchard was significantly influenced by the combination of propagation method and rootstock cultivar (Table 4). 'US-802' propagated by TC2 induced the largest tree size (113.8 cm); and 'US-897' propagated by SD, TC1 and TC2, and 'US-942' propagated by cuttings induced the smallest tree size.

Table 2. Biomass distribution of leaves, stem, roots, and other plant traits of field-ready, nursery-grown 'Valencia' trees grafted on different rootstocks propagated by seed (SD), cuttings (CT), and tissue culture (TC).

Factor	SC/RS trunk diam ratio	SC/RS biomass ratio	SLA (cm^2/g)	Leaf mass (%)	Stem mass (%)	Root mass (%)
Propagation method						
SD	0.71	2.60 b	163.4	26.0	46.3 b	27.7 a
CT	0.72	3.23 a	157.8	24.7	51.8 a	23.5 b
TC1	0.71	2.84 ab	165.2	26.2	48.0 b	25.8 ab
TC2	0.71	3.03 a	160.9	24.9	50.6 a	24.6 b
	$P = 0.4860$	$P = 0.0009$	$P = 0.1866$	$P = 0.3850$	$P < 0.0001$	$P = 0.0006$
Rootstock						
US-802	0.64 c	2.65 c	159.1	23.1 b	49.6	27.2 a
US-812	0.75 ab	2.78 bc	161.3	25.0 ab	48.7	26.3 ab
US-897	0.72 b	3.04 ab	161.6	26.5 a	49.0	24.5 bc
US-942	0.76 a	3.24 a	165.3	27.2 a	49.4	23.5 c
	$P < 0.0001$	$P = 0.0012$	$P = 0.3862$	$P = 0.0015$	$P = 0.7760$	$P = 0.0011$
Propagation method × rootstock						
SD × US-802	0.64 c	2.56	165.7	23.6	48.4	28.0
SD × US-812	0.74 a	2.42	159.5	26.9	43.9	29.2
SD × US-897	0.71 a	2.55	160.0	25.8	46.1	28.1
SD × US-942	0.78 a	2.94	168.5	27.8	46.9	25.3
CT × US-802	0.63 c	2.93	150.7	22.3	52.3	25.4
CT × US-812	0.75 a	2.67	159.7	21.0	51.8	27.2
CT × US-897	0.76 a	3.79	156.4	27.6	51.6	20.9
CT × US-942	0.76 a	3.85	164.4	28.1	51.4	20.6
TC1 × US-802	0.65 bc	2.51	164.3	25.9	46.1	28.0
TC1 × US-812	0.71 ab	2.84	162.6	26.6	47.5	25.9
TC1 × US-897	0.71 ab	2.99	169.8	25.5	49.6	24.9
TC1 × US-942	0.78 a	3.08	163.9	26.8	48.8	24.4
TC2 × US-802	0.63 c	2.62	155.8	20.7	51.7	27.6
TC2 × US-812	0.78 a	3.31	163.3	25.4	51.6	23.0
TC2 × US-897	0.71 ab	3.09	160.2	27.2	48.7	24.0
TC2 × US-942	0.74 a	3.22	164.2	26.1	50.3	23.6
	$P = 0.0104$	$P = 0.2161$	$P = 0.7219$	$P = 0.0991$	$P = 0.2515$	$P = 0.1942$

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. SC = scion; RS = rootstock; SLA = specific leaf area.

Table 3. Biomass distribution of large, medium, and fibrous roots, and other root traits of field-ready, nursery-grown 'Valencia' trees grafted on different rootstocks propagated by seed (SD), cuttings (CT), and tissue culture (TC).

Factor	Number of primary roots	Large size root mass (%)	Medium size root mass (%)	Fibrous root mass (%)	SRL (m/g)
Propagation method					
SD	1.4 d	55.8 a	20.6 b	23.6	24.1
CT	4.9 a	32.4 b	40.3 a	27.3	22.3
TC1	2.3 c	54.4 a	21.9 b	23.7	22.3
TC2	3.6 b	42.8 b	34.2 a	23.1	22.4
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P = 0.0616$	$P = 0.0903$
Rootstock					
US-802	3.0	0.493	0.282	0.225	20.1 b
US-812	2.8	0.435	0.323	0.242	20.9 b
US-897	2.7	0.487	0.272	0.242	26.1 a
US-942	3.6	0.439	0.293	0.268	24.0 a
	$P = 0.0651$	$P = 0.3502$	$P = 0.6398$	$P = 0.1055$	$P < 0.0001$
Propagation method × rootstock					
SD × US-802	1.5	49.2	27.1	23.7 ab	20.5
SD × US-812	1.8	54.4	22.0	23.6 ab	23.4
SD × US-897	1.0	60.9	18.8	20.3 ab	27.1
SD × US-942	1.2	58.9	14.5	26.6 a	25.3
CT × US-802	4.8	34.8	36.3	28.9 a	19.9
CT × US-812	4.3	31.5	43.8	24.7 ab	19.3
CT × US-897	4.3	34.6	38.8	26.6 a	26.4
CT × US-942	6.0	28.7	42.2	29.1 a	23.5
TC1 × US-802	1.8	60.4	16.6	23.0 ab	21.1
TC1 × US-812	2.0	48.8	26.8	24.5 ab	20.2
TC1 × US-897	2.0	63.2	16.4	20.3 ab	23.9
TC1 × US-942	3.3	45.2	27.8	27.0 a	23.9
TC2 × US-802	3.8	52.9	32.9	14.2 b	18.8
TC2 × US-812	3.2	39.4	36.6	24.1 ab	20.5
TC2 × US-897	3.5	35.9	34.7	29.4 a	26.9
TC2 × US-942	3.8	42.8	32.6	24.6 ab	23.3
	$P = 0.4167$	$P = 0.2714$	$P = 0.7925$	$P = 0.0130$	$P = 0.5045$

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. SRL = specific root length.

Canopy spread was significantly influenced by propagation method and rootstock cultivar. Trees on seed- and cuttings-propagated rootstocks were wider (92.1 cm and 89.9 cm) than trees on tissue culture-propagated rootstocks (83.4–83.7 cm). Among the rootstock cultivars, 'US-812' produced had the widest canopy (90.4 cm) and 'US-897' the narrowest (84.2 cm).

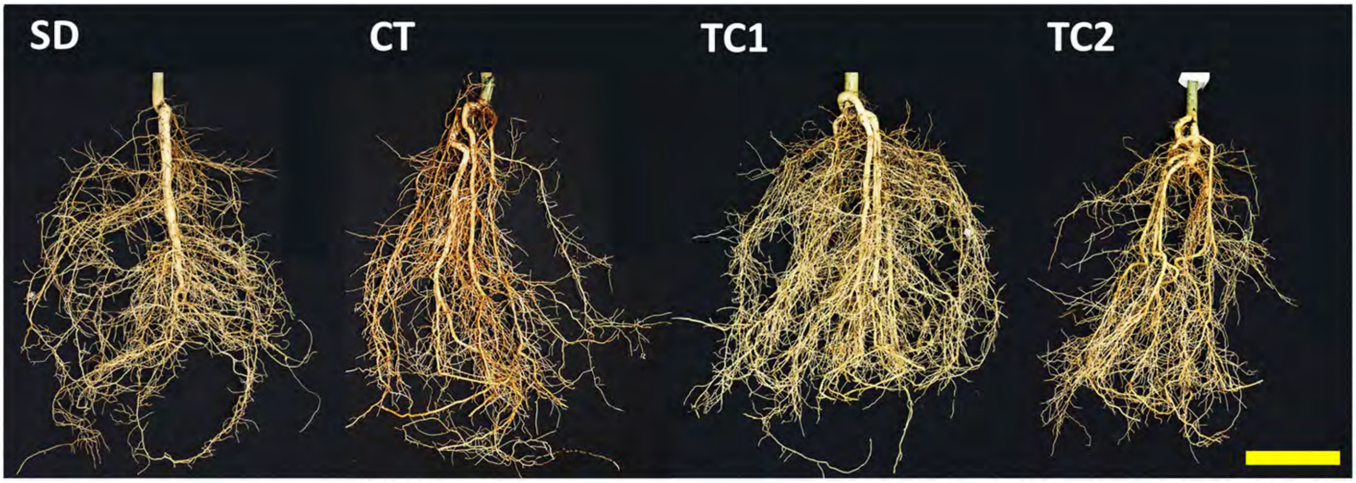

Fig. 1. Root systems of nursery-grown, field-ready 'Valencia' trees grafted on 'US-897' rootstock propagated by seed (SD), cutting (CT), and tissue culture (TC1 and TC2). The yellow bar represents a scale of 5 cm.

Table 4. Tree height, canopy spread, and other traits of field-grown 'Valencia' trees grafted on different rootstocks propagated by seed (SD), cuttings (CT), and tissue culture (TC).

Factor	Tree ht (cm)	Canopy spread (cm)	RS trunk diam (mm)	SC trunk diam (mm)	SC/RS trunk diam ratio
Propagation method					
SD	104.9	92.1 a	35.1 a	24.2 ab	0.69 b
CT	105.8	89.9 a	34.3 ab	24.9 a	0.72 a
TC1	107.3	83.4 b	33.8 ab	23.3 b	0.69 b
TC2	107.1	83.7 b	33.0 b	23.7 ab	0.72 a
	$P = 0.4185$	$P < 0.0001$	$P = 0.0274$	$P = 0.0067$	$P < 0.0001$
Rootstock					
US-802	108.2 a	87.3 ab	38.0 a	23.7 bc	0.62 c
US-812	107.7 a	90.4 a	35.1 b	26.0 a	0.74 a
US-897	103.4 b	84.2 b	31.5 c	22.5 c	0.71 b
US-942	105.7 ab	87.3 ab	31.7 c	24.0 b	0.76 a
	$P = 0.0111$	$P = 0.0357$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Propagation method × rootstock					
SD × US-802	106.2 ab	93.1	39.1 a	24.2 bcde	0.62
SD × US-812	106.2 ab	95.7	36.8 ab	26.6 ab	0.72
SD × US-897	102.2 b	86.6	31.4 cd	21.3 e	0.68
SD × US-942	105.2 ab	92.8	33.2 bcd	24.9 abcd	0.75
CT × US-802	109.4 ab	89.4	39.1 a	24.5 abcde	0.63
CT × US-812	110.1 ab	95.2	35.8 abc	27.5 a	0.77
CT × US-897	100.9 b	87.6	32.4 bcd	24.3 abcde	0.75
CT × US-942	102.7 b	87.6	30.0 d	23.3 cde	0.78
TC1 × US-802	103.3 ab	80.5	35.7 abc	21.9 de	0.61
TC1 × US-812	110.8 ab	85.7	36.2 abc	25.5 abc	0.70
TC1 × US-897	108.9 ab	83.9	31.7 cd	22.2 de	0.70
TC1 × US-942	106.1 ab	83.3	31.7 cd	23.6 bcde	0.75
TC2 × US-802	113.8 a	85.9	38.3 a	24.3 abcde	0.63
TC2 × US-812	103.7 ab	84.7	31.3 cd	24.2 bcde	0.78
TC2 × US-897	101.4 b	78.6	30.5 d	22.2 de	0.73
TC2 × US-942	109.0 ab	85.4	31.7 cd	24.2 bcde	0.76
	$P = 0.0012$	$P = 0.6934$	$P = 0.0072$	$P = 0.0043$	$P = 0.0959$
Block					
	$P < 0.0001$	$P < 0.0001$	$P = 0.0007$	$P < 0.0001$	$P = 0.0003$
Block × rootstock					
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P = 0.0823$

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. RS = rootstock; SC = scion.

Rootstock and scion trunk diameters were significantly influenced by the combination of propagation methods and rootstock. 'US-802' propagated by seed, cuttings, and TC2 had the largest rootstock trunk diameter (38.3–39.1 mm), and 'US-897' propagated by TC2 and 'US-942' propagated by cuttings had the smallest (30.5 and 30.0 mm). Largest scion diameters were induced by 'US-812' propagated by cuttings and the smallest by 'US-897' propagated by seed.

Both propagation method and rootstock cultivar significantly influenced the scion-to-rootstock trunk-diameter ratio. Trees with rootstocks propagated by cuttings and by TC2 had a larger ratio (0.72) than trees on rootstocks propagated by seed and by TC1 (0.69). Among rootstock cultivars, US-812 and US-942 had the largest scion-to-rootstock trunk-diameter ratio (0.74 and 0.76), and US-802 had the smallest (0.62).

The trees in this experiment were not much affected by HLB after 1 year of growth in the orchard. HLB disease indices ranged from 1.10 to 1.24 and were neither influenced by rootstock propagation method nor by rootstock cultivar (Table 5). Canopy thickness and canopy color indices ranged from 3.13 to 3.41 and from 4.06 to 4.34, respectively, with no significant effect of propagation method or rootstock cultivar.

For all of the measured variables, block was a significant factor; and significant interactions with rootstock cultivar—but not propagation method—were observed.

Table 5. Huanglongbing (HLB) foliar disease symptoms, canopy thickness, and canopy color indices of field-grown 'Valencia' trees grafted on different rootstocks propagated by seed (SD), cuttings (CT), and tissue culture (TC).

Factor	Foliar HLB symptoms	Canopy thickness	Canopy color
Propagation method			
SD	1.18	3.36	4.32
CT	1.22	3.38	4.31
TC1	1.14	3.19	4.16
TC2	1.13	3.19	4.10
	P = 0.3248	P = 0.3542	P = 0.1892
Rootstock			
US-802	1.21	3.41	4.28
US-812	1.10	3.32	4.34
US-897	1.13	3.13	4.21
US-942	1.24	3.25	4.06
	P = 0.4674	P = 0.6402	P = 0.4725
Propagation method × rootstock			
	P = 0.7170	P = 0.1525	P = 0.1368
Block			
	P = 0.0775	P = 0.3914	P = 0.6020
Block × rootstock			
	P = 0.0003	P = 0.0008	P = 0.0072

Indices were determined by ratings on a scale of 1 to 5, with 1 being the best and 5 being the worst for foliar HLB symptoms and 1 being the worst and 5 being the best for canopy color and canopy density.

Discussion

To cope with the devastating effects of HLB in Florida, the demand for superior rootstocks has increased dramatically, exceeding the available supply of seeds to be used for propagation of some rootstocks. One of the rootstocks dramatically affected by this is 'US-942', for which the commercial demand for trees far exceeds the seed supply. During 2018, of the 846,608 trees propagated on 'US-942' in Florida, more than 82% of nursery trees made use of liners propagated by tissue culture or cuttings (FDACS records). This study presents information on the suitability and effects of alternative methods of rootstock propagation on the grafted tree growth in the nursery and during the first year of establishment in the field.

Many of the tree traits during the early weeks after grafting were influenced by the propagation method; these include the number of rootstock sprouts, the scion height, and the scion stem diameter. Although trees with seed-propagated rootstocks appeared to induce more vigorous scion growth compared with the other propagation methods, effects depended on the rootstock cultivar. This is not unexpected, as the four rootstocks used in this study are known for their differences in vigor (Bowman and Joubert, 2020; Bowman et al., 2016a, 2016b). Among the four rootstocks studied, 'US-802' is the highest vigor-inducing rootstock and 'US-897' the lowest.

Some nursery owners reported observing excessive sprouting of rootstock liners propagated by tissue culture (Nate Jameson, personal communication). More sprouting was also observed in our study, but only when liners were propagated by TC2, not by TC1. One of the differences between these two methods is the source of the plant tissue used as explant. Whereas in TC1, cultures were initiated from embryos, in TC2, they were initiated from buds of mature trees. The type or the maturity of the explant used for regeneration may have been a determining factor for the differences in sprout formation. Although in tissue culture and in mature trees, shoot regeneration or sprouting usually diminishes with age (Bond and Migley, 2003; Yildiz, 2012), Webster (1995) reported that in temperate fruit trees, micropropagated rootstocks frequently sucker profusely because of rejuvenation. Other dissimilarities between the two tissue culture methods in our study, such as the use of plant growth hormones and time in culture, may be responsible for the observed differences in sprouting. Grant and Hammat (1999) reported that total time in culture is the most important factor causing physiological changes in micropropagated apple and cherry. Additional research is necessary to determine the differences in rootstock sprouting of micropropagated citrus and ways of effectively limiting or managing this. In addition to the propagation method, sprouting was influenced by the rootstock cultivar. A cultivar-specific influence was also observed in micropropagated and conventional-propagated apple rootstocks (Jones and Hadlow, 1989).

The biomass distribution of the field-ready nursery plants was influenced both by rootstock propagation method and by rootstock cultivar. In general, plants on seed- and TC1-propagated rootstocks were similar for most traits and different from plants on cuttings- and TC2-propagated plants. Relative root masses were larger in the former, and the reverse was found for the relative stem masses. A similar trend was observed in our previous study on young rootstock liners (Albrecht et al., 2017b), suggesting the persistence of morphological traits throughout the grafted-tree stage in the nursery. A larger root system is generally associated with a greater ability to withstand wind. This is particularly important in Florida where citrus production is threatened by tropical storms and hurricanes. Studies on apple trees found that trees on seedling-propagated rootstocks were more upright after 5 years of exposure to westerly winds than trees on micropropagated or dwarfing rootstocks (Larsen and Higgins, 1993). Similarly, uprooting resistance of Eucalyptus trees was lower for micropropagated plants than for macropropagated plants (rooted cuttings) and seedlings after 16 months of field growth (Mokotedi et al., 2010). It must be noted that in the study on Eucalyptus, micropropagated trees had the lowest number of roots, which is different to our findings in citrus. Whether the propagation method influences uprooting resistance in field-grown citrus trees is currently being examined in our laboratory. The relative root mass was also influenced by the rootstock cultivars. In addition, rootstock influenced the relative leaf mass of the scion, with 'US-942' and 'US-897' having the largest. Both rootstocks are known for inducing production of high-quality fruit (Bowman and Joubert, 2020; McCollum and Bowman, 2017).

As previously reported for rootstock liners (Albrecht et al., 2017b), the number of primary roots of the grafted field-ready trees increased from seed- to vegetative-propagated rootstocks with the most (adventitious) roots observed for cuttings. Consequently, in seed-propagated rootstocks, the biomass fraction was larger for large-size roots and smaller for medium-size roots compared with the other propagation methods, except TC1. According to Mokotedi et al. (2010), the number of roots is a strong predictor of uprooting resistance. This would suggest a possible advantage of citrus trees on vegetative-propagated over seed-propagated rootstocks, should differences in the root number persist under field conditions.

It is surprising that TC1-propagated rootstocks were more similar in their root morphological traits to seed-propagated rootstocks than to TC2-propagated rootstocks. One possible reason for this finding is that rootstocks produced by seed and by TC1 originated from juvenile tissue (embryos). whereas rootstocks from cuttings and TC2 originated from stems and buds, respectively. As discussed above, this suggests an influence of the type or maturity of the explant on the morphological traits of the regenerated plant. The maturity of the explants was attributed to differences in morphological traits of field-grown plants in other tree species (Gupta et al., 1991). For example, Chinese fir plants regenerated from explants of juvenile trees were vigorous, whereas plants regenerated from explants of mature trees were slow and plagiotrophic in growth (Bigot and Engelmann, 1987). It is probable that other factors involved in the propagation process, such as composition of the culture medium, transition to the potting medium, and early growth conditions, were at least partially responsible for the different traits of plants in our study. In a study on Japanese persimmon, the field performance of micropropagated own-rooted trees was affected by the initial growing environment, supporting this notion (Tetsumura et al., 1998). This may also explain the varying reports on the effects of propagation methods in other tree crops.

Contrary to our previous study (Albrecht et al., 2017b), which focused on young rootstock liners, in this study the specific root length was not influenced by propagation method. This is explained by the fact that in the present study, the fibrous roots used to determine SRL only comprised a small portion of the root system; whereas in the previous study, roots of the young liners were mostly fibrous. The fibrous root mass fraction was not influenced by the propagation method, but by the combination of propagation method and rootstock cultivar. Because of their role in water and nutrient uptake, fibrous roots are the most important part of the root system (Anderson and Ingram, 1993). Eissenstat (1991) found that citrus rootstocks of high SRL had a faster growth rate of root proliferation and a greater rate of water extraction compared with rootstocks of lower SRL. Our findings suggest that the ability to uptake water and nutrients in a grafted citrus tree seems to be more influenced by the rootstock cultivar than by the method by which they are propagated. As previously reported (Albrecht et al., 2017b; Bowman and Albrecht, 2017), 'US-942' and 'US-897' had the largest SRL, which may contribute to their positive influence on the juice total soluble solids content.

Tree survival and establishment during the first year of field growth is important for the future success of the orchard. In our field study, only two trees did not survive the first year in a commercial citrus orchard; both had rootstocks propagated by TC2. This is a small percentage of trees and does not necessarily suggest an inferiority of this propagation method, as a small percentage of tree death is expected in any new citrus planting. Tree height after 1 year of field growth was determined by the combination of propagation method and rootstock but not by propagation method alone. Interactions were also found for rootstock and scion trunk diameters. In contrast, rootstock propagation method influenced canopy spread and scion-to-rootstock trunk-diameter ratios, but these traits were also influenced by the rootstock cultivar. A larger influence of the genotype than the propagation method on tree growth and root morphology were also observed in Eucalyptus trees (Bell et al., 1993). Marín et al. (2003) compared peach and nectarine trees grafted on cuttings- and tissue culture-propagated rootstock and found no influence of propagation method on the field performance during the first year of growth. Seed-propagated rootstocks were not included in these studies.

The similarity of root traits between seed- and TC1-propagated rootstocks observed in the field-ready nursery trees did not transfer to aboveground traits during 1 year of growth in the field. This suggests that in the absence of seeds, vegetative propagation may provide a viable alternative for the rapid production of true-to-type rootstocks.

In conclusion, our results suggest that despite differences in the root architectures associated with the propagation method, growth of young grafted citrus trees appears to be affected more by the rootstock cultivar than by the propagation method. Whether this trend continues throughout the productive years is the subject of continuing investigations. The differences in some of the morphological traits of plants regenerated from different tissue culture methods provide an opportunity for further research on the role of the source and/or maturity of the explant in the short- and long-term growth traits of citrus trees.

Literature Cited

Albrecht, U. and K.D. Bowman. 2011. Tolerance of the trifoliate citrus hybrid US-897 (*Citrus reticulata* Blanco × *Poncirus trifoliata* L. Raf.) to Huanglongbing. HortScience 46:16–22.

Albrecht, U. and K.D. Bowman. 2012. Tolerance of trifoliate citrus rootstock hybrids to *Candidatus* Liberibacter asiaticus. Scientia Hort. 147:71–80.

Albrecht, U., I. Tripathi, and K.D. Bowman. 2019. Rootstock influences the metabolic response to Candidatus Liberibacter asiaticus in grafted sweet orange trees. Trees 1–27, doi: 10.1007/s00468-019-01925-3.

Albrecht, U., M. Zekri, and J. Williamson. 2017a. Citrus propagation. University of Florida IFAS Extension (edis.ifas.ufl.edu), publication HS1309.

Albrecht, U., M. Bordas, B. Lamb, B. Meyering, and K.D. Bowman. 2017b. Influence of propagation method on root architecture and other traits of young citrus rootstock plants. HortScience 52:1569–1576.

Anderson, J.M. and J.S.I. Ingram. 1993. Tropical soil biology and fertility: A handbook of methods. CAB International, Wallingford, UK, University of Arizona Press, Tuscon, AZ.

Asaah, E.K., T.N. Wanduku, Z. Tchoundjeu, L. Kouodiekong, and P. Van Damme. 2012. Do propagation methods affect the fine root architecture of African plum (*Dacryodes edulis*)? Trees 26:1461–1469.

Barcelos Bisi, R., U. Albrecht, and K.D. Bowman. 2020. Seed and seedling nursery characteristics for 10 USDA citrus rootstocks. HortScience 55:528–532.

Bell, D.T., P.G. van der Moezel, I.J. Bennett, J.A. McComb, C.F. Wilkins, S.C.B. Marshall, and A.L. Morgan. 1993. Comparisons of growth of *Eucalyptus camaldulensis* from seeds and tissue culture: Root, shoot and leaf morphology of 9-month-old plants grown in deep sand and sand over clay. For. Ecol. Mgt. 57:125–139.

Bigot, C. and F. Engelmann. 1987. Vegetative propagation *in-vitro* of *Cunninghamia lanceolata* (Lamb) Hook, p. 146–158. In: J.M. Bonga and D.J. Durzan (eds.). Cell and tissue culture in forestry, vol. 3. Martinus Nijhoff Publishing, Dordrecht, The Netherlands.

Bond, W.J. and J.J. Migley. 2003. The evolutionary ecology of sprouting in woody plants. Int. J. Plant Sci. 164:S103–S114.

Bowman, K.D. and U. Albrecht. 2017. Efficient propagation of citrus rootstocks by stem cuttings. Scientia Hort. 225:681–688.

Bowman, K.D. and J. Joubert. 2020. Citrus rootstocks, p. 105–127. In: M. Talon, M. Caruso, and F.G. Gmitter (eds.). The genus citrus. Woodhead Publishing, Cambridge, MA.

Bowman, K.D., R.D. Hartman, A.E. Lamb, and H.K. Wutscher. 1997. Enhancing development of improved rootstocks by tissue culture propagation and field performance of selected rootstocks. Proc. Annu. Meet. Fla. State Hort. Soc. 110:10–13.

Bowman, K.D., G. McCollum, and U. Albrecht. 2016a. Performance of 'Valencia' orange (*Citrus sinensis* [L.] Osbeck) on 17 rootstocks in a trial severely affected by huanglongbing. Scientia Hort. 201:355–361.

Bowman, K.D., F. Faulkner, and M. Kesinger. 2016b. New citrus rootstocks released by USDA 2001–2010: Field performance and nursery characteristics. HortScience 51:1208–1214.

Castle, W.S. 1977. Effect of method of propagation and scion cultivar on the root system of 'Milam' rootstock. J. Amer. Soc. Hort. Sci. 102:435–437.

Castle, W.S. 1995. Rootstock as a fruit quality factor in citrus and deciduous tree crops. N. Z. J. Crop Hort. Sci. 23(4):383–394.

Castle, W.S. 2010. A career perspective on citrus rootstocks, their development, and commercialization. HortScience 45:11–15.

Castle, W.S. and C.O. Youtsey. 1977. Root system characteristics of citrus nursery trees. Proc. Annu. Meet. Fla. State Hort. Soc. 90:39–44.

Eissenstat, D.E. 1991. On the relationship between specific root length and the rate of root proliferation: A field study using citrus rootstocks. New Phytol. 118:63–68.

Ferguson, J., M. Young, and J. Halvorson. 1985. The propagation of citrus rootstocks by stem cuttings. Proc. Annu. Meet. Fla. State Hort. Soc. 98:39–42.

Grant, N.J. and N. Hammat. 1999. Increased root and shoot production during micropropagation of cherry and apple rootstocks: Effect of subculture frequency. Tree Physiol. 19:899–903.

Gupta, P.K., R. Timmis, and A.F. Mascarenhas. 1991. Field performance of micropropagated forestry species. In Vitro Cell. Dev. Biol. 27P:159–164.

Halma, F.F. 1931. The propagation of citrus by cuttings. Hildgardia 6:131–157.

Jones, O.P. and W.C.C. Hadlow. 1989. Juvenile-like character of apple trees produced by grafting scions and rootstocks produced by micropropagation. J. Hort. Sci. 64:395–401.

Killiny, N., M.F. Valim, S.E. Jones, and F. Hijaz. 2018. Effect of different rootstocks on the leaf metabolite profile of 'Sugar Belle' mandarin hybrid. Plant Signal. Behav. 13:e1445934.

Koltunow, A.M., K. Soltys, N. Nito, and S. McClure. 1995. Anther, ovule, seed, and nucellar embryo development in *Citrus sinensis* cv. Valencia. Can. J. Bot. 73:1567–1582.

Kumar, K.M. and U.M. Rani. 2013. Techniques to differentiate zygotic and nucellar seedlings in polyembryonic fruit crops. IJAEB 6:344–350.

Larsen, F.E. and S.S. Higgins. 1993. Growth and fruit production of young micropropagated apple (*Malus domestica* Borkh.) trees. Scientia Hort. 53:205–211.

Marín, J.A., M. Castillo, E. García, and P. Andreu. 2003. Field performance of grafted fruit-tree rootstocks was not affected by micropropagation. Acta Hort. 616:295–299.

McCollum, G. and K.D. Bowman. 2017. Rootstock effects on fruit quality among 'Ray Ruby' grapefruit trees grown in the Indian River district of Florida. HortScience 52:541–546.

Mokotedi, M.E.O., M.P. Watt, and N.W. Pammenter. 2010. Analysis of differences in field performance of vegetatively and seed-propagated *Eucalyptus* cultivars II: Vertical uprooting resistance. South. For. 72:31–36.

Morgan, K.T., R.E. Rouse, and R.C. Ebel. 2016. Foliar application of essential nutrients on growth and yield of 'Valencia' sweet orange infected with Huanglongbing. HortScience 51:1482–1493.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15:473–497.

Mylavarapu, R., W. Harris, and G. Hochmuth. 2016. Agricultural soils of Florida. University of Florida IFAS Extension (edis.ifas.ufl.edu), publication SL441.

Palma, B., H. Bravo, and M. Castro. 1997. Comparative study of root systems in *Citrus macrophylla* seedlings and microcuttings propagated in vitro. Acta Hort. 447:591–596.

Savage, E.M., W.C. Cooper, and R.B. Piper. 1945. Root systems of various citrus rootstocks. Proc. Annu. Meet. Fla. State Hort. Soc. 58:44–48.

Stansly, P.A., H.A. Arevalo, J.A. Qureshi, M.M. Jones, K. Hendricks, P.D. Roberts, and F.M. Roka. 2014. Vector control and foliar nutrition to maintain economic sustainability of bearing citrus in Florida groves affected by huanglongbing. Pest Mgt. Sci. 70:415–426.

Tetsumura, T., H. Yukinaga, and R. Tao. 1998. Early field performance of micropropagated Japanese Persimmon trees. HortScience 33:751–753.

Yildiz, M. 2012. The prerequisite of the success in plant tissue culture: High frequency shoot regeneration, p. 64–90. In: A. Leva and L.M.R. Rinaldi (eds.). Recent advances in plant in vitro culture. IntechOpen, doi: 10.5772/51097.

Webster, A.D. 1995. Temperate fruit tree rootstock propagation. N. Z. J. Crop Hort. Sci. 23:355–372.

Webster, A.D. 1997. A review of fruit tree rootstock research and development. Acta Hort. 451:53–73.

Wutscher, H.K. and K.D. Bowman. 1999. Performance of 'Valencia' orange on 21 rootstocks. HortScience 34:622–624.

Wutscher, H.K. and L.L. Hill. 1995. Performance of Hamlin orange on 16 rootstocks in east central Florida. HortScience 30:41–43.

This paper was presented during the 2019 FSHS Annual Meeting and originally published in *HortScience* [HORTSCIENCE 55(5):729–737. 2020. https://doi.org/10.21273/HORTSCI14928-20]. The paper is included in this Proceedings as a reprint (with permission).

Proc. Fla. State Hort. Soc. 132:102–107. 2019.

Best Management Practice Selection for Florida Vegetable and Agronomic Crop Producers

Moonwon Soh[1,2], Tara Wade*[1,2], and Tatiana Borisova[2]

[1]*University of Florida/IFAS, Southwest Florida REC, 2685 State Rd. 29 North, Immokalee FL 34142*

[2]*University of Florida/IFAS Food and Resource Economics Department, P.O. Box 110370, Gainesville, FL 32611*

Additional index words. multiple best management practice adoption, water conservation, nutrient management

Agricultural best management practices (BMPs) can provide several private and public benefits. Private benefits can include reductions in costs (due to reduced inputs such as fuel, labor, and fertilizer) and improved yields (due to improved soil productivity). Public benefits can include sustained or improved water quality and water flows and levels (due to reduced nutrient losses, increased water conservation, and reduced soil erosion). The Florida Department of Agriculture and Consumer Services' (FDACS) Notice of Intent (NOI) checklist allows FDACS staff to identify practices that are applicable to specific agricultural operations, and for the producers to indicate the intent to implement the BMPs. The NOI checklist is based on FDACS BMP manuals, and the process is a part of the Florida water quality policy to protect and restore water resources. We use the NOI dataset to examine the selection of specific nutrient management and water conservation BMPs by Florida's vegetable and agronomic crop growers. Given that adopting multiple BMPs can have cumulative benefits increasing both private and public gains, we seek to examine the frequency of multi-practice selection. As discussed in agricultural economics literature, identifying frequent BMP bundles can lower the costs of disseminating information to encourage BMP adoption. The analysis of the BMP bundles could also assist in estimating potential water quality improvements. We expect the BMP bundles to vary throughout the state depending on the commodities produced, size of the operation, hydrogeological features, and regional policy priorities (e.g., spring or estuary protection). The study results indicate that for practices we associated with nutrient management (soil surveys, soil pH tests, cover crops (CC), controlled release fertilizer, optimum fertilizer management, and tissue tests), approximately 99% of the NOIs identified two or more BMPs accounting for 99% of sampled acres. For practices we associated with water conservation (CC, irrigation scheduling, irrigation system maintenance, and pesticide equipment calibration), 96% of the NOIs indicated a selection of two or more practices accounting for 98% of the sampled acres. Overall, of the nine practices examined, approximately 72% of the growers listed in the NOI database selected at least five practices accounting for 280,630 acres.

Water is one of the most important resources for human well-being. Access to water shapes agricultural practices and directly affects profitability of agriculture and many other industries (EPA, 2013; UN, 2018). Globally, about 70% of fresh water is used by the agriculture sector (UNESCO, 2001). In the United States, irrigation withdrawals accounts for approximately 40% of total freshwater use (USGS, 2018). In Florida, the agricultural sector is also a major user of fresh water: in 2015, 2.2 billion gallons of freshwater per day were withdrawn for crop irrigation, which is comparable with the total freshwater withdrawals in Florida for public water supply (USGS, 2019). By 2035, agriculture water use is expected to further increase by 6% (EDR, 2019). Improving the efficiency of irrigation water and fertilizer use is closely tied with improving and protecting water quality, particularly, the reduction of nonpoint sources of nitrogen and phosphorus (Houlbrooke et al., 2003).

Several studies indicate that agricultural best management practices (BMPs) can alleviate the negative impact on water quality and quantity by reducing nutrient losses and increasing water conservation (Braune and Wood, 1999; D'Arcy and Frost, 2001; Strauss et al., 2007; Chaubey et al., 2010; Shrestha et al., 2010; Lam et al., 2011). In this study, we focus on the Florida agricultural best management practice program intended to protect and restore water resources. As a part of the program, producers can schedule a meeting with a BMP coordinator from the Florida Department of Agriculture and Consumer Services (FDACS), who then provides the producers with state-adopted BMP manuals, which are relevant to the producers' crops, along with other BMP-related information. The FDACS BMP coordinator then assesses the producer's operation to determine what BMPs are applicable. The assessment is based on nutrient and irrigation regimes of the operation, as well as production-related activities near poorly drained areas, conveyances that discharge offsite, and water resources (such as wetlands, streams, and springs). Next, the BMP coordinator confirms which parcels of land the producer wishes to enroll in the BMP program. Producers fill out the BMP

We appreciate the data and the feedback for the study provided by Yesenia Escribano, Norman Doxford, and other personnel at the Florida Department of Agriculture and Consumer Services. We also appreciate help from Sayid Shah, formerly a research assistant at UF/IFAS SWREC-Immokalee. This research was partially funded by the U.S. Department of Agriculture, National Institute of Food and Agriculture under award no. 2016-67024-24755 and the multistate Hatch project W4133.

*Corresponding author. Email:tara.wade@ufl.edu

checklist and sign the Notice of Intent (NOI) to implement the BMPs. The producer keeps a copy of the checklist and signed NOI. To maintain a presumption of compliance with state water-quality standards the producer must implement and maintain the applicable BMPs and keep adequate records (FDACS, 2019).

In this study, we use the FDACS NOI data to investigate the selection of practices that improve water quality and conserve water. We find that growers simultaneously use multiple practices that help to reduce their impact on the environment. Adopting multiple BMPs can have cumulative benefits for the producer (e.g., increasing the yields and reducing the input costs) and water quality protection (e.g., by significantly reducing nutrient losses from the fields). Further, Amacher and Feather (1997) and Cooper (2003) reported that the identification of the BMP bundles—that, is practices that are commonly used together—can reduce the cost of educational programs and government incentive programs set up to encourage BMP adoption. Specifically, instead of discussing or incentivizing the individual practices, the focus could shift to a bundle of BMPs, such as the use of soil surveys (SS), soil tests, optimal fertilizer management, and (CC). In addition, many research projects focus on examining the effectiveness of BMP adoption (see FDACS, 2019b). While some projects focus on examining individual practices, others focus on examining effectiveness of "typical" practices used by the growers. The analysis of the BMP bundle selection in the NOI process can give the researchers additional information on the BMP bundles more frequently used in specific geographic locations and for specific crops.

In this initial analysis we only looked at NOI responses, parcel size, and county information. Other field characteristics such as soil, crop, and proximity to a body of water were not considered. In the future, we plan to expand the analysis and examine other characteristics of the operation that are used to determine the applicability of specific practices.

This study examines a limited list of practices listed in the FDACS 2006 Vegetable and Agronomic Crop BMP manual. We chose to examine these specific practices based on their relevance to the nationwide BMP programs (USDA, 2019; Chesapeake Bay Program, 2018) and the expectation of potential cumulative benefits from their joint implementation. Out of 51 practices listed in the manual, we examined nine. We report the study results using two general categories of the practices selected. The first category are the practices that are judged by the authors to primarily improve nutrient use efficiency and reduce or prevent nutrient water pollution: SS, soil pH tests (SPT), (CC), controlled release fertilizer (CRF), optimum fertilizer management (OFM), and tissue tests (TT). The second category is the practices that are judged to primarily improve water conservation or provide additional water quality protection or improvement: CC, irrigation scheduling (IS), irrigation system maintenance (ISM), and pesticide equipment calibration (PEC). The data show that growers intend to use most of these BMPs together, suggesting the existence of a complementary relationship between practices (Manda et al., 2016).

Data Description

The Florida Department of Agriculture and Consumer Services (FDACS) provides a list of recommended BMPs that help to mitigate agriculture's effect on the environment. The Notice of Intent (NOI) is the grower's formal notification that s/he is planning to implement selected BMPs. Note that some of the practices listed on the NOIs may already be implemented on the growers' fields. The Vegetable and Agronomic Crop manual was first published in 2006 and updated in 2015. This study uses parcel-level FDACS NOI data for the 2006 Vegetable and Agronomic Crop manual only. Because the practice descriptions in the 2006 manual are distinct from those in the 2015 manual, the two cannot be combined. We therefore examine a subset of vegetable and agronomic crop growers who filed NOIs: 739 growers or 639,260 acres. The data cover 46 counties and the average farm size is 864 acres. These data were collected in April 2019 and represent the population of growers still enrolled under the 2006 program/manual.

We examine nine BMPs, widely discussed nationally (USDA, 2019; Chesapeake Bay Program, 2018) to improve water quality and quantity. These are grouped into nutrient management BMPs that assist with mitigating nutrient losses, and water conservation BMPs that reduce water use and help mitigate leaching events. For practices associated with nutrient management, we examine the use of SS, SPT, TT, CC, CRF, and OFM. SS identify soil types and characteristics which growers can use to better understand their fields' irrigation and fertilizer needs. SPT are important tools that help growers understand what nutrients are available for the plants. Growers can then use this information to improve crop nutrient uptake. TT identify the nutritional status of the plant and can help optimize nutrient application rates and timing. CRF provides nutrients when the plant needs it, thereby increasing the efficiency of nutrient uptake and reducing leaching. OFM is the practice of applying the optimum rate to meet realistic yield goals, at the time the plant requires, and with correct placement (i.e., targeting plant's root zones). CC can help reduce nutrient losses to the environment by scavenging for excess nutrients in the soil, increasing soil tilth and soil organic matter, and minimizing sediment losses from soil erosion. CC can also play a role in improving water quantity.

For practices associated with water conservation and further improving or protecting water quality, we examined CC, (IS), ISM, and PEC. CC (can help improve soil organic matter and root networks, keeping sandy soils more compact and increasing soil water holding capacity to make water available to the plants when needed. IS represents the bundle of a technical systems that improves irrigation efficiency by improving the timing and amount of irrigation water used. PEC can lead to more cost-effective applications by maintaining the accuracy of the application; preventing over application of pesticides, thereby reducing losses to the soil and potential leaching.

Empirical Results

Below we examine grower selection of single and multiple water conservation and nutrient management BMPs. Except for CRF, which only 122 growers identified for 11% of acres, each of the other eight BMPs selected by 352 to 567 growers, covering at least a third of the total acreage (Table 1). Among the nine BMPs, SPT is the most dominant practice with 567 growers selecting it for 45% of acres. The average NOI area for the BMPs examined are from 511 acres to 615 acres. This narrow range of average acreage implies that, for this sample, the BMP selections do not depend on the size of their farm. Also, the average size per NOI for CRF is 572 acres; this indicates that the relatively small share of acres for which this practice is selected is because of the small number of growers who indicate they intend to use the practice.

Each water conservation BMP is identified for more than 36% of acres (Table 1). County-level intent to use water conservation BMPs is shown in Fig. 1. Growers in six counties (Suwannee,

Table 1. Notice of intent to use best management practices (BMPs) among producers who filed notices of intent (NOIs) for 2006 Vegetable and Agronomic Crop BMP Manual.

BMP type	BMP[z]	Selection rate (% acres)	Average NOI acres	Selection rate (% growers)
Water/Nutrient	CC	38.9	518.3	65.0
Water conservation	IS	36.3	551.7	57.0
	ISM	36.9	597.6	53.5
	PEC	44.5	513.4	75.0
Nutrient management	SS	40.1	514.6	67.4
	SPT	45.3	511.2	76.7
	CRF	10.9	571.7	16.5
	OFM	43.0	552.1	67.4
	TT	33.9	615.5	47.6
Total area		639,260 acres		
Total growers		739		

[z]CC = cover crops, CRF = controlled release fertilizer, IS = irrigation scheduling, ISM = irrigation system maintenance, OFM = optimum fertilizer management, PEC = pesticide equipment calibration, SS = soil survey, SPT = soil pH test, TT = tissue test.

Source: Florida Department of Agriculture and Consumer Services, Office of Agricultural Water Policy, Apr. 2019.

Madison, Jackson, Manatee, Collier, and Martin) selected at least one of these BMPs for over 9303 acres. These are also the counties with large vegetable and agronomic crop acreage, which explains the sizable area of the BMP selection. Table 2 shows that a few growers selected one practice while 45% selected all 4 water conservation practices for 28% of acres. Figure 2 illustrates the distribution of acres where four water conservation BMPs were selected. Growers in the six counties intend to adopt these BMPs on over 111,000 acres; this is greater than the combined acres for all other counties (about 70,000 acres) where growers selected four water conservation BMPs.

Table 3 shows observed choices when growers selected two water conservation BMPs. CC and PEC were selected by 16% of growers for 6% of acres while other bundles examined were selected for less than 1% of acres. Bundles of three water conservation practices are shown in Table 4. While Table 3 shows

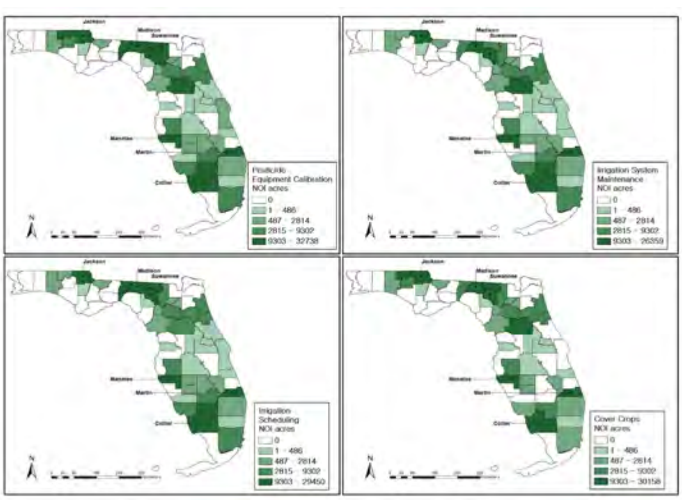

Fig. 1. Distribution of acres where growers intend to use water conservation best management practices (BMPs). Source: Florida Department of Agriculture and Consumer Services, Office of Agricultural Water Policy, Apr. 2019

Table 2. Notice of intent to use multiple water conservation best management practices (BMPs).

	Number of water conservation BMPs			
	1	2	3	4
Area	1.3%	6.7%	9.4%	28.4%
Growers	3.9%	18.3%	10.4%	44.7%
Total area	639,260 acres			
Total growers	739			

Source: Florida Department of Agriculture and Consumer Services, Office of Agricultural Water Policy, Apr. 2019.

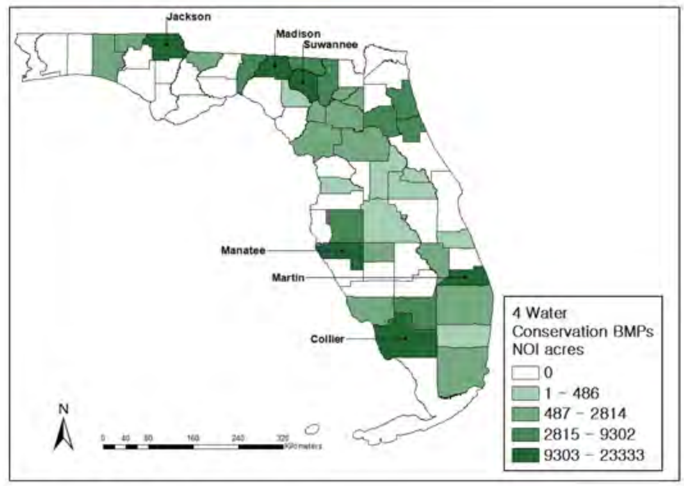

Fig. 2. Distribution of acres where growers intend to use four water conservation best management practices (BMPs). Source: Florida Department of Agriculture and Consumer Services, Office of Agricultural Water Policy, Apr. 2019

Table 3. Notice of intent to use two water conservation best management practices (BMPs)[z].

BMP	CC	IS	ISM	PEC	Area	Grower
Bundle 2-1	Yes	Yes	No	No	0%	0%
Bundle 2-2	Yes	No	Yes	No	0%	0%
Bundle 2-3	Yes	No	No	Yes	5.6%	15.6%
Bundle 2-4	No	Yes	Yes	No	0.7%	0.9%
Bundle 2-5	No	Yes	No	Yes	0.3%	1.4%
Bundle 2-6	No	No	Yes	Yes	0.1%	0.4%
Total area			639,260 acres			
Total grower			739			

[z]CC = cover crops, IS = irrigation scheduling, ISM = irrigation system maintenance, PEC = pesticide equipment calibration.

Source: Florida Department of Agriculture and Consumer Services, Office of Agricultural Water Policy, Apr. 2019.

Table 4. Notice of intent to use three water conservation best management practices (BMPs)

BMP	CC[z]	IS	ISM	PEC	Area	Grower
Bundle 3-1	Yes	Yes	Yes	No	0.6%	0.9%
Bundle 3-2	Yes	Yes	No	Yes	1.7%	3.0%
Bundle 3-3	Yes	No	Yes	Yes	2.6%	0.5%
Bundle 3-4	No	Yes	Yes	Yes	4.5%	6.0%
Total Area			639,260 acres			
Total Grower			739			

[z]CC = cover crops, IS = irrigation scheduling, ISM = irrigation system maintenance, PEC = pesticide equipment calibration.

Source: Florida Department of Agriculture and Consumer Services, Office of Agricultural Water Policy, Apr. 2019.

that these growers do intend to use CC with IS or CC with ISM alone, Table 4 shows that there are a few cases where the three practices were selected together (0.6% of acres). All other combinations of three BMPs were selected at more than twice the rate of CC–IS–ISM (bundle 3-1). All other combinations included PEC. This could be a result of the widespread use of PEC (75% of growers; Table 1). It could also imply that the PEC has a complementary relationship with other BMPs. Also, if we exclude outliers, we see that the range of those who selected three BMPs (2.8%) is narrower than that seen among those who selected two BMPs (4.9%).

County-level nutrient management BMP selection is illustrated in Fig. 3. Except for CRF, each nutrient management BMP is identified for more than 34% of NOI acres (see Table 1). Central Florida lags behind the other Florida regions, potentially due to differences in the types of crops produced (Fig. 3). Like water conservation BMP selection, we find that most acres are identified in only a few counties. Growers in each of seven counties (Jackson, Madison, Suwannee, Marion, Manatee, Martin, and Collier) selected nutrient management BMP on at least 9300 acres.

BMPs thought to improve nutrient management are listed in Table 5. Like water conservation BMPs, a few growers intend to use a single BMP while others intend to use multiple practices. The data indicate that most growers (30%) selected four nutrient management BMPs while combinations of five nutrient management BMPs were selected for 22% of acres. Overall, more than 50% of growers intend to use four or more nutrient management BMPs.

Figure 4 illustrates the distribution of combinations of four nutrient management BMPs over 31 counties. Suwannee County reports the highest selection area with 9915 acres. Figure 5 illustrates acreage for those who intend to use five nutrient management BMPs. Among 34 counties, Jackson, Madison, Marion, Manatee, and Martin counties each identified five practices on totaling 14,134 acres.

At 11% selection rate, CRF is applicable to only a small subset of producers (Table 1), so we have excluded it from further discussions (Tables 6–8). Tables 6, 7, and 8 provide selection rates when two, three, and four nutrient management BMPs are selected, respectively. Like water conservation practices, growers

Table 5. Notice of intent to use multiple nutrient management best management practices (BMPs)

	Number of nutrient management BMPs					
	1	2	3	4	5	6
Area	0.1%	1.7%	3.5%	12.3%	22.1%	6.4%
Growers	0.3%	3.2%	8.8%	29.9%	17.3%	8.5%
Total Area	639,260 acres					
Total Growers	739					

Source: Florida Department of Agriculture and Consumer Services, Office of Agricultural Water Policy, Apr. 2019.

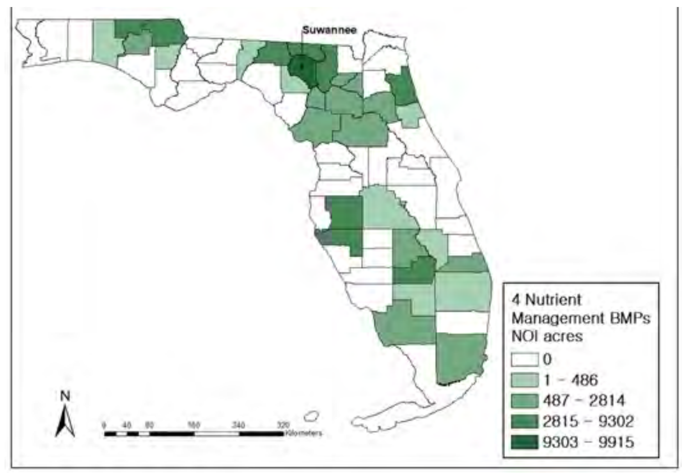

Fig. 4. Distribution of acres where growers intend to use four nutrient management best management practices (BMPs). Source: Florida Department of Agriculture and Consumer Services, Office of Agricultural Water Policy, Apr. 2019.

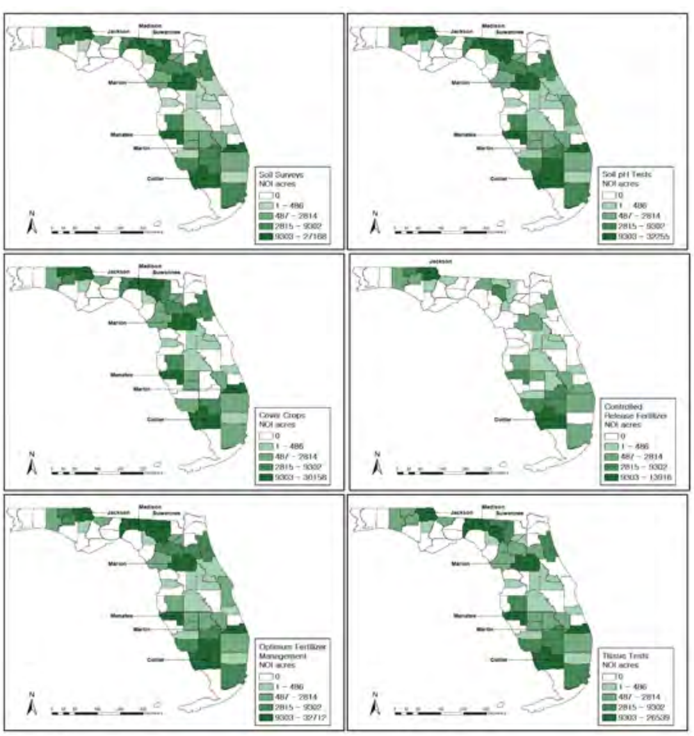

Fig. 3. Distribution of acres where growers intend to use nutrient management best management 390 practices (BMPs).

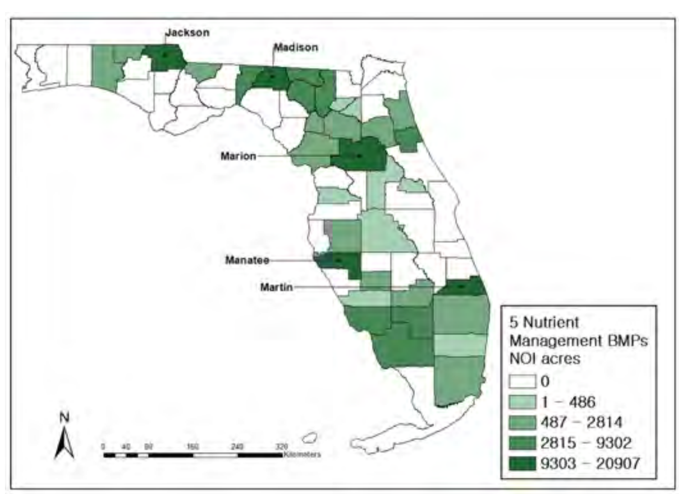

Fig. 5. Distribution of acres where growers intend to use five nutrient management best management practices (BMPs) Source: Florida Department of Agriculture and Consumer Services, Office of Agricultural Water Policy, April 2019.

Table 6. Notice of intent to use two nutrient management best management practices (BMPs).[z]

BMP	SS	SPT	CC	OFM	TT	Area	Grower
Bundle 2-1	Yes	Yes	No	No	No	0.1%	0.1%
Bundle 2-2	Yes	No	Yes	No	No	0%	0%
Bundle 2-3	Yes	No	No	Yes	No	0%	0%
Bundle 2-4	Yes	No	No	No	Yes	0%	0%
Bundle 2-5	No	Yes	Yes	No	No	0.1%	0.3%
Bundle 2-6	No	Yes	No	Yes	No	0.9%	1.8%
Bundle 2-7	No	Yes	No	No	Yes	0%	0%
Bundle 2-8	No	No	Yes	Yes	No	0.7%	0.9%
Bundle 2-9	No	No	Yes	No	Yes	0.1%	0.1%
Bundle 2-10	No	No	No	Yes	Yes	0%	0%
Total Area			639,260 acres				
Total Grower			739				

[z]CC = cover crops, OFM = optimum fertilizer management, SPT = soil pH tests, SS = soil survey, TT = tissue tests.

Source: Florida Department of Agriculture and Consumer Services, Office of Agricultural Water Policy, Apr. 2019.

Table 7. Notice of intent to use three nutrient management best management practices (BMPs).[z]

BMP	SS	SPT	CC	OFM	TT	Area	Grower
Bundle 3-1	Yes	Yes	Yes	No	No	0.5%	1.4%
Bundle 3-2	Yes	Yes	No	Yes	No	1.0%	3.4%
Bundle 3-3	Yes	Yes	No	No	Yes	0.1%	0.1%
Bundle 3-4	Yes	No	Yes	Yes	No	0.1%	0.1%
Bundle 3-5	Yes	No	Yes	No	Yes	0%	0%
Bundle 3-6	Yes	No	No	Yes	Yes	0%	0%
Bundle 3-7	No	Yes	Yes	Yes	No	1.6%	3.4%
Bundle 3-8	No	Yes	Yes	No	Yes	0%	0%
Bundle 3-9	No	Yes	No	Yes	Yes	0.2%	0.3%
Bundle 3-10	No	No	Yes	Yes	Yes	0%	0%
Total Area			639,260 acres				
Total Grower			739				

[z]CC = cover crops, OFM = optimum fertilizer management, SPT = soil pH tests, SS = soil survey, TT = tissue tests.

Source: Florida Department of Agriculture and Consumer Services, Office of Agricultural Water Policy, Apr. 2019.

indicated intent to use multiple nutrient management practices, with most choosing four practices. Of these four, 14% selected SS, SPT, CC, and OFM for 6% of acres. TT selection was much lower. The intent to use SS, CC, OFM, and TT was not observed.

Conclusion

Growers intend to use multiple BMPs. Of the 739 growers in our sample, 73% selected two or more water conservation BMPs for 44% of acres and 68% selected two or more nutrient management BMPs for 46% of acres. PEC (water quality BMP) and SPT (nutrient management BMP) are the most widely selected practices of the nine practices considered in this study. One potential explanation of the high selection rate of these BMPs is higher compatibility of these practices with various crops and other characteristics of the surveyed operations.

The choice of practices to be implemented jointly varies among the growers, providing some evidence of the complementary relationship among certain BMPs. Most growers selected all four

Table 8. Notice of intent to use four nutrient management best management practices (BMPs).[z]

BMP	SS	SPT	CC	OFM	TT	Area	Grower
Bundle 4-1	Yes	Yes	Yes	Yes	No	5.7%	14.5%
Bundle 4-2	Yes	Yes	Yes	No	Yes	1.9%	7.3%
Bundle 4-3	Yes	Yes	No	Yes	Yes	2.1%	3.1%
Bundle 4-4	Yes	No	Yes	Yes	Yes	0%	0%
Bundle 4-5	No	Yes	Yes	Yes	Yes	1.9%	2.7%
Total Area			639,260 acres				
Total Growers			739				

[z]CC = cover crops, OFM = optimum fertilizer management, SPT = soil pH tests, SS = soil survey, TT = tissue tests.

Source: Florida Department of Agriculture and Consumer Services, Office of Agricultural Water Policy, Apr. 2019.

water conservation BMPs (i.e., CC, IS, ISM, and PEC). Among those growers who selected only two water conservation BMPs, CC in combination with PEC is the dominant bundle. CC, IS, ISM, and PEC seem to have a complementary relationship. For the six nutrient management BMPs considered, growers intend to use four or five practices. SPT and OFM seem to have a complementary relationship with the other nutrient management BMPs.

The NOI data used is a small sample of statewide intent. Though informative, it will be important to expand this analysis to a wider grower population. While we assume the data identifies practice selection based on the applicability to specific operations, it does not include verification information. Similar analysis of verification data is necessary to better understand drivers of conservation practice adoption. Future work will include the effect of land use, soil characteristics, weather characteristics, and other factors identified in the literature that may affect BMP adoption.

Literature Cited

Amacher, G.S. and P.M. Feather. 1997. Testing producer perceptions of jointly beneficial best management practices for improved water quality. Appl. Econ. 29(2):153–159.

Braune, M.J., and A. Wood. 1999. Best management practices applied to urban runoff quantity and quality control. Water Science and Technol. 39(12):117–121.

Chaubey, I., L. Chiang, M.W. Gitau, and S. Mohamed. 2010. Effectiveness of best management practices in improving water quality in a pasture-dominated watershed. J.Soil and Water Conservation.65(6):424–437.

Chesapeake Bay Program. 2018. Chesapeake Bay Program Quick Reference Guide for Best Management Practices (BMPs): Nonpoint Source BMPs to Reduce Nitrogen, Phosphorus and Sediment Loads to the Chesapeake Bay and its Local Waters. CBP DOC ID.12 July 2019. <https://www.chesapeakebay.net/documents/BMP-Guide_Full.pdf>

Cooper, J.C. 2003. A joint framework for analysis of agri-environmental payment programs. Amer. J. Agri. Econ. 85:976–987.

D'Arcy, B., and A. Frost. 2001. The role of best management practices in alleviating water quality problems associated with diffuse pollution. Science of the Total Environment 265(1–3):359–367.

Economic and Demographic Research (EDR). 2019. Annual assessment of Florida's water resources and conservation lands. 2019 Edition. 12 July 2019. <http://edr.state.fl.us/Content/natural-resources/LandandWaterAnnualAssessment_2019Edition.pdf>

Florida Department of Agriculture and Consumer Services (FDACS). 2019. Office of Agricultural Water Policy.12 July 2019. <https://www.freshfromflorida.com/Divisions-Offices/Agricultural-Water-Policy>

Florida Department of Agriculture and Consumer Services (FDACS). 2019b. BMP Research. 12 July 2019. https://www.freshfromflorida.com/Agriculture-Industry/Water/Agricultural-Best-Management-Practices/BMP-Research>

Houlbrooke, D.J., D.J. Horne, M.J. Hedley, J.A. Hanly, and V.O. Snow. 2003. The impact of intensive dairy farming on the leaching losses of nitrogen and phosphorus from a mole and pipe drained soil. Proc. N. Z. Grassl. Assoc. 65:179–184.

Lam, Q.D., B. Schmalz, and N. Fohrer. 2011. The impact of agricultural Best Management Practices on water quality in a North German lowland catchment. Environmental Monitoring and Assessment 183(1-4):351–379.

Manda, J., A.D. Alene, C. Gardebroek, M. Kassie, and G. Tembo. 2016. Adoption and impacts of sustainable agricultural practices on maize yields and incomes: Evidence from rural Zambia. J. Agri. Econ. 67(1):130–153.

Shrestha, R.K., L.R. Cooperband, and A.E. MacGuidwin. 2010. Strategies to reduce nitrate leaching into groundwater in potato grown in sandy soils: Case study from North Central USA. Amer. J. Potato Res. 87(3):229–244.

Strauss, P., A. Leone, M.N. Ripa, N. Turpin, J.M. Lescot, and R. Laplana. 2007. Using critical source areas for targeting cost-effective best management practices to mitigate phosphorus and sediment transfer at the watershed scale. Soil Use and Management 23:144-153.

United Nations (UN). 2018. Sustainable Development Goal 6. Synthesis Report 2018 on Water and Sanitation. New York. United Nations. 12 July 2019. <https://sustainabledevelopment.un.org/content/documents/19901SDG6_SR2018_web_3.pdf >

United Nations Educational, Scientific and Cultural Organization (UNESCO). 2001. Securing the Food Supply. World Water Assessment Programme. United Nations Education Scientific and Cultural Organization, Paris

United States Department of Agriculture (USDA). 2019. NRCS Payment Schedules. 12 July 2019. <https://www.nrcs.usda.gov/wps/portal/nrcs/detail/national/programs/financial/?cid=nrcseprd1328426>

United States Environmental Protection Agency (EPA). 2013. The importance of water to the U.S. Economy. Synthesis Report.

United States Geological Survey (USGS). 2018. Estimated use of water in the United States in 2015: U.S. Geological Survey Circular 1441:65.

United States Geological Survey (USGS). 2019. Title: Water Use Data for Florida. 4 July 2019. <https://waterdata.usgs.gov/fl/nwis/water_use>

Proc. Fla. State Hort. Soc. 132:108–110. 2019.

Improved Productivity and Economic Advantages of Advanced Cabbage Production on Plasticulture

Wendy Mussoline*[1], Bonnie Wells[1], Gary England[1], and Lincoln Zotarelli[2]

[1]*Hastings Agriculture Extension Center, Institute of Food and Agricultural Sciences, 9500 Cowpen Branch Rd., Hastings, FL 32145*

[2]*Horticultural Sciences Department, University of Florida/IFAS, P.O. Box 11690 Gainesville, FL 32611*

Additional index words. black rot, drip irrigation, 'Bravo', high density, nutrient leaching

Advanced production technologies are essential to improve marketable yields from existing agricultural lands and meet the nutritional demands of a growing human population. Cabbage production using advanced plasticulture (i.e. plastic mulch combined with drip irrigation) was compared with conventional, bare ground production in Northeast Florida. Advanced plots consisted of plastic mulch with four drills per bed with in-row spacings of 0.25 m (two outer drills) and 0.35m (two inner drills), resulting in 73,753 plants/ha. The marketable yield (58 Mg/ha) was 93% higher than bare ground planting (30 Mg/ha) and 45% higher than the statewide average in Florida (40 Mg/ha). An economic evaluation showed that additional earnings of $9,951/ha are possible using the high-density configuration with plasticulture compared to conventional methods. Environmental advantages include twice the productivity using the same land area and more efficient use of valuable inputs including nutrients and water.

Agriculture is competing with residential development projects and highway infrastructure expansion as well as facing erratic weather patterns, increasing environmental regulations, and economic imbalances. These occur at a time when input costs are continually increasing but outputs remain constant. Advanced technologies are necessary to improve production while reducing inputs and adapting to a changing climate. One advanced approach for cabbage production is high-density planting on plastic mulch, also known as plasticulture. Some of the advantages of plasticulture include increased yields, reduced water loss from evaporation, reduced nutrient leaching, less weed pressure, and extended effectiveness of pre-plant fumigant treatments in the soil (Lamont, 1993).

According to the latest USDA Census of Agriculture, 9156 acres of cabbage were harvested in Florida in 2017 compared to 7338 acres reported in 2012 (USDA, 2017). Half this production was within the Tri-County Agriculture Area (TCAA) including Putnam, Flagler and St. Johns Counties. Cabbage producers in the TCAA primarily use seepage irrigation, which requires excessive water use during dry periods, poses a risk for flooding during wet years, and results in non-uniform distribution of water across the field. Plasticulture with drip irrigation can provide a more uniform distribution of water and has been shown to increase water savings.

Several configurations of high density cabbage plantings under plastic were compared during the 2011–12 season in Hastings, and results showed that marketable yields were not significantly different when comparing three-row to four-row configurations (Barrett et al., 2015). However, when using a four-row configuration, inter-row spacing of 0.25, 0.30, and 0.35 m resulted in significantly higher yields than spacing of 0.15 and 0.20 m (Barrett et al., 2015). In the former studies, optimal N fertilization application rates for high-density cabbage plantings using plasticulture in Hastings were 400 to 570 kgN/ha (Barrett et al., 2018b); however, these are much higher than typical rates used by local cabbage producers in the TCAA.

The objectives of this project were to: 1) design an optimized cabbage spacing configuration for high density cabbage planting on plasticulture; 2) measure marketable yields associated with advanced cabbage production compared with conventional methods while maintaining fertilizer application rates consistent with local practices; and 3) evaluate and quantify the economic and environmental advantages associated with advanced cabbage production.

Materials and Methods

Cabbage planting

Field experiments were conducted using the commercial cabbage cultivar 'Bravo' grown during the 2018–19 season at the Hastings Agriculture Extension Center. Conventional planting methods used by local growers were compared with high-density, advanced plasticulture methods. Cabbage was transplanted on 17 Oct. 2018, as previous studies showed higher yields with a fall planting date (i.e. 25 Oct.) compared to a winter planting date (i.e. 6 Dec.) (Barrett et al., 2015). Conventional plots were consistent with cultural practices which include seepage irrigation, bare ground, raised beds on 1-m centers and in-row spacing of 0.2 m, resulting in a planting density of 45,555 plants/ha. Advanced plots consisted of 1.2-m-wide raised beds with black plastic mulch (1.8 m width, 1.25 mm thickness, VIF film; Polygro, LLC, Safety Harbor, FL) and two drip tapes (Aqua-Traxx model EA5081222,

*Corresponding author. Email: wmussoli@ufl.edu

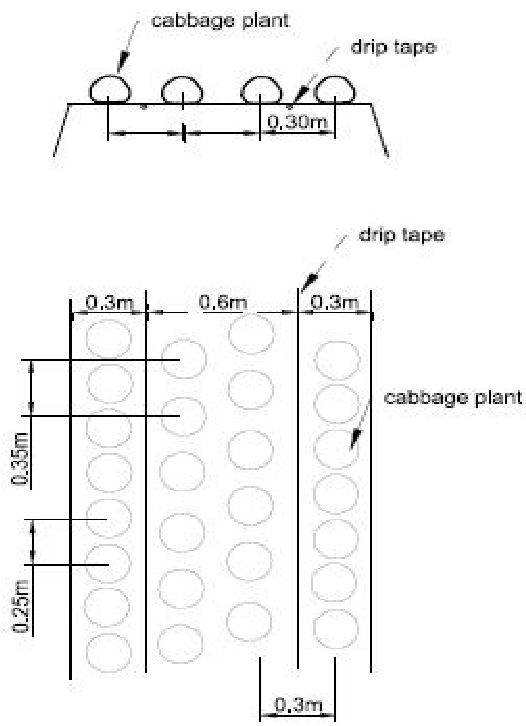

Fig. 1. Configuration of the plasticulture system used during the 2018–19 season at the Hastings Agriculture Extension Center including a transect view (top) and an aerial view (bottom). The bed spacing and in-row plant spacings are included in the aerial view.

16 mm diameter, 0.3 m emitter spacing, 0.5 $L \cdot h^{-1}$ at 55 kPa; Toro Agricultural Irrigation, El Cajon, CA). Four drills were planted in each bed, as shown in Fig. 1, with an in-row spacing of 0.25 m on the two outer drills and 0.35 m on the two inner drills resulting in 73,753 plants/ha. The goal was to maximize both planting density and marketable yield.

The fertilization scheme was consistent with cultural practices among local cabbage producers. Pre-plant fertilizer for both plots consisted of N–P–K rates (kg/ha) of 28–56–28. Three side dress applications totaling 196N–0P–196K were applied to the conventional plots in granular form, and five fertigation applications totaling 196N-0P-196K were applied to the advanced plots in liquid form. Supplemental calcium nitrate (34 kg/ha) was added to the conventional plot prior to the initial harvest.

Cabbage harvest

Cabbage harvest was initiated when the majority (>50%) of the plants from both plots reached maturity. A commercial cabbage producer from the surrounding area provided equipment, a crew and oversight for the harvest, as well as distribution and marketing of the product. The harvest equipment consisted of a Kubota M6H-101 pulling a single-axle field packing unit aligned with a flat-bed truck for loading and hauling. The 18-person crew consisted of 8 field cutters, 8 packers and 2 equipment drivers. The crew extracted marketable heads from both plots on 29 Jan. 2019 (103 days after transplanting (DAT)), 19 Feb 2019 (124 DAT) and 1 Mar. 2019 (134 DAT) and packed each box in the field.

Table 1. Marketable cabbage yield for conventional compared with high-density plasticulture.

Harvest	Days after transplanting (DAT)	Conventional (Mg/ha)	Plasticulture (Mg/ha)
1st pass	103	18.8	22.6
2nd pass	124	10.9	25.9
3rd pass	134	0	9.5
Total		29.7	58.0

Results and Discussion

Marketable yields

Marketable yields were consistently higher in the plasticulture plot on the 1st, 2nd and 3rd passes as shown in Table 1. Black rot affected the entire bare ground cabbage plot shortly after the second pass; therefore, no marketable cabbage was extracted from this plot during the third pass (134 DAT). The majority (63%) of the marketable cabbage was harvested during the 1st pass (103 DAT) for the conventional plot. The cabbage heads in the high-density plasticulture plots required multiple passes is order to size up and maximize the marketable yield. Only 39% of the total marketable yield was harvested during the 1st pass, while 45% was harvested during the 2nd pass, and 16% was harvested during the 3rd pass. The extended harvest season is an advantage for marketability while it serves the same purpose as staggered planting dates. The increase in total marketable harvest yield within the plasticulture plot is attributed to both higher density planting and less disease pressure. Cabbage planted in plasticulture had a 62% increase in planting population but a 93% increase in marketable yield per hectare. The harvest season was extended in the plasticulture plots since the crop was still marketable and black rot was not evident, even after 134 days of an excessively wet season. The total marketable yield from the current season is slightly above average (53.0 Mg/ha) that was determined by plasticulture trials conducted from 2010 to 2015 (Barrett et al., 2018a).

Economic evaluation

According to the 2019 State Agriculture Overview, average cabbage yields were 360 cwt/acre (40 Mg/ha), resulting in an overall production value of $58 million in Florida, with an average price per unit of $20/cwt or $440/Mg (USDA, 2019). The key to promoting adoption of the advanced plasticulture technology among local cabbage growers is to demonstrate the economic benefits. Although plasticulture is more expensive (36% higher costs) for advanced cabbage production, projected profits are 57% higher due to higher marketable yields (Barrett et al., 2018a). Additional costs as well as additional earnings from the 2018–19 plasticulture plots are shown in Table 2. The measured marketable yields and the latest market value for cabbage in

Table 2. Economic evaluation based on marketable cabbage yield during the 2018–19 season for conventional compared with high-density plasticulture.

	Conventional ($/ha)	Plasticulture ($/ha)
Extra transplants	0	–918
Drip tape + fertigation	0	–1,084
Plastic mulch + labor	0	–716
Supplemental fertilizer	–217	0
Gross earnings from yield	13,068	25,520
Total gross earnings	$12,851/ha	$22,802/ha

Florida ($20/cwt or $440/Mg) according to USDA were used to evaluate economic benefits. The economic analysis based on actual yields and market price shows that an additional $9951/ha was earned using the high density, four-drill configuration with plasticulture compared to conventional methods.

Environmental considerations

The primary benefit is the increased productivity for cabbage production. When using advanced plasticulture, the same amount of land could be used to produce nearly twice the yield. From a regulatory perspective, the system inherently reduces nutrient leaching and requires less fertilizer compared with bare ground. A total of 34 kg/ha of N was saved using the plasticulture compared with bare ground. Drip irrigation combined with plastic mulch is more efficient compared to conventional seepage irrigation methods and water savings are clearly documented in former studies. Cabbage production with plasticulture provides more efficient crop moisture distribution and water savings ranging from 11 to 33%, as demonstrated in the Hastings trials during the 2012-2013 and 2013-2014 growing seasons (Barrett et al., 2018b). Results from three consecutive years of data collection in India showed that cabbage yields increased an average of 62% when using drip irrigation when compared to seepage, and yields increased an average of 70% when using drip plus plastic mulch when compared to seepage (Tiwari et al., 2003).

Conclusion

Cabbage production using advanced plasticulture with a configuration that maximizes both planting density and marketable yield has been demonstrated. The yield (58 Mg/ha) was 93% higher than bare ground planting (30 Mg/ha) and 45% higher than the statewide average in Florida (40 Mg/ha). Based on market prices in 2018, additional earnings of $9951/ha are possible using the high density, four-drill configuration with plasticulture compared to conventional methods. Environmental advantages include twice the productivity using the same land area and more efficient use of valuable inputs including nutrients and water. The primary challenge is with grower adoption of advanced technologies since it will require modification of planting configurations and additional equipment and labor to install.

Literature Cited

Barrett, C.E., L. Zotarelli, L.G. Paranhos, P. Dittmar, C.W. Fraisse, and J. Vansickle. 2018a. Economic feasibility of converting from a bare ground system with seepage irrigation to plasticulture for cabbage production: Where is the risk? HortScience 53:875–881.

Barrett, C.E., L. Zotarelli, L.G. Paranhos, P. Dittmar, C.W. Fraisse, and J. Vansickle. 2018b. Optimization of irrigation and N-fertilizer strategies for cabbage plasticulture system. Sci. Hort. 234:323–334.

Barrett, C.E., L. Zotarelli, L.G. Paranhos, B.S. Taylor, P. Dittmar, C.W. Fraisse, and J. Vansickle. 2015. Optimum planting configuration for high population plasticulture grown cabbage. HortScience. 50:1472–1478.

Lamont, Jr., W.J., 1993. Plastic mulches for the production of vegetable crops. HortTechnology 3:35–39.

Tiwari, K. N., A. Singh, and P. K. Mal. 2003. Effect of drip irrigation on yield of cabbage (*Brassica oleracea* L. var. *capitata*) under mulch and non-mulch conditions. Agric. Water Mgt. 58:19–28.

USDA 2017. Agriculture Census for Florida: Table 36. 25 Mar. 2020. <https://www.nass.usda.gov/Publications/AgCensus/2017/Full_Report/Volume_1,_Chapter_1_State_Level/Florida/st12_1_0036_0036.pdf>

USDA 2019. State Agriculture Overview. 25 Mar. 2020. <https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=FLORIDA>

Proc. Fla. State Hort. Soc. 132:111–114. 2019.

Northeast Florida Artichoke Demonstration

Gary K. England*[1] and Shinsuke Agehara[2]

[1]*University of Florida/IFAS, Hastings Agricultural Extension Center, P.O. Box 728, Hastings, FL 32145-0728*

[2]*University of Florida/IFAS, Gulf Coast Research and Education Center, 14625 CR 672, Wimauma, FL 33598*

Additional index words. buds, fertigation, mulch

Historically, commercial growers in the Tri-County Agricultural Area (TCAA) encompassing Flagler, Putnam and St. Johns Counties have focused on the production of two key crops, potatoes and cabbage. These growers, especially producers of potatoes for the chip manufacturing segment, have had increasing challenges in marketing their crops. During the past decade, many of these "chip potato" producers have shifted to growing potato cultivars intended for the fresh market. Seeing no obvious expansion of marketing opportunities for either potato or cabbage production, TCAA growers have inquired about alternative crops they could evaluate for inclusion into their market offerings. Based upon the work of Shinsuke Agehara at the University of Florida, IFAS (UF/IFAS) Gulf Coast Research and Education Center, it was decided that artichoke would be a good crop to evaluate in the TCAA. A small planting was established in Fall 2016 at the UF/IFAS Hastings Agricultural Extension Center (HAEC), but production problems limited any usable data. A demonstration planting of three cultivars (Imperial Star, Colorado Red Star, and Green Globe Improved) was initiated in November 2017 and cumulative yields of 10,033, 6298 and 8021 lb/acre, respectively, were documented from weekly harvests beginning on 14 March and ending on 2 May 2018. Another demonstration comprised of the same cultivars plus Opal was established at HAEC in November 2018. Other factors included in this trial are comparisons of controlled release fertilizer versus fertigation and in-row plant spacing. Harvest of this demonstration resulted in cumulative yields of 11,260, 4922, and 3858 lb/acre for 'Imperial Star', 'Colorado Red Star', and 'Green Globe Improved', respectively.

The Tri-County Agricultural Area (TCAA), comprised of Flagler, Putnam and St. Johns County in northeast Florida, has a history of being a significant producer of commercial vegetable crops for over 130 years. During this time, potato and cabbage have been the major crops produced. In the past 20 years, some growers have diversified their offerings by growing other vegetable crops such as broccoli and Asian mixed vegetables.

Competition from other production regions has resulted in significant reduction in marketing opportunities for TCAA growers producing potato and to some extent cabbage. These growers have asked UF/IFAS Extension Faculty working in the TCAA to gather information on alternative crops to replace the void created by diminished marketing opportunities for "traditional" crops produced within the region.

Other *Brassica* crops such as cauliflower, Brussels sprouts and some of the Asian mixed vegetable selection have begun to be produced in the TCAA and UF/IFAS Faculty have initiated Extension demonstration and research projects focused on the potential viability and profitability of adding such crops to the list of offerings. Other crops under evaluation in the region are sweet potato, citrus and sweet corn. Upon learning about the work of Shinsuke Agehara at the UF/IFAS Gulf Coast Research and Education Center (GCREC) with Artichoke, the UF/IFAS Hastings Agricultural Extension Center (HAEC) faculty and staff decided artichoke is worth evaluating as a potential alternative crop for commercial vegetable producers in the TCAA.

Methods and Materials

In Fall 2016, an Extension demonstration focused on three cultivars of artichoke already being studied at GCREC was attempted. Challenges with transplant and field production resulted in little useful information being generated, other than identifying several practices that needed to be improved.

2017–2018

In Fall 2017, at the HAEC Cowpen Branch Rd. Extension Demonstration and Research Farm (farm) a 61 lb/acre rate of Telone II nematicide was applied to Bed 318. After the 14-day post-application waiting period, a 14–6–12 granular fertilizer at a rate of 1500 lb/acre [210 lb nitrogen (N), 90 lb phosphorus (P), 180 lb potassium (K)] was incorporated into a 400-foot row shaped to a height of 6 inches and width of 48 inches. A roll of 72-inch wide black plastic mulch and a line of pressure compensating drip irrigation tape with 8-inch emitter spacing was applied to the row.

On 7 Nov. 7 2017, six to eight-week-old transplants of three artichoke cultivars [Colorado Red Star (CRS), Green Globe Improved (GGI), and Imperial Star (IS)] grown in the greenhouse at GCREC were planted in a single row on 3 ft centers. The plants were watered in and additional irrigation was provided with a drip irrigation system on an as-needed basis.

*Corresponding author. Email: gke@ufl.edu

Once most of the plants were in 4–6 true leaf stage, an application of ProGibb at 20 ppm active was applied on 13 Jan. 2018 in a sprayer calibrated to apply 32 gallons per acre. A second application of the same rate and spray volume of ProGibb was made on 23 Jan. 2018. No pest management product applications were made on this planting.

When significant numbers of the artichoke buds began to mature, 8 weekly harvests were initiated on 14 Mar., ending on 2 May 2018. To determine any size differences between cultivars, total number and weight of mature artichoke buds were measured and recorded in four size designations; 2.25-, 2.75-, 3.25-, and 3.5-inch diameter buds. These data were converted to weight per acre for the total harvest period per cultivar.

2018–2019

In Fall 2018, seeds of three artichoke cultivars (CRS, GGI and IS) were sewn at the HAEC farm greenhouse. A 61 lb/acre rate of Telone II nematicide was applied to Bed 306 and after the 14 day post-application waiting period, a 16–6–11 four-month controlled release (CR) granular fertilizer at a rate of 1250 lb/acre (200 lb N, 75 lb P, 138 lb K) was incorporated into three 400 foot rows shaped to a height of 6 inches and width of 48 inches and set on 6 ft centers (controlled release rows). A 4–8–4 granular fertilizer at a rate of 625 lb/acre (25 lb N, 50 lb P, 25 lb K) was incorporated into three similar 400-ft rows (fertigation rows). In the controlled release rows, 72-inch-wide black plastic mulch and a line of pressure compensating drip irrigation tape with 8-inch emitter spacing was applied to each row. In the fertigation rows, 72-inch-wide black plastic mulch and two lines of pressure compensating drip irrigation tape with 8-inch emitter spacing was applied to each row to increase the distribution of nutrients applied with each fertigation event. Timing of any irrigation cycles were adjusted to apply the same total water volume to all six rows in the demonstration bed.

The initial design of this demonstration block had 5 plots of the three cultivars in one row × 24 plants (3 ft. in-row plant spacing, 72 ft plot length) in each of the fertilizer regimes. Due to mechanical problems in the farm greenhouse exacerbated by extremely hot and dry weather there were not enough transplants to plant the plots as designed.On 19 Nov. 2018, available six to eight-week-old transplants of two artichoke cultivars (GGI and IS) were planted. On 29. Nov. 2018, two plots per fertilizer treatment of CRS, Opal and additional GGI and IS transplants from the greenhouse at GCREC were planted to complete the demonstration.

Once most of the plants were in 4–6 true leaf stage, ProGibb at 20 ppm active was applied on 22 Jan. 2019 in a sprayer calibrated to apply 32 gallons per acre. A second application of the same rate and spray volume of ProGibb was made on 31 Jan. 2019. During the season, two pyrethroid and one neoniconitoid insecticide applications were applied following initial bud formation to manage leafooted bugs and stink bugs. To mimic the release period of the CR fertilizer, 11 equal applications of a liquid 8–0–8 applied through the drip irrigation system began on 14 Dec. 2018 and ended 1 Mar. 2019 applying an additional 175 lb/acre each of N and K in the fertigation area.

When significant numbers of artichoke buds began to mature, 10 harvests were initiated on 21 Mar. and ending on 10 May 2019. Harvests were completed on 10 representative plants in each plot. To determine any size differences between cultivars, total number and weight of mature artichoke buds were measured and recorded in four size designations; 2.25-, 2.75-, 3.25-, and 3.5-inch diameter buds.

During the term of the demonstration, quality of plants varied significantly between plots of GGI and IS. For this reason, the two most uniform plots for each fertility regime for these two cultivars were selected for comparisons. All four CRS plots appeared uniform and were also utilized for varietal comparison. Potential statistical differences between fertilizer regimes and cultivars was evaluated using Excel ANOVA.

Results and Discussion

2017–2018

All three cultivars progressed through the season and had significant amounts of artichoke buds to harvest beginning on 14 Mar. 2018. There was one significant freeze event during the demonstration where low temperatures dipped into the 20s °F with more than 20 h at or below freezing and significant frost formed on two consecutive mornings (Fig. 1). No damage was observed in the HAEC planting, but the same weather system caused some damage at the GCREC trials during a freeze event there (Agehara, personal communication, 2018). It is possible that the more northerly location of the HAEC demonstration resulted in the plants being somewhat more acclimated to cooler temperatures.

All three artichoke cultivars in the demonstration planting produced well, although there were significant differences in total yield and size distribution. IS had the highest weight yield per acre at 10,033 lb followed by GGI and CRS at 8021 and 6298 lb respectively (Fig. 2). GGI had nearly 40% of harvest weight yield in the upper two size cultivars followed by IS and CRS at 23.5% and 12.5% respectively.

2018–2019

When comparing differences between the CR fertilizer and fertigation regimes, the analysis of variance (ANOVA) indicated P-values of 0.40, 0.63, and 0.73 for CRS, GGI, and IS, respectively. Since there were no significant differences between

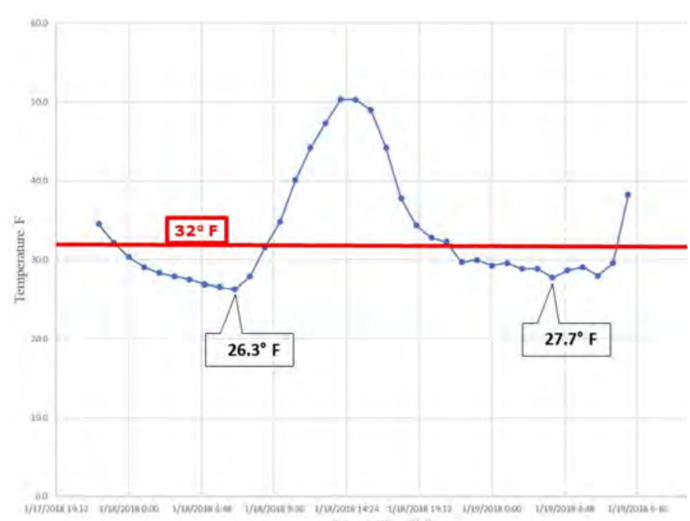

Fig. 1. UF/IFAS Hastings Agricultural Extension Center FAWN Temperatures (2 ft) 18–Jan. 2018.

Cultivar	Harvest Date	21/4		23/4		31/4		31/2	
		Number	Weight	Number	Weight	Number	Weight	Number	Weight
Colorado Red Star	3/14/18	23	5.3	6	2.9	8	5.2	4	3
	3/21/18	36	10.7	13	5.45				
	3/27/18	30	7.4	4	1.55				
	4/4/18	46	9.95	66	25.65	11	6.05	1	0.8
	4/11/18	174	26.1	26	8.9	7	3.21	1	0.8
	4/17/18	84	13.95	22	5.8	7	2.65		
	4/24/18	62	24.1	29	7.6	2	0.75	2	0.9
	5/2/18	50	2.88	15	1.6	2	0.36		
Grand Total LBS./Acre	Running Total:	505	100.38	181	59.45	37	18.22	6	4.6
6,298									
			55.0%		32.5%		10.0%		2.5%
		21/4		23/4		31/4		31/2	
		Number	Weight	Number	Weight	Number	Weight	Number	Weight
Green Globe Improved	3/14/18	16	3.7	9	3.15	8	4.05	2	1.5
	3/21/18	18	5.1	5	1.95			1	0.6
	3/27/18	15	4	27	9.7	3	1.3	2	1.05
	4/4/18	27	6.4	41	13.35	19	8.75	16	8.55
	4/11/18	10	2.2	57	16.15	56	22.42	4	2.4
	4/17/18	39	6.7	53	14.95	18	7.05	5	2.55
	4/24/18	39	5.95	40	9.45	25	8.5		
	5/2/18	20	1.46	24	2.6	6	0.94		
Grand Total LBS./Acre	Running Total:	184	35.51	256	71.3	135	53.01	30	16.65
8,021									
			20.1%		40.4%		30.0%		9.4%
	Harvest Date	21/4		23/4		31/4		31/2	
Imperial Star	3/14/18	9	2.35	6	3.15	16	9.05	4	2.6
	3/21/18	6	1.65	59	20				
	3/27/18	12	2.95	12	4.35	5	2.9		
	4/4/18	20	4.55	114	36.05	1	0.4	2	1
	4/11/18	20	3.55	52	14.7	26	11.1	4	2.2
	4/17/18	58	10.6	29	7.35	3	1.1		
	4/24/18	37	6	75	17.5	25	7.45	6	3.3
	5/2/18	20	1.36	20	2.18	6	0.98	1	0.22
Grand Total LBS./Acre	Running Total:	182	33.01	367	105.28	82	32.98	17	9.32
10,033									
			18.3%		58.3%		18.3%		5.2%

Fig. 2. 2018 UF/IFAS Hastings Agricultural Extension Center artichoke demonstration harvest.

Fig. 3. Analysis of variance (ANOVA) table comparing total weight yield of three artichoke cultivars.

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
CRS	4	80.7167	20.17917	7.624747		
GG	4	63.27837	15.81959	9.539137		
IS	4	184.6625	46.16563	47.87971		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2153.579	2	1076.789	49.66467	1.3732E-05	4.256495
Within Groups	195.1308	9	21.6812			
Total	2348.71	11				

CRS = 'Colorado Red Star', GGI = 'Green Globe Improved', and IS = 'Imperial Star'.

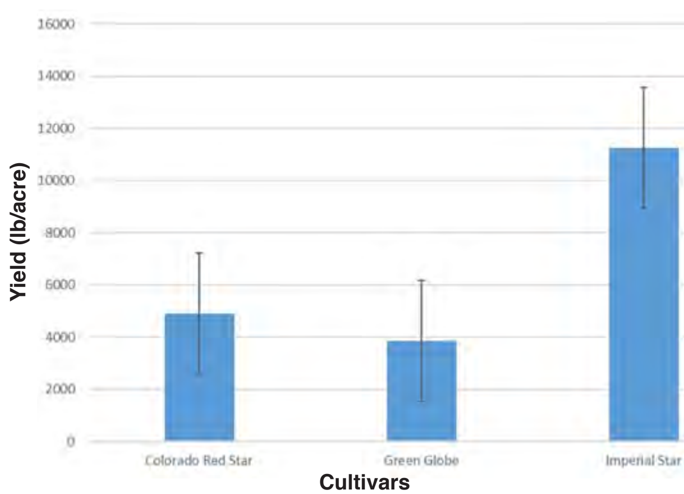

Fig. 4. 2019 artichoke yield comparison.

fertilizer regimes, all four selected plots for each of the three cultivars were compared with ANOVA for total yield converted to lbs/acre. The results of the ANOVA (Fig. 3) indicates a significant difference between yields of the cultivars and those differences are depicted in Fig. 4.

To depict number and total weight for each size category at each harvest date for each of the three cultivars, data from the four plots was averaged and placed in Fig. 5. IS had the highest weight yield per acre at 11,260 lb followed by CRS and GGI at 4922 and 3858 lb, respectively. IS had a slightly higher total weight yield per acre than in 2018, while CRS and GGI were significantly lower. This suggests that from season to season, IS may be less dependent on chill accumulation, GA application or other factors.

GGI had nearly 37% of harvest weight yield in the upper two size cultivars followed by IS and CRS at 32.5% and 27.6%, respectively. Both CRS and IS had increased yield percentages in the upper two categories versus the previous year.

Cultivar	Harvest Date	21/4		23/4		31/4		31/2	
		Number	Weight	Number	Weight	Number	Weight	Number	Weight
Colorado Red Star	3/21/19	4	0.5	2	0.4	4	1.1	0	0.0
	3/28/19	2	0.3	6	1.9	1	0.4	2	1.0
	4/2/19	6	1.1	1	0.4	0	0.0	0	0.0
	4/9/19	5	0.9	8	2.6	0	0.0	0	0.0
	4/12/19	0	0.0	0	0.0	0	0.0	0	0.0
	4/16/19	12	2.3	6	1.9	12	4.0	0	0.0
	4/22/19	16	3.6	10	3.0	1	0.4	1	0.5
	4/29/19	31	5.9	20	5.6	12	4.7	1	0.6
	5/8/19	21	3.6	43	10.2	2	0.7	2	1.3
	5/10/19	25	3.7	41	10.7	17	5.5	5	2.3
Grand Total LBS./Acre	Running Total:	76	14.54	53	15.62	30	10.51	2	1
4,922									
			34.9%		37.5%		25.2%		2.4%
		21/4		23/4		31/4		31/2	
		Number	Weight	Number	Weight	Number	Weight	Number	Weight
Green Globe Improved	3/21/19	3	0.3	0	0.0	0	0.0	0	0.0
	3/28/19	2	0.4	0	0.0	1	0.4	0	0.0
	4/2/19	7	1.6	10	2.3	2	0.5	0	0.0
	4/9/19	3	0.6	17	4.7	0	0.0	1	0.6
	4/12/19	0	0.0	0	0.0	0	0.0	0	0.0
	4/16/19	10	2.1	4	1.4	16	5.5	2	0.8
	4/22/19	27	5.5	6	1.5	12	4.3	0	0.0
	4/29/19	20	4.0	22	5.9	15	5.5	0	0.0
	5/8/19	10	1.8	12	3.0	2	0.7	0	0.0
	5/10/19	24	5.2	11	3.1	5	2.0	0	0.0
Grand Total LBS./Acre	Running Total:	72	14.32	59	15.7	46	16.2	3	1.35
3,858									
			30.1%		33.0%		34.1%		2.8%
	Harvest Date	21/4		23/4		31/4		31/2	
Imperial Star	3/21/19	8	0.9	20	3.1	3	0.6	0	0.0
	3/28/19	14	4.3	23	5.9	1	0.3	1	0.7
	4/2/19	16	3.8	17	5.4	8	3.2	1	0.5
	4/9/19	2	0.4	42	10.6	4	1.4	4	2.0
	4/12/19	30	5.3	46	10.7	3	0.9	1	0.4
	4/16/19	9	1.9	12	2.9	2	0.7	1	0.5
	4/22/19	23	4.6	19	4.6	19	5.4	1	0.0
	4/29/19	49	10.4	77	19.7	71	23.7	12	5.4
	5/8/19	2	3.3	51	9.7	1	0.4	2	1.4
	5/10/19	36	6.0	60	14.8	24	8.2	3	1.5
Grand Total LBS./Acre	Running Total:	151	31.38	256	62.78	111	36.112	20.8	9.25
11,260									
			22.5%		45.0%		25.9%		6.6%

Fig. 5. 2019 UF/IFAS Hastings Agricultural Extension Center artichoke demonstration harvest.

Conclusion

In two seasons, results from demonstrations at the HAEC farm indicated that three artichoke cultivars produced fair to excellent yields. These results justify further investigation of artichoke as a potential alternative crop for producers in the TCAA to consider as replacement for reduced demand for their core crops of potato and cabbage.

Several local growers observed the HAEC artichoke demonstrations and at least two are considering small pilot plantings on their farms. Future work on this crop should look closer at fertilizer and gibberellic acid management programs to enhance production. Additionally, other UF/IFAS Extension Specialists and Agents should be consulted to determine best practices for postharvest handling and marketing of this crop.

Proc. Fla. State Hort. Soc. 132:115–116. 2019.

Florida Central and South Florida Small Farms Hydroponic Program

Francisco Rivera*[1], Jonael Bosques[2], E. Vanessa Campoverde[3], German Sandoya[4], Lorna Bravo[5], and Jiangxiau Qiu[6]

[1]*Hillsborough County Extension, University of Florida/IFAS, 5339 County Rd. 579, Seffner, FL 33584*

[2]*Hardee County Extension, University of Florida/IFAS, 507 Civic Center Dr., Wauchula, FL 33873*

[3]*Miami-Dade County Extension, University of Florida/IFAS, 18710 SW 288th St., Homestead, FL 33030*

[4]*Everglades Research & Education Center, 3200 Palm Beach Rd., Belle Glade, FL 33430*

[5]*Broward County Extension, University of Florida/IFAS, 3245 College Ave., Davie, FL 33314*

[6]*Fort Lauderdale Research and Education Center, University of Florida/IFAS, 3205 College Ave., Davie, FL 33314*

Additional index words. indoor farming, hydroponics, leafy vegetables, urban agriculture

It is essential that small and beginning farmers identify niche markets, develop sale strategies, and take corrective measures to achieve a sustainable operation. Both groups can benefit greatly from hands-on learning experiences including basic use of equipment. Producing crops using hydroponic systems is an alternative that may yield high quality profitable specialty crops such as herbs and greens, as well as cut flowers in places that are unsuited for extensive agricultural operations. University of Florida, Institute of Food and Agricultural Sciences Extension faculty provided training for 457 people during 2017–18 across six Florida counties: Alachua, Broward, Hardee, Hillsborough, Miami-Dade, and Osceola. Three hundred ninety-three (393) participants responded to program evaluation surveys that showed knowledge gain in the areas of hydroponic growing systems (48%; n = 189), hydroponic growing media (20%; n = 79) and nutrient solution management (25%; n = 98). Overall, 91% (n = 358) of participants plan to implement the information received in these workshops. Participants also reported increased confidence in answering questions and locating online science-based information about hydroponic production. Fifty-eight percent (n = 228) were inspired to build hydroponic systems with floating beds (63%; n = 248) preferred over nutrient film technique (37%; n = 145) systems. Due to this growing interest, faculty expanded hydroponic outreach workshops and implemented strategies to develop a regional initiative for small and beginning farmers, homeowners, students, small business entrepreneurs, and Master Gardeners in central and south Florida.

The State of Florida's current population is 21.6 million with an estimated growth rate of 1.6% (30,000 people) per year (United States Census Bureau, 2019). This represents an increase in consumer demands for housing, food, and industrial development that threatens the availability of land for agriculture. Small and beginning farmers are exploring cost-effective ways to enter and/or remain in agriculture due to challenges such as increasing land costs and availability.

Beginning, small, and specialty farmers also need to learn how to identify niche markets, develop sales strategies, and make any needed changes to their operations so they can become sustainable, including using natural resources more efficiently. There is also an increase in protected horticulture operations such as greenhouses, high tunnels, and vertical farms, some of which are in urban or peri-urban areas. All these operations use methods for producing crops for self or local consumption that require less space while staying profitable on a long-term basis.

Hydroponic production systems are an alternative that can yield high quality profitable specialty crops such as herbs, greens, and cut flowers in urban and peri-urban settings that are not suited for other kinds of agricultural production. Hydroponic farming also has the potential to increase per square foot production capacity while conserving significant amounts of water compared to traditional growing methods (Barbosa et. al., 2015).

County Extension Agents and State specialists in several disciplines developed a program in five counties in Central and South Florida called the "Urban Agriculture Initiative for Florida." A main component is the use of hydroponics for urban agriculture. Educational programs about hydroponic systems were developed to teach small and beginning farmers as well as homeowners basic concepts of and differences between the kinds of protected culture and their viability as production systems.

*Corresponding author. Email: friveramelendez@ufl.edu

Materials and Methods

Cooperative Extension agents from the University of Florida/ Institute of Food and Agricultural Sciences (UF/IFAS) from Broward, Hardee, Hillsborough, Palm Beach, and Miami-Dade counties were involved in a pilot hydroponic production program.

Workshops topics included the following: basic knowledge of hydroponics; nutrient solution management; building hydroponic systems in protected structures; and integrated pest management (IPM). Participants received hands-on experience on building the following systems: flood and drain system (a.k.a. ebb and flow), nutrient film technique (NFT), aeroponics, and vertical gardening. They also learned: how to select an optimum growing medium; about nutrient solution issues (use of pH and/or electrical conductivity meters and fertilizer blends); seed germination; and plant monitoring. Since its implementation, the program has been offered in six counties (Alachua, Broward, Hardee, Hillsborough, Miami-Dade, and Osceola).

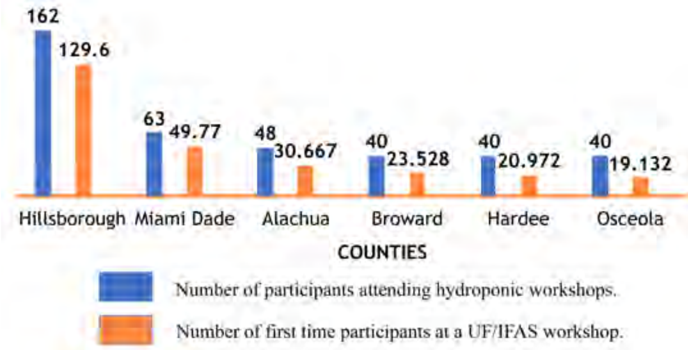

Fig. 1. Number of small farms hydroponic program participants by county in Central and South Florida (n = 393).

Results

A total of 393 people [by county: Hillsborough (162), Miami-Dade (63), Alachua (48), Broward (40), Hardee (40), and Osceola (40)] attended a one-day workshop in one of six counties in Central and South Florida. Sixty-nine percent (69%) of participants indicated that this was the first time they had attended a hydroponic workshop (Fig. 1).

The initial assessment showed that participants were mostly homeowners (84%), with the rest farmers (11%), and students (5%). The homeowner–student group who expressed an interest in becoming growers in urban and peri-urban settings have the potential to help increase food production in their communities.

Of the 393 participants, 306 (78%) are willing to use what they learned in their farm or at home; at present 286 (93% of these 306) do not currently have a hydroponic system, with 277 (97% of those without systems) willing to build a hydroponic system. The preferred systems are floating bed [81% (247/306)] and NFT [47% (145/306)] compared to systems such as aeroponics and flood and drain.

Implications. Science-based educational materials and experiences for small and beginning farmers is needed to successfully start a new operation those with little to no experience. Homeowner and student participation in these workshops show that Floridians are interested in grow their own food, especially in urban settings.

Conclusion

Based on results of this initiative, hydroponic workshops should be implemented in more counties across the state, reaching small and beginning farmers in rural and urban areas. This audience will likely also include homeowners, students, and small business entrepreneurs. This initiative has the potential to reduce food deserts in Florida.

Literature Cited

Barbosa, G.L., F.D.A. Gadelha, N. Kublik, A. Proctor, L. Reichelm, E. Weissinger, G.M. Wohlleb, and R.U. Halden. 2015. Comparison of land, water, and energy requirements of lettuce grown using hydroponic vs. conventional agricultural methods. Intl. J. Environ. Res. Public Health, 12(6):6879–6891.

United States Census Bureau. 2019. Quick facts Florida. <https://www.census.gov/quickfacts/fact/table/FL,US>

Proc. Fla. State Hort. Soc. 132:117–119. 2019.

Seed Piece Spacing for Table Stock Potatoes

Luis E. Gomez-Pesantes[1], Lincoln Zotarelli*[1], Mario H.M.L. Andrade[1], and Gary K. England[2]

[1]*Horticultural Sciences Department, University of Florida/IFAS, P.O. Box 110690 Gainesville, FL 32611*

[2]*Hastings Agricultural Extension Center, University of Florida/IFAS, Hastings, FL 32145*

Additional index words. potato cultivar; tuber yield; tuber size distribution

In Florida potato commercial farm operations, the seed represents 18 to 34% of the total production cost of producing potato (*Solanum tuberosum*). Growers can reduce costs and improve revenues by modifying seed piece spacing without affecting potato marketable yield and tuber quality. The objective of this study was to evaluate the effect of seed piece spacing on total and marketable yield, tuber size distribution of six table stock cultivars (Red LaSoda, Satina, Natascha, Envol, Purple Majesty, and Actrice) at four different seed piece spacing (6, 8, 10, and 12-inches) in a randomized complete–block design. A field experiment was carried out between January and May 2018 in Hasting, FL. Seed piece spacing and cultivars affected total and marketable yields, but there was no interaction between seed piece spacing and cultivar. The 8–inch seed piece spacing resulted in a higher total (289 cwt/acre) and was statistically different than the other seed piece spacing treatments. 'Red LaSoda' had the highest marketable yield (223 cwt/acre), while 'Purple Majesty' had the lowest marketable yield of tubers larger than 1.8-inch diameter but produced higher yield of B and C tuber size (1.9–0.5-inch diameter). Therefore, seed piece spacing for each cultivar can be adjusted by growers to meet market preferences and/or to receive premium prices different tuber size class.

In 2017, Florida contributed with 7.17 million hundred weight (cwt) of potatoes cultivated in 29,000 acres. Ranking thirteenth in U.S production and valued in $86.6 million (USDA, 2018). A total of 20,000 acres of potato are cultivated in Northeast Florida, representing 68% of Florida total cultivated potato area (USDA, 2018). In Northeast Florida commercial potato operations, seed costs account for 18 to 34% of total production costs. Seed costs are determined by the total amount of seed needed per acre. Therefore, growers can reduce seed costs and improve revenue by adjusting seed piece spacing without affecting potato marketable yield and tuber quality (Krupek et al., 2018). Currently, the standard potato seed piece spacing for farming operations in Florida is between 7–10 inches (Zotarelli et al., 2017) however, this range does not consider the cultivar. Potato cultivars respond differently to seed piece spacing for total and marketable yield, tuber size distribution and quality. Krupek et al. (2016) reported that total and marketable yield increases when reducing seed piece spacing. In contrast, wider seed piece spacing increases total and marketable yield. Potato cultivar performance may vary when combining different within-row spacings and growing area environmental conditions (Magnani et al., 2015). The objective of this study was to evaluate the effect of seed piece spacing on total and marketable yield, tuber size distribution and tuber external and internal quality of six table stock cultivars (Red LaSoda, Satina, Natascha, Envol, Purple Majesty, and Actrice) at four different seed piece spacings (6, 8, 10, and 12 inches within-rows) in northeast Florida.

*Corresponding author. Email: lzota@ufl.edu

Materials and Methods

A field trial was conducted during the Spring 2018 potato season (January–May) at the University of Florida, Hastings Agricultural Extension Center Research Farm located in Hastings, FL. The experiment was established in a randomized complete block design with two factors, cultivar and seed piece spacing, and replicated four times. The trial was planted on 9 Feb. 2018 and harvested when the plants reached 96 days after planting (DAP) on 16 May 2018. Aboveground biomass samples were collected throughout the season at 40 and 72 DAP. Tubers were mechanically harvested and total and marketable yield, tuber size distribution, specific gravity, and internal and external quality were evaluated. Tubers were graded and classified into categories according to USDA standards (USDA, 1997). The data were analyzed using the PROC MIXED procedures of SAS version 9.4 (SAS Institute, Cary, NC). An analysis of variance (ANOVA) was used to determine seed piece spacing and cultivar main effects and interactions. For means separation, Least Square means (LS-means) comparison was applied with the Holm-Tukey adjusted at a P value of 0.05.

Results and Discussion

Aboveground biomass accumulation measured at 40 DAP responded to seed piece spacing. There was greater aboveground biomass accumulation with 6-inch seed spacing (915 lb/acre) compared to 12 inches (706 lb/acre). At 72 DAP, the 6 inch seed piece spacing resulted in 37% greater aboveground biomass compared to the 12-inch seed spacing biomass increased by 56%.

Total yield was significantly affected by both seed spacing (P = 0.035) and cultivar (P = 0.045) (Table 1). There were no

Table 1. Effects of seed piece spacing and cultivar on total, marketable yield and tuber size distribution.

Planting spacing (inches)	Tuber yield Total	Tuber yield Marketable	Tuber size distribution[z] A1	A2	A3	B	C
	---- cwt/acre ----						
6	268.9 AB[y]	178.7	177.5 AB	6.66	–	65.1 A	18.47 A
8	289.4 A	210.4	204.5 A	10.43	–	54.5 B	15.55AB
10	238.9 B	169.2	166.9 B	8.89	–	48.8 B	12.39 B
12	240.2 B	172.0	168.8 B	8.92	–	45.9 B	11.85 B
Cultivars	---- cwt/acre ----						
Actrice	278.0 AB	213.0 AB	221.5 A	2.7 BC	–	43.4 C	10.3 C
Envol	264.7 ABC	215.7 A	212.0 AB	8.4 B	–	36.0CD	7.4 C
Natascha	290.7 A	185.3 B	182.5 B	2.4 BC	–	86.4 B	18.9 B
Purple Majesty	218.2 C	75.8 C	75.8 C	0.0 C	–	103.8 A	38.5 A
Red LaSoda	265.8 ABC	223.5 A	202.4 AB	20.4 A	–	25.1 D	6.2 C
Satina	238.8 BC	193.6 AB	182.3 B	18.5 A	–	27.3 D	6.1 C
Spacing vs. cultivar	NS	NS	NS	NS	***	NS	NS

[z]Tuber size distribution: A4 (> 4.0 inches), A3 (3.3–4.0 inches), A2 (2.5–3.3 inches), A1 (1.9–2.5 inches), B (1.5–1.9 inches), C (0.5-1.5 inches)
[y]Mean values within a column followed by same letters are not statistically different at $P < 0.05$ according to the Holm Tukey test. Absence of letter means not significant.
*** = Significant at $P < 0.001$, NS = nonsignificant.

significant differences between 6–8 inch seed piece spacing treatments. However, the average total yield of 6 and 8 inches seed piece spacing was significantly higher (14%) than the average from the 10- and 12-inch spacings for total yield. Marketable yield was significantly affected by cultivar ($P = < 0.001$). 'Red LaSoda' and 'Envol' procuded 223.5 and 215.7 cwt/ac, respectively which was significantly higher than 'Natascha' (185.3 cwt/acre) and 'Purple Majesty' (75.8 cwt/acre) (Table 1). Overall, seed piece spacings of 6 and 8 inches resulted in 23% higher marketable yield than the 10- and 12-inch spacings.

Tuber size distribution was significantly affected by seed piece spacing (Fig. 1). There was an increase of smaller tuber sizes (C and B's) when reducing seed spacing from 10 and 12 inches to 6 inches. The amount (weight) of A1 (1.9- to 2.5-inch) tubers at the 10- and 12-inch seed piece spacings was significantly lower compared to the 6- and 8-inch spacings (Fig. 1). The A2 (2.5–3.3") tuber sizes class did not responded to seed piece spacing treatments, and represented 3% of total yield (Fig 1).

Cultivar affected tuber size distribution (Fig. 2). 'Purple Majesty' had a smaller overall tuber size profile than the other varieties with 65% (142 cwt/ac) of the tubers classified as C (0.5–1.5") and B's (1.5-1.9"); the remaining 45% (75.8 cwt/ac) was classified as A1 (1.9-2.5"). Overall 'Actrice', 'Envol', 'Satina', and 'Red LaSoda' produced larger tuber sizes. For these cultivars, the A1 (1.9–2.5") tuber size represented 76 to 80% of total yield though 'Red LaSoda' and 'Satina' produced significantly higher amount of A2 (2.5–3.3") tubers (Fig. 2) compared to the other cultivars.

For tuber external quality, there was a higher presence of tuber greening (1.1%) at the 12-inch seed piece spacing. The incidence of rotten tubers was greater than 3% for 'Natascha', 'Red LaSoda',

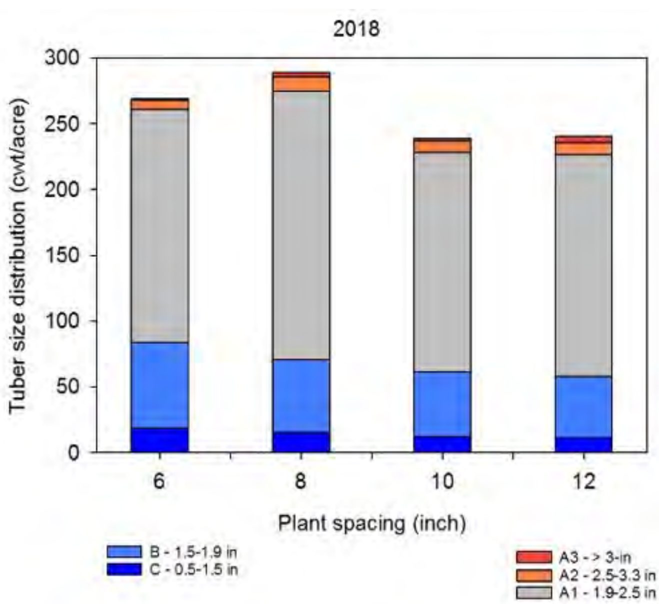

Fig. 1. Main effect of seed piece spacing treatments on tuber size distribution of table stock potato cultivars cultivated in Florida.

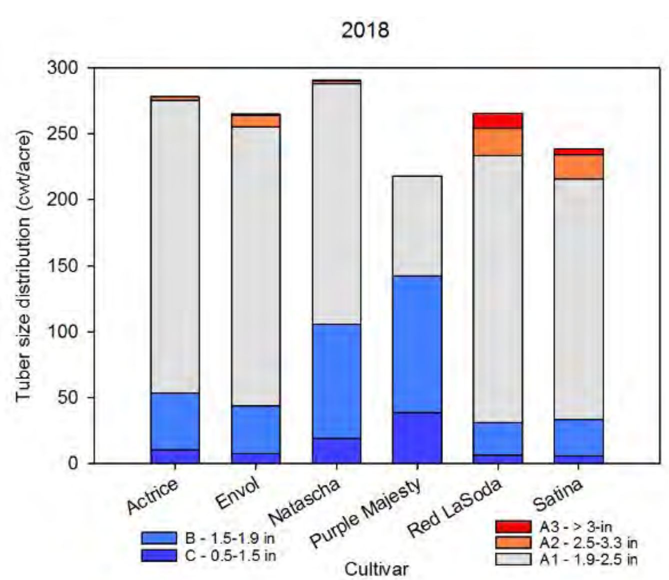

Fig. 2. Main effect of cultivars on total and marketable yield and tuber size distribution of table stock potato cultivars cultivated in Florida.

'Satina' and 'Actrice'. Tuber specific gravity was significantly affected by seed piece spacing and cultivar treatments. The 12-inch spacing treatments resulted in higher tuber specific gravity than 6-inch spacing. 'Purple Majesty' had statistically highest specific gravity compared to other cultivars.

Conclusion

In conclusion this study demonstrated that potato total and marketable yield, tuber size distribution, specific gravity responded differently to seed piece spacing and cultivars. Potato seeds spaced between 6 and 8 inches resulted in higher total and marketable yields compared to 10 and 12 inches seed pieces spacing. Tuber size distribution can be effectively managed by adjusting seed piece spacing according to growers proffered tuber size according to market preferences and price.

Literature Cited

Food Science Resource Economics Department. 2018b. Table potatoes: estimated production costs in the Hastings areas, 2008-2009. University of Florida. 25 Sept. 2018. <https://fred.ifas.ufl.edu/pdf/iatpc/files/HastingsTablePotato09.pdf>

Krupek, F.S., C.T. Christensen, C.E. Barrett and L. Zotarelli. 2017. Seed Piece Spacing for Spring Chipping Potato Cultivars in Florida. HortScience 52:230–235. doi:10.21273/hortsci11431-16.

Krupek, F.S., S.A. Sargent, P.J. Dittmar, and L. Zotarelli. 2018. Seed piece adjustment for Florida chipping potato. IFAS HS1317. Gainesville: University of Florida Institute of Food and Agricultural Sciences, 2018. 25 Sept. 2018. <http://edis.ifas.ufl.edu/hs1317>

Magnani, R., U Mazarura, A.M. Tuarira, and A.I. Shayanowako. 2015. Growth, yield, and quality responses to plant spacing in potato (*Solanum tuberosum*) varieties. Afr. J. Agr. Res. 10:571–578. https://doi.org/10.5897/AJAR2014.8665

U.S. Department of Agriculture (USDA). 1983. Soil Survey of St. Johns County, Florida. Natural Resources Conservation Service, U.S. Department of Agriculture. 24 Feb. 2015. <http://www.nrcs.usda.gov/Internet/FSE_MANUSCRIPT/florida/FL109/0/StJohns.pdf>

USDA. 1997. United States standards for grades of potatoes for chipping. USDA, Agric. Marketing Serv. <www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5050437 (accessed 10 Jul. 2017).

Zotarelli, L., P.J. Dittmar, P.D. Roberts, and S.E. Webb. 2016. Potato production, p. 233–251. In: J. Dittmar , J.H. Freeman, and G.E. Vallad (eds.). Vegetable production handbook of Florida. Vance Publishing, Lincolnshire, IL.

Proc. Fla. State Hort. Soc. 132:120–122. 2019.

Low Cost High Tunnel versus Open Field Production of Organically Grown Strawberries in North Florida: Second Year Evaluation

Alex Bolques*[1], Gilbert Queeley[2], Fanny Ospina[1], and Vonda Richardson[2]

[1]*Florida A&M University, Research and Extension Center, 4259 Bainbridge Hwy., Quincy, FL 32352*

[2]*Florida A&M University, Cooperative Extension Programs, 215 Perry Paige, Bldg. South., Tallahassee, FL 32307*

Additional index words. protected agriculture, high tunnel, farm profitability

Crop production in north Florida can be limited by freezing temperatures during winter months. Many fresh market organic and small scale producers utilize season extension techniques such as floating row covers, low tunnels, and high tunnels to protect specialty crops from damaging frost. In this study, we present the second year evaluation of strawberries grown in low cost high tunnel versus open field production systems. The four strawberry (*Fargaria ×ananassa*) cultivars used in this evaluation were Sensation™, brand (Florida 127), Winterstar™, (FL 05-107), Florida Radiance and Florida Beauty. Within each production system, 16 subplots, each containing 16 strawberry plants per cultivar, were arranged in a randomized complete-block design with 4 replications. Yield data were collected weekly from 27 Dec. 2017 through 4 May 2018. Descriptive statistics were generated using SAS ver. 9.4 and an independent samples *t* test was used to compare treatment means.

A low cost high tunnel (LCHT) is a temporary protective structure that is well suited for high-value low to medium canopy size horticultural crops. They are commonly installed in the field, over an existing crop, to help moderate weather condition extremes as well as the effects of freezing temperatures. This practice has been shown to not only maintain fruit quality but to extend the crop growing season resulting in higher yields (Bolques, et al., 2016 and 2018). Unlike a typical commercial grade high tunnel, LCHTs are commonly constructed using polyvinyl chloride (PVC) pipes and covered with polyethylene plastic. Commercially available high tunnels can be costly and may be financially out of reach for the small farm producer.

In this paper we discuss the findings from two years of field evaluations on low cost high tunnel production of strawberries in north Florida. We continue to evaluate the feasibility of producing organically grown strawberries in a LCHT as an alternative crop enterprise for small scale farmers, inclusive of urban and organic producers.

Materials and Methods

Experimental design. This study was conducted at the Florida A&M University, Research and Extension Center in Quincy, FL. For the 15 Oct. 2016 to 18 Apr. 2017 growing season, four strawberry cultivars Sensation™ (Florida 127) (Whitaker et al, 2014), Winterstar™ (FL 05-107) (Whitaker et al., 2012), Florida Radiance, and Strawberry Festival were evaluated for their performance within two 12 ft (3.6 m) wide × 100 ft (30 m) low cost high tunnels and one 12 ft (3.6 m) wide × 100 ft (30 m) open field arrangement. Likewise, for the 2017–18 growing season, the cultivars were Sensation™, Winterstar™, Florida Radiance, and Florida Beauty. The objectives, methods and data analytical procedures were the same as those utilized in the 2016–17 study (Bolques, et al., 2018).

The soil at the study site is an Orangeburg loamy sand with moderate permeability and water holding capacity (USDA NRCS, 2017). Soil preparations consisted of growing a sunn hemp cover crop, *Crotalaria juncea*, during the late summer (Aug. to Sept.) for both years. The cover crop was selected for its ability to fix nitrogen and when incorporated into the soil; sunn hemp can release allelopathic compounds toxic to plant parasitic nematodes (Wang, 2008). Following cover crop incorporation, 5 lb (2.3 kg) of preplant fertilizer, Nature Safe 8–3–5 Super Fine organic fertilizer (Nature Safe, Irving, TX), was incorporated into each production bed prior to forming the plastic mulch raised beds inclusive of two 5/8 (1.6 cm) T-Tape drip irrigation lines per bed. Two rows were designated for the open field (OF) treatment and other four for the low cost high tunnel (LCHT). Commercially available strawberry plant plugs were used for the evaluations with plugs planted using a randomized complete block design with 4 replications. Each replication covered an area of 50 ft (15 m) along the production bed with two blocks per 100 ft of row for a total of 4 replications per treatment. Each replication contained 16 plants per cultivar. Within the production bed, the planting arrangement consisted of double rows spacing 18 in (45) cm × 18 in (45) cm apart. At each end of a production bed, four strawberry plugs were planted as a buffer.

The crop was watered daily for an hour the first week and then three times a week or as needed. At mid-season, individual plants were top dressed with a teaspoon of Nature Safe 8–3–5 Super Fine organic fertilizer every three weeks. The crop was scouted

This research was funded by an USDA/NIFA/Organic Agriculture Research and Extension Imitative (OREI) Grant.

*Corresponding author. Email: alejandro.bolques@famu.edu

weekly and applied control measures were recorded. Ripe (red) fruit were sampled weekly from 8 plants/cultivar/replication/treatment. Marketable fruit collected was weighed.

Low cost high tunnel. The LCHT was constructed based on design specifications by Coolong (2012) with modifications to structure anchoring (Bolques et al., 2016). Otherwise, LCHT construction were the same as Bolques (2018).

Results and Discussions

The results from the two-year evaluation of organically grown strawberry cultivars in OF and LCHT production systems are shown in Tables1, 2, and 3 respectively. First we present the results for each year of the study (Tables 1 and 2) then an overall evaluation of the two production systems using the average of the two years of data for each of the cultivars evaluated (Table 3). In Tables 1 and 2, the treatment means compared are the mean yields of the cultivars within each production system. In the overall evaluation (Table 3), the comparisons are between production systems (OF vs. LCHT). This comparison allows for the determination of whether the LCHT provided any yield advantage over the OF (control). Only three cultivars (Florida Radiance, Winterstar, and Sensation) were used in the overall comparison. This is due to the substitution of 'Strawberry Festival' for 'Florida Beauty' in year two.

Evaluation of strawberry cultivars in the OF (2016/17). In the OF, mean yield ranged from 524 lb/acrere (587 kg/ha) for 'Florida Radiance' to 1682 lb/acrere (1885 kg/ha) for 'Winterstar'. For the 2016 growing season, 'Florida Radiance' produced significantly lower yield compared to the other 3 cultivars in the OF (Table 1). 'Winterstar™' was notably the highest producer in the OF. When compared to 'Strawberry Festival' and 'Sensation™' however, its yield was not significantly higher than the other two cultivars (Table 1).

Evaluation of strawberry cultivars in the LCHT (2016): In the LCHT, mean yield ranged from 1640 lb/acrere (1838 kg/ha) for 'Florida Radiance' to 3426 lb/acrere (3840 kg/ha) for 'Strawberry Festival'. The yield from 'Florida Radiance' in the LCHT was significantly lower compared to the other 3 cultivars (Table 1). 'Strawberry Festival' produced significantly higher yield compared to the other cultivars in the LCHT. There was no significant difference between the yield obtained from

Table 1. Mean yield[z] (lb/ace) of four organically grown strawberry cultivars gown in open field and in a low cost high tunnel protective structure from Fall 2016 to Spring 2017 in north Florida.

Treatment	Cultivar	Min	Max	Mean	±SE
Open field	Strawberry Festival	0.00	5101.4	1326.4a	131.3
	Florida Radiance	0.00	3464.3	524.1b	54.0
	Winterstar	0.00	6463.1	1682.2a	158.6
	Sensation	0.00	6099.6	1361.8a	136.5
Low cost high tunnel	Strawberry Festival	232.7	8623.1	3426.1a	165.3
	Florida Radiance	67.3	6151.1	1640.2c	94.6
	Winterstar	0.00	7013.7	2598.5b	135.0
	Sensation	0.00	10164.1	2835.1b	125.3

[z]Means within each production system with the same letter are not significantly different at $\alpha = 0.05$.

Table 2. Mean yield[z] (lb/acre) of four organically grown strawberry cultivars gown in open field and in a low cost high tunnel protective structure from Fall 2017 to Spring 2018 in north Florida.

Treatment	Cultivar	Min	Max	Mean	±SE
Open field	Florida Beauty	0.00	3575.2	776.92b	105.1
	Florida Radiance	0.00	9267.8	1810.3a	195.5
	Winterstar	0.00	4669.6	1108.9b	111.8
	Sensation	0.00	5400.4	1224.2ab	139.2
Low cost high tunnel	Florida Beauty	0.00	4888.4	922.3b	60.8
	Florida Radiance	57.4	4866.6	1504.7a	62.1
	Winterstar	107.0	4628.0	1437.7a	76.9
	Sensation	0.00	4595.3	1232.8a	71.0

[z]Means within each production system with the same letter are not significantly different at $\alpha = 0.05$.

Table 3. Mean yield[z] (lb/acre) of 3 organically grown strawberry cultivars gown in open field and in a low cost high tunnel protective structure from Fall 2016 to Spring 2018 in north Florida.

Treatment	Cultivar	Min	Max	Mean	±SE
Open field	Florida Radiance	0.0	9267.8	1529.0a	166.9
	Winterstar	0.0	6463.1	1915.9a	160.2
	Sensation	0.0	5400.4	1526.6a	140.8
Low cost high tunnel	Florida Radiance	57.4	6151.1	1686.0a	79.5
	Winterstar	107.0	7013.7	1988.2a	118.1
	Sensation	0.00	6956.3	1730.5a	113.1

[z] Means between production systems with the same letter are not significantly different at $\alpha = 0.05$.

'Winterstar™' and 'Sensation™' in the LCHT. In 2016, the best yield was observed for 'Winterstar™' in the OF and 'Strawberry Festival' in the LCHT (Table 1).

Evaluation of strawberry cultivars in the OF (2017–18). In 2017, mean yield in the OF ranged from 777 lb/acre (871 kg/ha) for 'Florida Beauty' to 1810 lb/acre (2029 kg/ha) for 'Florida Radiance'. Mean yield from 'Florida Radiance' in the OF was significantly higher compared to the other 3 cultivars. The yields from 'Florida Beauty', 'Winterstar™', and 'Sensation™' were not significantly different (Table 2).

Evaluation of strawberry cultivars in the LCHT (2017). In the LCHT, mean yield ranged from 922 lb/acre (1033 kg/ha) for 'Florida Beauty' to 1504 lb/acre (1686 kg/ha) for 'Florida Radiance'. The yield obtained from 'Florida Beauty' in the LCHT was significantly lower compared to the other 3 cultivars. The yields obtained from 'Florida Radiance', 'Winterstar™' and 'Sensation™' were not significantly different (Table 2). In 2017, 'Florida Radiance' was the best overall performer in both the OF and the LCHT (Table 2).

Overall evaluation of strawberry cultivars in the OF and LCHT (2016–18). Over the two-year duration of the study, mean yield in the OF ranged from 1526 lb/acre (1710 kg/ha) for 'Sensation™' to 1915 lb/acre (2146 kg/ha) for 'Winterstar™'. In the LCHT, mean yield ranged from 1686 lb/acre (1890 kg/ha) for 'Florida Radiance' to 1988 lb/acre (2222 kg/ha) for 'Winterstar™'. The yield obtained from 'Winterstar™' was notably higher in both the OF and LCHT. However, after two years of evaluation, there was no statistically significant difference in yield for the strawberry cultivars evaluated in the OF and LCHT (Table 3). We therefore conclude that the LCHT did not provide any statistically significant yield advantage over the OF. It must be mentioned however, that the LCHT provided other benefits such as: protection from frost and insect damage and limiting fruit contamination from dust particles. All of these benefits can contribute to enhanced fruit quality. However, limited resource farmers will still have to balance the additional cost of constructing a LCHT with the benefits it can provide before making a decision regarding whether or not to use it as an alternative production system for organically grown strawberries.

Literature Cited

Bolques, A., G. Queeley, F. Ospina, and V. Richardson. 2016. Season extension and overwintering of scotch bonnet hot pepper in north Florida. Proc. Fla. State Hort. Soc. 129:134–136.

Bolques, A. Steps to create a low cost high tunnel [Video file]. 4 Oct. 2017. <https://www.youtube.com/watch?v=ILkWswp9jBM>.

Bolques, A., G. Queeley, F. Ospina, and V. Richardson. 2018. Low cost high tunnel versus open field production of organically grown strawberries in North Florida. Proc. Fla. State Hort. Soc. 131:106–109.

Coolong, T. 2012. Low cost high tunnel construction. eXtension article, eOrganic 2311. 4 June 2016. <http://articles.extension.org/pages/18356/low-cost-high-tunnel-construction>.

United States Department of Agriculture, Natural Resources Conservation Service. 2007. Soil Survey of Gadsden County, Florida. Revised 2009. <http://soils.usda.gov/ survey/printed_surveys/>.

Wang, K-H. 2008. Sunn hemp and its allelopathic compounds for vegetable production in Hawaii and beyond. Western SARE Hawaii Conference. 15 Aug. 2018. <https://www.westernsare.org/Learning-Center/Conference-Materials/Western-SARE-Subregional-Materials/Hawaii-Conference/Hawaii-Posters-and-Handouts>.

Whitaker, V.M., C.K. Chandler, B.M. Santos and N.A. Peres. 2012. Winterstar™ Brand (Florida 05-107) Strawberry. HS1198. Horticultural Sciences Dept., UF/IFAS Extension, Gainesville, FL.

Whitaker, V.M., C.K. Chandler and N.A. Peres. 2014. Sensation™ Brand 'Florida127' Strawberry. HS1256. Horticultural Sciences Dept., UF/IFAS Extension, Gainesville, FL.

Proc. Fla. State Hort. Soc. 132:123–124. 2019.

Effects of Biostimulants on Potato Growth and Production

Muhammad Shahid and Guodong Liu*

Horticultural Sciences Department, University of Florida/IFAS, P.O. Box 110690, Gainesville, FL 32611-0690

Additional index words. petiole sap nitrate content, photosynthetic activity, potato tuber yield

The study was conducted to investigate the effects of individual biostimulants on potato (cv. 'Atlantic') growth and yield. Five different biostimulants including CP1, CP2, BS, CP2+BS, and Competitor plus water as the control were foliar-applied at two different stages: 30, and 55 days after plating. Competitor and CP2 significantly increased yield, photosynthetic activity, leaf greenness and nitrate concentration in leaf petioles. Statistically, Competitor and CP2 showed the greatest augmentation in above mentioned attributes. Based on the findings of this study, application of Competitor or CP2 enhances nitrogen use efficiency and increases tuber yield for potato production.

Agricultural biostimulants are biological fertilizer additives and similar products. These products are used to enhance plant nutrition, health, growth, and productivity for crop production. The term "agricultural biostimulant" includes a diverse group of ingredients with different modes of action and are derived from microorganisms, plant extracts, or other organic matter. Agricultural biostimulants are applied to plants or soils to improve crop growth, tolerance to abiotic stresses, yield, and quality. Biostimulants also foster plant growth and development throughout the crop life cycle from seed germination to plant maturity in a number of demonstrated ways, including: (a) facilitating nutrient assimilation, translocation and use (b) using water more efficiently, and (c) enhancing soil fertility, especially by fostering the development of complementary soil microorganisms. The objectives of this study was: 1) identifying the best of the biostimulants tested; 2) evaluating effects of the biostimulants tested on tuber yield; and 3) assessing effects of the biostimulants on nutritional quality of potato tubers.

Materials and Methods

A field trial was conducted at the University of Florida/IFAS (UF/IFAS) Hastings Agricultural Extension Center in 2017. The plot size was 533.3 ft² (40' × 13.3'). Fertilizers applied included 200 lb/acre N as ammonium nitrate, 120 lb/acre P_2O_5 as triple superphosphate, and 200 lb/acre K_2O as potassium chloride (muriate of potash, MOP). A quarter of the 200 lb/acre N, 120 lbs/acre P_2O_5 and 50 lb/acre K_2O were applied before planting. At emergence, 100 lb/acre N and 100 lb/acre K_2O were sidedressed.

At the beginning of tuber initiation, the final 50 lb/acre each of N and K_2O were sidedressed. The pre-mixed fertilizer was provided by ICL Specialty Fertilizers (Dublin, OH). All 24 plots were randomly assigned to 6 treatments (Table 1) of biostimulants with 4 replications. The treatments were applied to the foliage using handheld sprayers 30 and 55 d after planting. Potato ('Atlantic') seed pieces were planted on 31 Jan. 2017. The tubers were dug out on 15 May 2017. A linear pivot irrigation system was used. Regular field observations were done to ensure good growing conditions and maintain plant health based on the UF/IFAS recommendations (Liu et al., 2020; Zotarelli et al., 2020).

Petiole sap testing was used to measure nitrate levels in the plants. Thirty leaf samples were taken from each plot and put in a zip lock bag. Petioles were separated from the leaves, cut into small pieces and mixed. A lemon squeezer was used to squeeze the sap. Nitrate content in the sap was measured using a LAQUA Twin Nitrate Meter (Spectrum Technologies, Inc., Aurora, IL). The nitrate meter was calibrated with the standard solutions (300 and 2000 mg/L) provided by Spectrum Technologies, Inc., and then samples were processed. The petiole sap nitrate was measured 4 d after either (a) the first spray or (b) the second spray.

The harvest was done 105 d after planting. Tubers were graded as A1, A2, A3, A4, B, and C using a potato grader based on the USDA standard for grading potato tubers.

Physiological characteristics like leaf greenness and photosynthetic activity were measured by using SPAD (502Plus, Konica Minolta, Tokyo, Japan) and Li-Cor (LiCor-6400, Lincoln, NE) meters, respectively.

Field data were plotted with Excel. All data analysis was completed using STATISTICA 9.0 (Stat-Soft, Inc., Tulsa, OK). However, SigmaPlot 11 (Systat Software, Inc., San Jose, CA) was used to make the graphics.

Table 1. Treatment name and description.

Treatment	Rate
CP Rate 1	182 mL/acre (2 mL/L)
CP Rate 2	364 mL/acre (4 mL/L)
CP+BS	900 mL/acre + 0.9 lbs /acre (4 mL/L + 4.5 g/L)
BS	0.9 lbs/acre (4.5 g/L)
Competitor	473 mL/acre (5.2 mL/L)
Water	4700 mL

ICL Specialty Fertilizers, Dublin, Ohio provided the biostimulants. University of Florida/IFAS Hastings Agricultural Extension Center, Hastings, FL helped with land, labor, and materials. Mr. Jonathan Denison reviewed the manuscript.

*Corresponding author. Email: guodong@ufl.edu

Results and Discussion

The nitrate content in plant petioles treated with Competitor and CP2 showed the highest level of nitrate followed by CP+BS and BS (Fig. 1). However, the CP1 treatment and the control (water only) did not show any significant differences.

Leaf greenness also showed significant differences in response to foliar application of biostimulants (Fig. 2). Plants treated with Competitor and CP2 had maximum SPAD readings compared with each of the other treatments and the control (only water sprayed) had minimum greenness. The treatments CP1, BS, and CP+BS were between the extreme values.

All the biostimulant treatments showed significant effect on photosynthetic activity (Fig. 3). Plants treated with Competitor and CP2 maintained the greatest photosynthetic rate compared with each of the other treatments and the control.

Tuber yield showed marked differences in response to the biostimulant treatments with respect to the control. Plants sprayed with Competitor CP+BS, and CP2 gave greater tuber yields in comparison to the control. However, the plants treated with water had the lowest tuber yield (Fig. 4). The findings of this investigation showed that biostimulants (especially Competitor and CP2) had beneficial impacts on growth and productivity of potato plants.

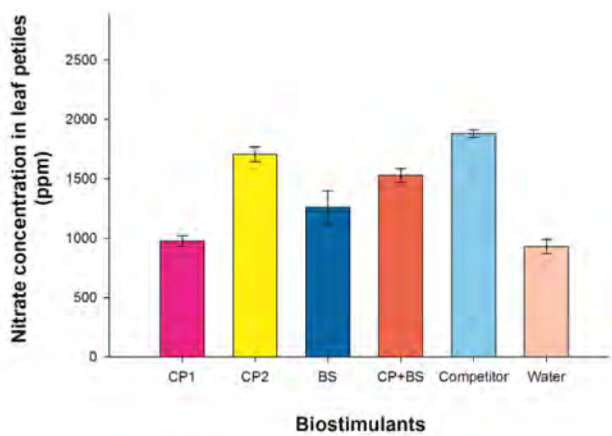

Fig. 1. Effect of biostimulants on petiole nitrate concentration of chipping potato, 'Atlantic'

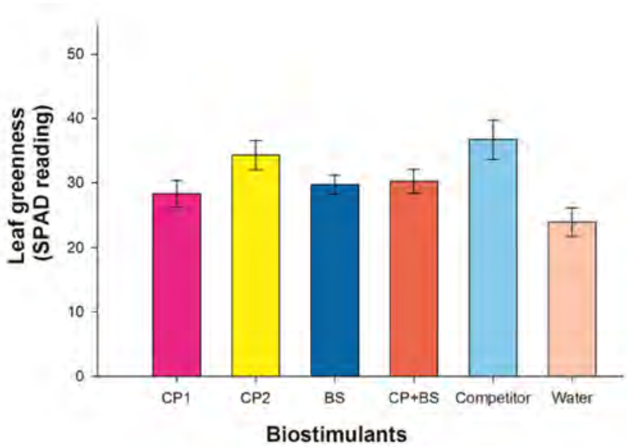

Fig. 2. Effect of biostimulants on leaf greenness of chipping potato,

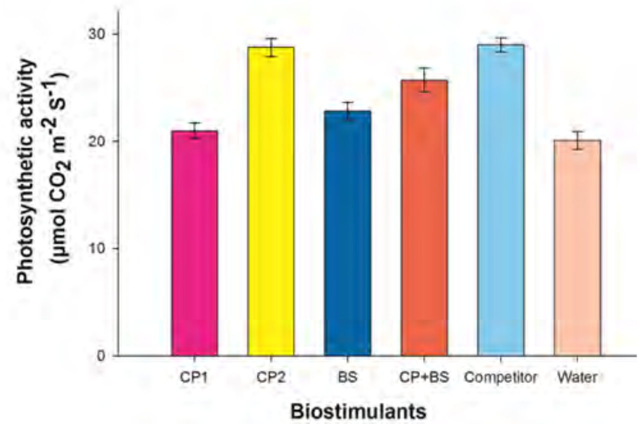

Fig. 3. Effect of biostimulants on photosynthetic activities of chipping potato, 'Atlantic'.

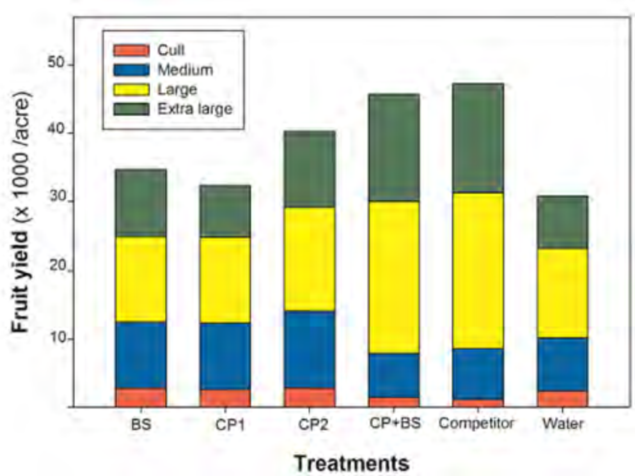

Fig. 4. Effect of biostimulants on tuber yield of chipping potato, 'Atlantic'.

Conclusions

The field study was completed with a chipping potato variety, 'Atlantic' with 5 biostimulants plus a control (with water only). The preliminary results showed that CP2 and Competitor had significant effects on petiole nitrate concentration, leaf greenness, photosynthesis and tuber yield than the other treatments. These two biostimulants may be used for enhancing nitrogen use efficiency for potato production in Florida.

Literature Cited

Liu, G.D. E.H. Simonne, K.T. Morgan, G.J. Hochmuth, S. Agehara, and R. Mylavarapu. 2020. Chapter 2. Fertilizer management for vegetable production in Florida. In: Vegetable Production Handbook of Florida (2019–2020 edition). P. Dittmar, J. Freeman, M. Peret, and H. Smith (eds). CV296. Gainesville: University of Florida Institute of Food and Agricultural Sciences. 17 Apr. 2020. <https://edis.ifas.ufl.edu/cv296>

Zotarelli, L., P.J. Dittmar, P.D. Roberts, J.Desaeger, J.W. Noling, and B. Wells. 2020. Chapter 14. Potato production. In: Vegetable Production Handbook of Florida (2019–2020 edition) P. Dittmar, J. Freeman, M. Peret, and H. Smith (eds). HS733. Gainesville: University of Florida Institute of Food and Agricultural Sciences. 17 Apr. 2020. <https://edis.ifas.ufl.edu/cv131>

USDA-AMS. 2011. United States Standards for Grades of Potatoes. <https://www.ams.usda.gov/sites/default/files/media/Potato_Standard%5B1%5D.pdf>

Proc. Fla. State Hort. Soc. 132:125–127. 2019.

Demonstration of Drone Technology to Improve Crop Management in Vegetable Production

Qingren Wang*

University of Florida/IFAS Extension Miami-Dade County, 18710 SW 288th St., Homestead, FL 33030

Additional index words. crop management, drone, multispectral sensing, NDRE, NDVI, NIR, RGB.

Drone technology has shown high potential implementations in many fields including agriculture. This paper describes a demonstration of a drone associated with corresponding technologies in field surveys to assess plant growth, missing spots, plant growth status with different cultural practices, and biomass. With the development of artificial intelligence (AI), an autonomous setup for flight, manipulation, data collection, image mapping, and data processing have made the technology practical to accept and implement. The results show that drone technology can provide real-time information to growers with images of: seed germination rates; proportions of healthy and unhealthy areas of the field; crop dead spots; growth status with different practices, such as configuration of plastic mulch; amounts of crop biomass accumulation; and pest damage. It can help growers with timely decision-making, practice changes, and precise management plan adjustments to reduce yield losses.

With the rapid development of artificial intelligence (AI), drone technology has been applied in many areas including the agriculture industry. A few examples include: survey and mapping soil properties (Abbas et al., 2013); estimating canopy leaf area (Mathews and Jensen, 2013); crop biomass with nutrient levels (Bendig et al., 2014; Vega et al., 2015); detecting and mapping a field for weeds (Pena et al., 2015); plant nitrogen status (Agüera et al., 2011); assessing drip irrigation efficacy (Zarco-Tejada et al., 2012); and in livestock management (Daneshkhu, 2016).

All these applications are based on specific device types and models, setup of flight parameters, data collection and processing. For example, a high definition (HD) camera preinstalled on a drone can take a large number of high quality color pictures across the entire field. The number of pictures taken depends on field size, flight height, speed, and overlaps between any two pictures. Color is based on red, green, and blue (RGB) colors. All the individual pictures must be put together (stitching) to generate an integrated field map, called an RGB map. Only a very narrow band of light spectrum with a wavelength of 400–700 nm is visible to human eyes. The Near-Infrared (NIR) band beyond 700 nm is invisible to humans, but is correlated to plant chlorophyll content. NIR sensors have been developed and are widely used in agriculture but NIR images are in black and white.

Based on visible and NIR wavelengths, some multispectral sensors have been developed to derive a Normalized Difference Vegetation Index (NDVI), and a Normalized Difference Red Edge (NDRE), which have great advantages in sensitivity based on color changes. As in processing RGB pictures, these individual images of NIR, NDVI, or NDRE captured by separate sensors preinstalled on the drone also must be stitched together to form a single mosaic mapping image for an overview of the field with corresponding software, such as FieldAgent™ or Drone Deploy. This paper provides examples of drone flight setup, data collection, data processing for applications to vegetable crops to improve crop management for sustainable development.

Materials and Methods

A drone with a model DJI Phantom 4 Pro with an HD camera and double 4k multispectral sensor preinstalled on the drone was used in field surveys for various vegetable crops in the Miami-Dade agriculture area. The crops were green beans, okra, tomato, and sweet corn. Field size ranged from approximately 1/2 acre to more than 40 acres. With specific software downloaded to either an iPhone or an iPad, autonomous flights were set up with appropriate parameters such as picture overlap, flight altitude, speed, and orientation based on the objectives of the survey, the crop, growth stage, and type of stresses. Once a field was marked at each corner, the system displayed the field size, the number of pictures that would be taken, the number of batteries needed, flight duration, picture pixel size, and Ground Survey Distance (GSD) in inches or centimeters (cm) per pixel. The flight would complete automatically and the drone would return to the takeoff spot once the job was completed. In a large field, when one battery was unable to complete the job, the drone would fly back to the starting spot for battery replacement when the battery level dropped to 35%. Once fitted with a new battery, the drone would fly back to the spot where it had stopped and continued to finish the job. For instance, in the following flight, an ~40-acre okra field at flowering was selected to evaluate plant health and possible problems in the field. The parameters chosen were, picture overlap: 80% or DSM/Ortho flight mode; flight altitude: 200 feet above the ground; flight speed: 20 miles per hour (MPH), and

The author expresses his sincere appreciation to FDACS for the financial support to conduct the project, Dr. Kelly Morgan and Mr. James Fletcher for their coordination of the Statewide BMP Program, and Mr. Kern Carpenter and other growers for agreeing to allow their land to be used for these surveys.

*Corresponding author. Email:grwang@ufl.edu

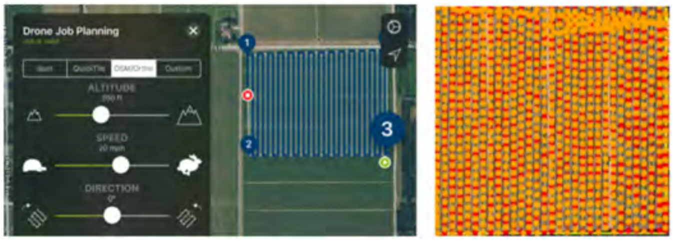

Fig. 1. Setup of the flight (left) with iPhone or iPad app and photos taken (right) by both the camera (red) and the sensor (yellow) after the flight.

flight orientation: south to north against or following the wind direction, wind speed should be less than 15 MHP (Fig. 1 left).

Once the survey was completed, a field map was created with a large number of photo dots distributed in the selected area of the field (Fig. 1 right). Each dot represented a picture taken by either the HD camera or the sensor. The collected information during the flight was stored in two separate micro SD cards, one for the drone camera and the other for the multispectral sensor. The data from both cards were transferred to a computer for processing with specific software, such as FieldAgent™ or DroneDeploy. In either case, it needed to upload individual pictures ranging from a few hundred to >1000 to its cloud system for stitching and processing. When finished, a series of Orthomosaic (from Drone Deploy) or Quicktile mosaic (FieldAgent™) images were viewable, such as RGB (Red, Green and Blue) or color, NIR (Near-Infrared), NDVI (Normalized Difference Vegetation Index), and NDRE (Normalized Difference Red Edge) to show the uniformity or difference of the crop field.

Results and Discussion

A few examples of the types of information that can be obtained by using drones is listed below.

Field usage and missing plant spots in green beans. After data processing, several mosaic images were created, such as RGB, NIR, NDVI, and NDRE. For example, a NDVI mosaic image (Fig. 2 left) from a green bean field showed the general image of crop growth in the field, in which, green was healthy plants, red was either a driveway or bare land, yellow or brown was unhealthy plants, which could be viewed for details in a particular area or spot (Fig. 2 middle and right).

Evaluation of the growth status of an okra field. Based on a mosaic image, a map of management zones (Fig. 3 left) was created. It clearly showed that most of the field had healthy plants (green color) but in the middle and on the west side of the field (left of the image), fewer healthy plants appeared (patches in other colors rather than green). To find out what the problem

Fig. 2. A quicktile mosaic image of the surveyed field of green beans (left), drive way and plastic mulch with other crop (middle) on the right side of the mosaic image in red, and a plant missing spot (right) of a yellow rectangular shape close to the mid-bottom of the mosaic image.

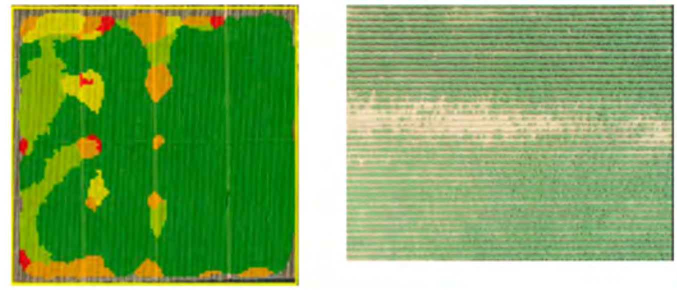

Fig. 3. Management zones showing healthy (green) and unhealthy areas (colors other than green) in a the field (left), and spot checking for low germination or missing plant patches (right).

was, when we clicked the corresponding photo dot in the yellow area, a detailed picture of that spot, it showed poor seed germination rate with missing plants (Fig. 3 right).

Evaluation of cultural practices in a tomato field. Using the same approach, a number of surveys were carried out in the agriculture area of Homestead, FL. For instance, a tomato field with different cultural practices, such as plastic mulch in a layout with white on black color vs. black on white close to the edge of the right side of the field to compare different growth status (Fig. 4).

The result from this field survey showed that the tomato plants grew better with plastic mulch in a layout of black on white as opposed to white on black. That might be why most tomato growers in this area preferred the cultural practice of black on white plastic mulch. A silver metallic plastic mulch was also tried and has the advantage of controlling pests due to its reflectivity, but the cost is a concern for most growers.

Estimation of plant biomass in a sweet corn field. The volume of sweet corn biomass from various areas in the same field measured with the drone technology showed that in two areas of 770 ft^2, the vigorously growing area had 52.9 $yard^3$ of biomass vs. only 18.2 $yard^3$ in an area of poor growth (Fig. 5).

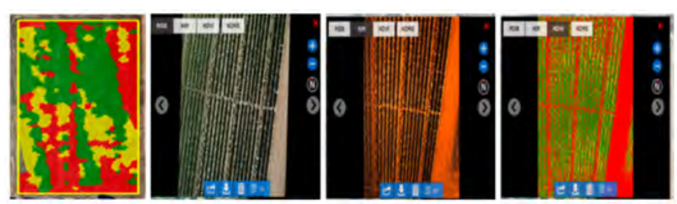

Fig. 4. A map of management zones in plant growth status with different plastic mulches, black on white (rows on left) vs. white on black color (rows on right). The images from left to right are: management zones, RGB at the right edge of the management zones with different plastic mulches, NIR flipped over from the RGB image, and NDVI flipped over from the same image of RGB or NIR (note that the black on white mulch in the two images on right is at the very left edge due to the position of the sensor on the drone: the HD camera is on one side and the sensor is on the other side of the drone), especially with a low flight height.

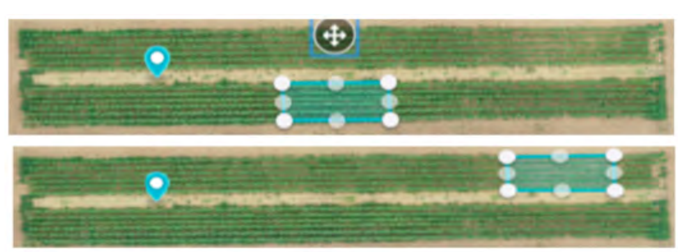

Fig. 5. Volume of biomass in a healthy area (top): 52.9 $yard^3$ vs. that of an unhealthy area (bottom): 18.2 $yard^3$ of a sweet corn field in 770 ft^2.

These results showed that biomass estimates can be made using remote technology and the correct software. This survey also demonstrated that drone technology can be used in either large or small fields, such as the two strips in this small scale sweet corn field.

Detection of plant diseases and creation of a field report. Tomato tospoviruses, especially tomato chlorotic spot virus (TCSV), and tomato yellow leaf curl virus (TYLCV) were detected in a tomato field with the drone technology by generating a mosaic image and confirmed with a ground truthing survey. A map of management zones was created with a field report to show the acreage distribution of healthy and unhealthy areas of the field, and a ground truthing survey showed disease incidence of both TCSV and TYLCV (photo) close to the top left corner of the field (Fig. 6).

Fig. 6. Map of management zones in a tomato field (left) with acreage distributions of healthy and unhealthy plants based on NDVI values (middle), and tomato plants infected with TCSV and TYLCV (right) a side by side close to the top left corner of the field (yellow area). Note that the red areas were mainly the field edge and driveways.

Conclusion

Drone technology has many advantages in conducting field survey with rapid, precise and reliable results. This technology can obviously save a lot of time and labor, and provide real-time information on plant health, seed germination rates, finding dead spots, comparing cultural practices, making biomass estimates, and detecting plant stress. The collected information through the field survey and data processing may allow growers to make timely management plan adjustments and practice changes to reduce yield and quality losses. With the rapid development of AI, more applications in this type of technology for agriculture will be available soon.

Literature Cited

Abbas, A., S. Khan, N. Hussain, M.A. Hanjra, and S. Akbar. 2013. Characterizing soil salinity in irrigated agriculture using a remote sensing approach. Physics and Chemistry of the Earth, Parts A/B/C. 55–57:43–52.

Agüera, F., F. Carvaja, and M. Perez. 2011. Measuring sunflower nitrogen status from an unmanned aerial vehicle-based system and an on the ground device. In: Proc. conference on unmanned aerial vehicle in geomatics, Zurich, Switzerland, Sept. 14–16, 2011, Vol. 38:(1/C22).

Bendig, J., A. Bolten, S. Bennertz, J. Broscheit, S. Eichfuss, and G. Bareth. 2014. Estimating biomass of barley using crop surface models (CSMs) derived from UAV-based RGB imaging. Remote Sensing. 6:10395–10412.

Daneshkhu, S. 2016. Drones part of leap in agriculture technology. Business Day. <http://www.bdlive.co.za/business/innovation/2016/01/19/drones-part-of-leap-in-agriculture-technology>

Zarco-Tejada, P.J., V. González-Dugo, and J.A.J. Berni. 2012. Fluorescence, temperature and narrow-band indices acquired from a UAV platform for water stress detection using a micro-hyperspectral imager and thermal camera. Remote Sens. Environ. 117:322–337.

Proc. Fla. State Hort. Soc. 132:128–130. 2019.

Biorational Insecticides for Controlling Diamondback Moth, *Plutella xylostella* (Lepidoptera: Plutellidae)

Dakshina Seal*, Rafia Khan, Catherine Sabines, and Shawbeta Seal

University of Florida/IFAS, Tropical Research and Education Center, 18905 SW 280th Street, Homestead, FL 33031

Additional index words. Diamondback moth, biorational insecticides, management, application time, insect growth regulator

Cabbage, *Brassica oleracea* var. *capitata* is a popular food item. It is attacked by several insect pests including diamondback moth, cabbage looper, beet armyworm, cabbage webworm, aphids, sweet potato whitefly and others. Diamondback moth, *Plutella xylostella* is the most common pest of cabbage. Insecticides are commonly used to control diamondback moth. We conducted three studies in Miami-Dade County to study management of diamondback moth using biorational insecticides. Entrust® alone and Xentari® in rotation with Knack® provided significant reductions of diamondback moth. Various biorational products including Azera®, Dipel®, and Spear® reduced diamondback moth larvae and pupae in the present study. This information can be used to control diamondback moth without using conventional insecticides with long residuals.

Cabbage is one of the most important vegetable crops used as a food globally. Cabbage is grown throughout the United States. Florida ranks third nationally in the production of fresh market cabbage and ranks second in terms of crop value. Other cabbage growing states are California, Wisconsin, New York, and Texas.

Cabbage originated in Europe where it started as a garden vegetable. Soon after its domestication, cabbage was grown as a leafy vegetable around Mediterranean. Cabbage is now grown all across the globe. In 2005 world production of cabbage was 62 million metric tons on 2.8 million ha. With the increase in production, cabbage became a preferred host of several insect pests. Diamondback moth is the most notorious of all insect pests of cruciferous crops in diverse climatic conditions (Ohbayashi et al., 1990). Growers use insecticides with various modes of action to control diamondback moth. The annual cost for managing diamondback moth has been estimated at U.S. $1 billion (Hill, 1993).

The diamondback moth feeds on plants belonging to crucifers that contains some stimuli in the form of mustard oils and their glucosides (Thorsteinson, 1953; Thorsteinson, 1955). Among cultivated crucifers, the most commonly reported ones are cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*), broccoli (*B. oleracea* var. *italica*), radish (*Raphanus sativus*), turnip (*B. rapa pekinesis*), Brussels sprouts (*B. oleracea* var. *gemmifera*), Chinese cabbage (*B. rapa* cv. gr. *pekinensis*), kohlrabi (B. *oleracea* var. *gongylodes*), mustard (*B. juncea*), rapeseed (*B. napus*), collard (*B. oleracea* var *acephala*), pak choi (*B. rapa* cv. gr. *pakchoi*), saishin (*B. rapa* cv. gr. *saishin*), watercress (*Nasturtium officinale*), and kale (*B. oleracea* var. *alboglabra*). In addition to cultivated plants, many weed hosts also support diamondback moth's feeding and reproduction including *Arabis glabra*, *Armoracia lapathifolia*, *Barbarea stricta*, *Barbarea vulgaris*, *Basela alba*, *Beta vulgaris*, *Brassica caulorapha*, *Brassica kaber* (*Sinapis arvensis, Brassica arvensis*), *Brassica napobrassica*, *Bunias orientalis*, *Capsella bursa-pastoris*, *Cardamine amara*, *Cardamine cordifolia*, *Cardamine pratensis*, *Cheiranthus cheiri*, *Conringa orientalis*, *Descurainia Sophia*, *Erysimum cheiranthoides*, *Galinsoga ciliata*, *Galinsoga parviflora*, *Hesperis matronalis*, *Iberis amara*,(*Satis tinctoria*), *Lepidium perfoliatum*, *Lepidium virginicum*, *Lobularia maritima*, *Mathiola incana*, *Norta* (*Sisymbrium*) *altissima*, *Pringlea antiscorbutica*, *Raphanus raphanistrum*, *Rorippa amphibia*, *Rorippa islandica*, *Sinapis alba*, *Sisymbrium austriacum*, *Sisymbrium officinale,* and *Thlaspi arvense* (Harcourt 1954, 1957; Kanervo, 1936; Louda, 1986; Rai and Tripathi, 1985).

The diamondback moth has a short life cycle of 25–30 days from egg to pupa. The adult lifespan extends from 12–16 days. During this short time, a female can lay 250–300 eggs. The egg stage lasts 5–6 days. Diamondback moths can have 12 generations in a year. Female moths start laying eggs immediately after mating. Peak oviposition period is between 7:00 to 8:00 p.m. The first instar starts feeding on the foliage soon after hatching.

Management of diamondback moth is principally based on synthetic pesticides with various modes of action. Due to its short life cycle and repeated use of the same effective insecticides, this moth has developed resistance to various older synthetic chemicals. Diamondback moth resistance to insecticides was first reported from Indonesia in 1953 (Ankersmit, 1953; Johnson, 1953). Later on, insecticide resistance was also reported from different cabbage producing countries. Due to the lack of effective alternative control programs and the availability cheaper insecticides, insecticides still remain as a major tool for controlling diamondback moth.

In the present study, we conducted various trials using biorational insecticides alone or in rotation with benign conventional insecticides. This approach will reduce the use of harsh long residual insecticide and promote the use of reduced risk biorational insecticides.

*Corresponding author. Email: dseal3@ufl.edu

Materials and Methods

All studies were conducted at the Tropical Research and Education Center, University of Florida/Institute of Food and Agricultural Sciences (UF/IFAS) in Homestead, FL. Cabbage transplants were planted with 12-inch spacing within the bed and 36-inch spacing between beds. The soil type is a Rockdale very gravelly loam, which consists of about 33% soil and 67% limestone pebbles (> 2 mm) (Noble et al., 1996). Raised beds, 6.0 inches high and 3.0 ft. wide, were formed and covered with black-white plastic mulch (0.9 mil, Canslit Inc. Victoriaville, Quebec, Canada). Two lines of drip tape (Ro Drip, USA) with a 12-inch emitter spacing were placed 6 inches apart parallel to the center of the bed. The drip irrigation system delivered 0.4 gal. (1.51 L) per 100 ft. per minute. Plants in each study were irrigated for one hour every day. The treatment plots, each 40 ft long, were arranged in a randomized complete block design with four replications in all studies.

Effect of reduced risk pesticides. The treatments used in the first study included a. Azera® (40 oz/acre, Azadrachtin + Pyrethrins, Valent); b. Dipel® (1.0 lb/acre, *Bacillus thuringiensis*, subsp. *Kurstaki* (BTK)); c. V-10433 (1.0 lb/acre, Experimental); d. Entrust® (4.0 oz/acre, spinosad, Dow AgroSciences); e. Xentari® in rotation with Knack® (1.0 lb, *Bacillus thuringiensis* subsp. *aizawai* (Bta) strain (ABTS-1857), Valent, rotated with 4.0 oz of pyriproxyfen, Valent) and f. an untreated control. All treatments were applied at weekly intervals for a total of four times. Treatments were evaluated by thoroughly checking 5 randomly selected plants/treatment plot 48 h after each application. The number of larvae and pupae on the whole plant was recorded.

In the second study, the treatments used were: a. Spear-C® + Bt-K (1.0 pt/acre+ 1.0 pt/acre); b. Spear-C® + Bt-K (2.0 pt/acre + 1.0 pt/acre); c. Bt-K (1.0 pt/acre); d. Entrust® (4.0 oz/acre), and e. an untreated control. MSO (methylated seed oil) was added to each treatment at 0.125% v/v. Treatments were applied weekly for four weeks. Evaluation of treatments were conducted using the method described for the first study.

In the third sudy, treatments included were: a. Knack® (5.0 lb/acre) applied in combination with Xentari® (1.0 lb/acre); b. Xentari® (1.0 lb/acre), c. Radiant® (8.0 oz/acre, spinetoram, Dow AgroScience) and d. an untreated control. Applications of Xentari® and Radiant® were made at weekly intervals for eight weeks, whereas, Knack® was applied every other weeks as a tank mix. Evaluation of treatments was made following the same method as described in the first study.

Statistical analysis. Data on the abundance diamondback moth of larvae and pupae, from treated samples were transformed using square-root of $X + 0.25$ before performing an analysis of variance (ANOVA). The transformed data were analyzed by least squares ANOVA (SAS Institute Inc., 2013). However, for ease of interpretation, the means of the original data are presented in the table. The Waller-Duncan *k-ratio t-test* was used to separate treatment means where significant ($P < 0.05$) differences occurred [Waller Duncan K-ratio test ($\alpha < 0.05$) using SAS, SAS Institute Inc. 2013].

Results and Discussion

First study. Diamondback moth abundance was high during this study (Fig. 1). Entrust® significantly reduced diamondback moth larvae/plant as compared to the untreated control. Xentari® in rotation with Knack® also provided significant reduction of diamondback moth larvae. Azera® and Dipel® treated plants had fewer larvae than the untreated control with some fluctuation in abundance on different sampling dates.

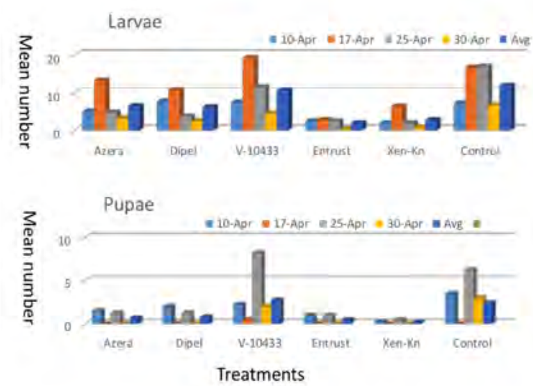

Fig. 1. Mean number of diamondback moth larvae and pupae/plant treated with various biorational insecticides.

Like diamondback moth larvae, mean numbers of pupae were significantly lower for all treatments than the untreated control, except V10433. (Fig. 1).

Second study. In the second study, diamondback moth abundance was also high (4-8 larvae/plant) (Fig. 2). Entrust® treated plants had significantly fewer larvae than the untreated control followed by Bt-K, Spear® in combination with Bt-K.

All insecticide treatments significantly reduced diamondback moth pupae on the first, second and third sampling dates. Mean numbers of pupae increased sharply on subsequent sampling dates on all treated plants except Entrust®.

Third study. The population abundance of diamondback moth larvae was high during this study (Fig. 3a). Mean numbers

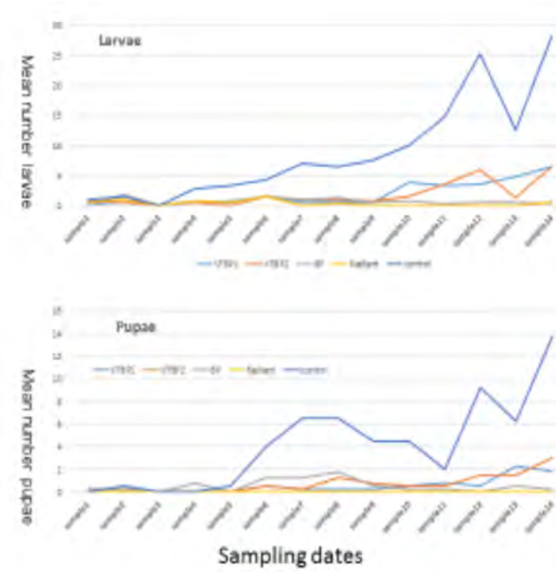

Fig. 2. Mean number of diamondback moth larvae and pupae on different sampling dates treaded with biorational insecticides.

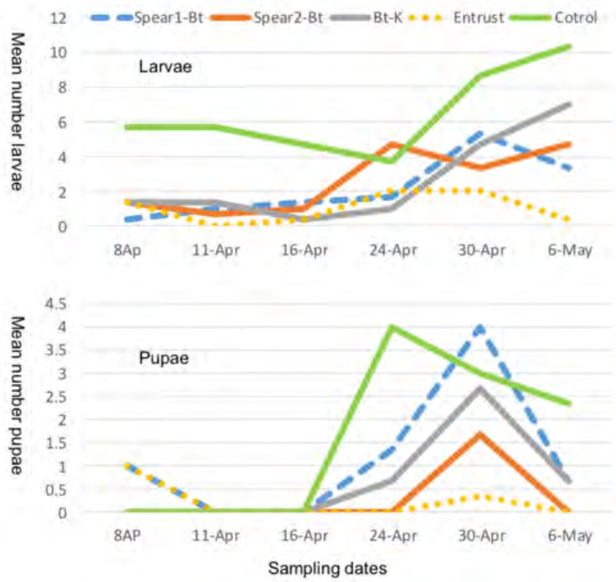

Fig. 3. Mean number of diamondback moth larvae and pupaereated with biorational insecticides.

of diamondback moth larvae on all treated plants were significantly lower than the untreated control. Insecticide treatments also significantly reduced diamondback moth pupae/plant (3b).

Diamond back moth is a serious pest of cabbage and other cruciferous plants. To protect cabbage and other plants, growers use various insecticides frequently to control diamondback moth. The diamondback moth has developed resistance to various insecticides (Hama, 1987). Tanaka and Kimura (1990) reported reduced susceptibility of *Bacillus thuringiensis* Berliner. Hama et al. (1990) reported that the resistance development in diamondback moth to *B. thuringiensis* is genetic. In the present study, we observed variation (high and low) in the effectiveness of *B. thuringiensis* based insecticides in different populations. Knack® is a growth regulator which provided significant reductions of diamondback moth larvae and pupae. In our previous study, Novaluron, another growth regulator, showed promise in controlling fall armyworm. These populations of diamondback moth were collected from the United States, Mexico, and Thailand.

Literature Cited

Ankersmit, O.W. 1953. DDT resistance in *Plutella maculipennis* (Curt.) (Lepidoptera) in Java. Bull. Entomol.Res. 44:421–25.

Gupta, P.D. and A.J. Thorsteinson.1960. Food plant relationship of diamondback moth [*Plutella maculipennis* (Curt.)]. I. Gustation and olfaction in relation to botanical specificity of larva. Entomol. Exp. Appl. 3: 241-50. https://doi.org/10.1111/j.1570-7458.1960.tb00454.x

Hama, H. 1987. Development of pyrethroid resistance in the diamondback moth, *Plutella xylostella* Linne (Lepidoptera: Yponomeutidae). Appl. Entomol. Zool., 22:166–175.

Hama, H., T. Suzuki, and H. Tanaka. 1990. BT resistance of diamondback moth. Cross-resistance and heredity. Proc. 34th Congress of Japanese Applied Entomol. Zool. p. 235 (in Japanese).

Harcourt, D.G. 1954. The biology and ecology of the diamondback moth, *Plutella maculipennis*, Curtis, in Eastern Ontario. PhD. thesis. Cornell Univ., Ithaca, N.Y. 107 pp.

Harcourt, D.G. 1957. Biology of diamondback moth, *Plutella maculipennis* (Curt.) (Lepidoptera: Plutellidae) in Eastern Ontario. II. Life history, behavior and host relationship. Can. Entomol. 89:554–64.

Hill, G. 1993. Diamondback moth and other crucifer pests. Proc. Second. Intl. Workshop, Tainan, Taiwan, 10-14 December 1990. N.S. Talekar (Ed.) Asian Vegetable Research and Development Center, 1992. 603 p.

Johnson, D. R. 1953. *Plutella maculipennis* resistance to DDT in Java. J. Econ. Enlomol. 46: 1-76.

Kanervo, V. 1936. The diamondback moth (*P. maculipennis* Curt.) as a pest of cruciferous plants in Finland. Valt. Maatalouskoetoiminnan Julk. 86:1–86 (In Finnish with English summary).

Louda, S.M. 1986. Insect herbivory in response to root-cutting and flooding stress on native crucifer under field conditions. Acta Oecol. Gen. 7:37–53.

Noble, C.V., R.W. Drew, and V. Slabaugh. 1996. Soil survey of Dade County Area, Florida. U.S. Dept. Agric., Natural Resources Conservation Serv., Washington D.C.

Ohbayashi, K., M. Anma, Y. Takai and H. Hayakawa. 1990. High-T_c (95 K) As-Grown superconducting Bi-Sr-Ca-Cu-O Thin Films. Japanese J. Appl. Physics 29: 2048-2052.

Rai, J. P. N., R. S. Tripathi. 1985. Effect of herbivory by the slug, *Mariaella dussumieri*, and certain insects on growth and competitive success of two sympatric annual weeds. Agric. Ecosyst. Environ. 13: 125-37.

Tanaka, H. and H. Kimura. 1990. Decrease in susceptibility to BT insecticide. Proceedings of the 34th Congress of Japanese Applied Entomology and Zoology, 235 (in Japanese).

Thorsteinson, A. J. 1953. The chemotacticresponses that determine host-specificity in an oligophagous insect (*Plutella maculipennis* (Curt.): lepidoptera). Can. J. Zool. 3 1: 52-72.

Thorsteinson, A. J. 1 955. The experimental study of the chemotactic basis of host specificity in phytophagous insects. Can. Entomol. 33: 49-57.

Proc. Fla. State Hort. Soc. 132:131–132. 2019.

The Current Status of Fungicidal Control on Foliar Blights of Sweet Corn

Christian F. Miller*[1], Eugene McAvoy[2], and Richard N. Raid[3]

[1]*University of Florida/IFAS, Palm Beach County Extension, 559 N. Military Trail, West Palm Beach, FL 33415*

[2]*University of Florida/IFAS, Hendry County Extension, P.O. Box 68, LaBelle, FL 33975*

[3]*University of Florida/IFAS, Everglades Research and Education Center, 3200 Palm Beach Rd., Belle Glade, FL 33430*

Additional index words. blight, fungicide, management, sweet corn

The objective of this research was to evaluate the efficacy of new and existing fungicides registered for use on sweet corn.

Florida leads the nation in fresh market sweet corn production, with the lion's share based in Palm Beach, Miami-Dade, Collier and Hendry Counties. Grown on an estimated 35,000 acres, the bulk of this $160 million crop is produced during the spring months, with peak harvests occurring March through May. A major expense for sweet corn growers is disease management. Two diseases of critical importance to Florida sweet corn growers are Southern Corn Leaf Blight (SCLB) and Northern Corn Leaf Blight (NCLB) caused by *Bipolaris maydis* and *Exserohilum turcicum* respectively. These diseases not only cause necrosis of the foliage, they also negatively affect the marketability of this fresh market crop by infecting ear flag leaves. Two fungicide field experiments were conducted during the Spring 2017 growing season using new and existing fungicides to manage NCLB, Florida's most important spring season foliar disease. Sweet corn of the NCLB susceptible variety 'AC7112R' was seeded in rows on 2.5-ft. centers with 8-in. in-row spacing. Experimental units were two rows by 25 ft. long, bordered on the ends by 7-ft. alleys. Trials were established in a randomized complete-block design with four replications. A minimum of three non-sprayed guard rows served as spreader rows between replications facilitating inoculum buildup for disease evaluation. Environmental conditions for disease development were excellent in both trials, with frequent dews providing for long periods of leaf wetness for profuse sporulation, and cool to moderate temperatures favoring good spore germination and infection. Fungicide sprays were applied using a CO_2 backpack sprayer equipped with a hand-held three nozzle boom, with one nozzle directed over the row, and two nozzles (TeeJet 11004s) on 16-in. drops directed inward for maximizing coverage. Rows were sprayed singly. All fungicides were applied at 30 psi using a nonionic surfactant at 0.025% by volume in a spray volume of 70 GPA. Fungicides included in the first trial were Quilt Xcel® (azoxystrobin + propiconazole), Priaxor® (pyraclostrobin + fluxapyroxad), Trivapro® (azoxystrobin + propiconazole + benzovindiflipyr), Prosaro® (prothioconazole + tebuconazole), and Viathon® (potassium phosphite + tebuconazole) (Table 1). Applied three times at 7–10 day intervals, all provided excellent disease control, reducing final NCLB severities by an average of 62%. Percentages of marketable ears were significantly improved with fungicide treatment, being improved by an average of 78% over the untreated check. In the second trial, treatments consisted of Quilt Xcel® + Epidyn® (pydiflumetofen), Headline Amp® (pyraclostrobin + metconazole), Priaxor®, Stratego YLD® (trifloxystrobin + prothioconazole), Aproach Prima® (picoxystrobin + cyproconazole), and Fortix® (fluxastrobin + flutriafol), and a check. Again, all provided for significant disease control, with Quilt® + Epidyn®, Headline Amp®, and Priaxor® providing optimal control, followed by Fortix®, Stratego YLD®, and

Table 1. Percentage of northern corn leaf blight severity and marketable ears resulting from fungicide treatments in two trials conducted during Spring 2017.

Treatment and rate	NCLB severity (%)			Marketable
of product/A	30 Mar.	7 Apr.	15 Apr.	ears (%)
Trial 1				
Untreated check	23 a[z]	34 a	78 a	14 c
Quilt Xcel SC 14.0 fl oz	7 b	11 bc	17 bc	92 ab
Priaxor SC 8.0 fl oz	5 b	13 b	15 bc	94 ab
Trivapro SE 10.5 fl oz	6 b	14 b	18 bc	91 ab
Viathon SE 3.0 pt	5 b	8 bc	8 c	95 a
Prosaro SC 8.0 fl oz	6 b	9 bc	22 b	89 b
Trial 2				
Untreated check	24 a	36 a	75 a	18 c
Quilt Xcel SC 10.5 fl oz + Epidyn SE 4.0 fl oz	3 b	5 c	6 d	98 a
Headline Amp 10.0 fl oz	3 b	4 c	6 d	97 a
Priaxor SC 4.0 fl oz	5 b	7 bc	9 cd	96 a
Stratego YLD SC 4.0 fl oz	5 b	7 bc	16 b	90 b
Aproach Prima SC 6.8 fl oz	7 b	10 b	19 b	88 b
Fortix SC 5.0 fl oz	5 b	6 c	11 c	90 b

[z]Numbers within a column followed by a letter in common are not significantly different as determined by Fishers LSD at $P < 0.05$.

*Corresponding author. Email: cfmiller@ufl.edu

Aproach Prima®, respectively. All provided for significantly higher percentages of marketable yield, improving marketable yield by an average of 75% over the untreated check. In summary, the levels of NCLB and the resultant low marketability observed in the untreated check demonstrate the destructive potential of this foliar disease if left unmanaged. The fungicides included in the two trials, combinations of strobilurins (FRAC group 11), triazoles (FRAC group 3), and carboxamides, (FRAC group 7), all performed well, providing season long NCLB management with only three applications under heavy disease pressure conditions. This should bode well for sweet corn growers, who should remain mindful of using host plant resistance when available, and rotating these premixtures with broad spectrum protectants such as mancozeb and chlorothalonil.

Proc. Fla. State Hort. Soc. 132:133–138. 2019.

Management of the Pepper Weevil, *Anthonomus eugenii* Cano using Biorational Insecticides and Aggregation Pheromone

Victoria O. Adeleye* and Dakshina Seal

Tropical Research and Education Center, University of Florida/IFAS, 18905 SW 280th St., Homestead, FL. 33031

Additional index words. Infestation, initiation, marketable, population, resistance, sustainable

Pepper, (*Capsicum annum* L.) is an important crop in Florida and several other states in the southern United States. Pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae), is the most harmful insect pest of pepper in Florida and other tropical and subtropical regions of North, Central and South America. We conducted studies using various biorational insecticides in the presence and absence of pheromones in 2018 and 2019. Pepper weevil adults were observed on all treated plants and did not differ from the untreated control. We recorded infested fruit in all treatments with higher numbers at the end of the season. Mean numbers of infested fruit were numerically fewer in Xpectro-treated plants than the untreated control. This information will provide insight in developing sustainable approach for management of pepper weevil.

Pepper, *Capsicum annum L.* is an important crop in Florida and several other states in the southern United States. Florida is the second largest producer of bell pepper in the United States. Total harvested U.S. acreage declined from 62,080 acres in 2000 to 40,900 acres in 2015, while during the same period it declined from 18,400 acres to 12,200 acres in Florida (National Agricultural Statistics Service 2017; Biswas et al. 2018). Peppers are grown in open fields using a raised bed system covered with plastic mulch with drip tubes for irrigation. Pepper is produced using conventional methods where growers depend mainly on the use of broad-spectrum insecticides to reduce pest populations that may cause damage to the crop, thereby increasing yield (Vasquez et al., 2005; Servin-Villegas et al., 2008).

Pepper is also often grown in greenhouses. In Florida, constraints at the level of production include attacks by pest insects and diseases. Insect pests are one of the limitations in growing pepper. Pepper weevil (PW), *Anthonomus eugenii* Cano (Coleoptera: Curculionidae), is the most harmful insect pest of pepper in Florida and other tropical and subtropical regions of North, Central and South America (Elmore et al., 1934; Riley and Sparks, 1995; Toapanta, 2001). High populations of this pest cause damage in fruit, flowers and buds through adult oviposition and larval feeding leading to a reduction in pepper fruit production (Rodriguez-Leyva, 2006). The major tool used by growers for the control of pepper weevil is the use of broad-spectrum insecticides (Andrews et al., 1986; Seal and Lamberts, 2012). The repeated use of broad spectrum insecticides has led to the development of resistance and elimination of pepper weevil's natural enemies (Andrews et al. 1986; Servin-Villegas et al. 2008). The search for alternative strategies for minimizing the use of broad-spectrum insecticides and reducing risks to both the environment and humans has been on the rise for decades. To avoid sole reliance on broad spectrum insecticides, it is important to integrate other control strategies, such as the use of biorational insecticides and the attract and kill method using pheromones with yellow sticky traps (Pedigo and Rice 2009).

Pheromones are an important tool used in pest management. They are semiochemicals that aid communication between organisms of the same species. Two common types of pheromones are sex and aggregation pheromone. Sex pheromones are released by an organism to signal to potential mates of the opposite sex while aggregation pheromones serve many functions including group aggregation to food sources, mass attack and mate selection (Tewari et al., 2014). Pheromones are used in pest management for monitoring, mass trapping, mating disruption and early detection of pests (Tewari et al., 2014). Aggregation pheromones produced by different species of the genus *Anthonomus* vary in abundance and distribution. The primary components of the aggregation pheromone in pepper weevil are the Z grandlure II, E grandlure II, grandlure III, grandlure IV, geraniol and geranic acid (Eller et al., 1994; Tewari et al., 2014). Pheromone based scouting is more efficient than visual scouting when pest populations are low, therefore it is a useful tool for monitoring and early detection (Witzgall et al., 2010).

Since highly toxic insecticides are a great threat to natural or beneficial organisms, the use of reduced risk insecticides could be a better approach in reducing the harmful effects of broad spectrum insecticides (Aktar et al., 2009). Reduced risk insecticides or biorational insecticides are natural or synthetic products with new modes of action which pose less risk to beneficial insects, other non-target organisms and the environment (Hara, 2000; Reddy et al., 2016). To our knowledge, there are only few studies on the management of pepper weevil using biorational insecticides (Addesso et al., 2014), but other studies have shown that biorational insecticides are promising for the control of insect pests and other weevils in different genera.

There is an immediate need to develop a sustainable strategy to control pepper weevil that does not enhance insecticide resistance. For this reason, the present research aims at developing alterna-

*Corresponding author. Email: vadeleye@ufl.edu

tive management strategies for the control of pepper weevil on jalapeño peppers under field conditions. Specifically, the main objective of this research was to evaluate the use of reduced risk insecticides and pheromones in suppressing pepper weevil. We also determined marketable yield of jalapeño pepper. We hypothesize that the biorational insecticides in combination with aggregation pheromone will significantly reduce PW adult population, reduce the number of infested fruit and there will be an increase in yield compared to the study with only biorational insecticides.

Materials and Methods

Study site and area. The study was carried out from Nov. 2017 to Mar. 2018 and Feb. 2019 to May 2019 at the Tropical Research and Education Center (TREC) in block 1 (25.513°N, –80.504°W) and block 3 (25.511°N, –80.501°W) research field plots. The soil type of the plot is a Krome gravelly loam soil classified as a loamy-skeletal, carbonatic hyperthermia lithic rendoll, which consists of 67% limestone pebbles (>2 mm) and 33% finer particles.

Plant material. Jalapeño pepper seeds were placed in Styrofoam seed starting trays (Seedling, Inc., Sun City, FL) filled with Pro-Mix growing medium in the greenhouse. Six weeks later, the seedlings were transplanted to the field.

Field preparation. Soil was prepared by ploughing field with a mold board plough (CASE IH agriculture) and then the field was disked using disking machine (Athens Plow Co Inc., TN). Raised beds were 91 cm wide and 15 cm high with 1.83 m between centers. Granular fertilizer (6 N–12 P–12 K) at the rate of 1344 Kg (1200 lb/acre) per ha was broadcast at planting and incorporated before covering the beds with plastic mulch (manufactured by Canslit Inc. Victoriaville, Quebec, Canada, and supplied by IMAFLEX USA Inc.) The raised beds were prepared with Kennco superbedders (Kenco Manufacturing Co. Inc., Atoka, OK). The polyethylene mulch was placed on the beds using a plastic layer (Kennco micro-combo, Kenco Manufacturing Co. Inc., Atoka, OK). At the time of laying the plastic, two drip tubes (RO-Drip) with emitters spaced 30 cm apart, one on each side of the plant row, spacing 15 cm were placed for irrigation. Holes (7 cm diameter) were made manually using a metallic hole digger maintaining standard spacing between each transplant. Jalapeño pepper transplants were planted in each plot for all studies with 31 cm (12 inch) spacing within the bed.

Experimental design and treatments. Five treatments including the control were arranged in a randomized complete block design and replicated four times. Each treatment plot was 6.096 m (20 ft) long with a 1.524 m (5 ft) unplanted buffer area separating treatments within the block with blocks separated by a 3.048 m (10 ft) unplanted area. The whole study was conducted in two blocks (1 and 3) separated 304.8 m (1000 ft) from one another. Block 1 had the study with biorational insecticides only while Block 3 had the study with biorational insecticides in combination with aggregation pheromone traps. The treatments evaluated in the field with the presence or absence of pheromone traps include: Asana® XL (esfenvalerate, DuPont™ IRAC group 3A Insecticide, 8.0 oz/acre) (synthetic pyrethroid and standard insecticide used for the experiment); Xpectro® (refined pyrethrum extract and *Beauveria bassiana* GHA strain, LAM International, 1.0 qt/acre); Venerate™ XC (heat-killed *Burkholderia* spp. strain A396 cells and spent fermentation media, 3 qt/acre); Spear®-T liquid concentrate (GS-Omega/Kappa-Hxtx-Hv1a, Vestaron Corporation, 4 lb/acre) and the control. Insecticides were applied on foliage by using a hand propelled backpack sprayer delivering 30 GPA at 30 psi.

The plots where pheromone traps were used had Trece Pherocon® traps consisting of pheromone septum and yellow sticky plastic cards to hold trapped weevils. Pheromone traps were placed at each corner of the field the same day jalapeño peppers were transplanted and was checked every three days for trapped pepper weevil adults.

Crop management. Pepper plants were subjected to recommended cultural practices (as mentioned in Florida Vegetable Hand Book) which include irrigation, weeding, fertilizer applications, etc. throughout the season. After planting, plots were subjected to close observation to scout for the presence and infestation of pepper weevil. Plants were irrigated twice a day (10: 00 am and 4:00 pm) for half an hour using the drip irrigation system already described. Immediately after transplanting, the base of each transplant was drenched with starter fertilizer solution (20 N–20 P–20 K) (0.75 oz/gal of water) (Diamond R Fertilizer Inc. Ft. Pierce, FL) using a back-pack sprayer without a nozzle tip. Granular fertilizer (N-P-K: 6:6:6) (Loveland Products Inc., Greely, CO) was applied every three weeks after planting. The fertilizer was applied 20 cm from and parallel to the side of the transplants or plants and incorporated within the top 15 cm of the soil.

Application and evaluation of insecticide treatments. Application of insecticide treatments was initiated four weeks after planting. The first application for Spring 2018 was on 29 Dec. and on 29 Feb. for Spring 2019. Applications continued at seven days intervals for eight weeks in 2018 and seven weeks in 2019. Evaluation of insecticide treatments were made by thoroughly checking randomly selected five plants for PW adults in each treatment plot 24 h after each spray application. All counting was done between 10 am and 12 pm. On the same day, all infested fruits were also collected. We also checked each pheromone trap with a yellow sticky card for the number of trapped pepper weevil adults. At harvest all fruit/plot were collected and graded as marketable and unmarketable based on U.S. grade standards. The marketable fruit were counted and weighed. Trapped PW adults were counted every three days and traps were replaced every two weeks.

Statistical analysis. Data were analyzed using the Statistical Analysis System. Data were square root transformed before analysis to meet the assumption of normality and to fit the model. The non-transformed means were reported while transformed data were analyzed using analysis of variance [ANOVA (PROC GLM, SAS Institute Inc. Cary, NC, 2013)]. For adults on plants and number of fallen fruit, when the F-value was significant, differences among means were separated using Tukey´s HSD (honestly significant difference) Test procedure in SAS. All data were analyzed at the 5% level of significance.

Results

Abundance of pepper weevil adults on jalapeño pepper plants

Pepper weevil adults were not recorded on any plants, irrespective of treatments, on the first three sampling dates in the field in absence of pheromone traps (Table 1). Pepper weevil adults were first recorded in this field on the fourth sampling date. Mean number of adults increased on subsequent sampling dates on all treated plants although there were no significant differences between treatments on any sampling date. When means across the sampling dates were considered, numerically higher numbers of adults were recorded on plants treated with Spear®-T followed by the untreated control.

In the presence of the pheromone, pepper weevil adults were recorded on all sampling dates except the first (Table 2). Mean numbers of adults on treated plants did not differ statistically on any of the sampling date when compared with the untreated control. When means across the sampling dates were compared, numerically higher number of adults were observed on plants treated with Asana® XL followed by Venerate™ XC.

Abundance of pepper weevil infested jalapeño pepper fruit

Infested fallen fruits in the absence of the pheromone in 2018. Like the adult PW, infested fruits were almost absent on the first four sampling dates in the absence of the pheromone treatment in 2018 (Table 3). Mean numbers of fallen fruit increased thereafter with the peak number of infested fruits recorded on the last sampling date, 14 Mar. Mean numbers of infested fruits in treated plots, irrespective of treatment, did not differ statistically from the untreated control.

Infested fallen fruits in the presence of the pheromone in 2018. In the presence of pheromone traps in 2018, pepper weevil infested fruits were recorded on all sampling dates with the peak on the last sampling date 14 Mar. (Table 4). On this sampling date, numerically fewer infested fruits were recorded in all plants treated with biorational insecticides. Xpectro®, Spear®-T and Venerate™ XC reduced the number of PW infested fruits by 37.4%, 14.25% and 7.91% respectively as compared to the control. The means across all sampling dates also showed numerically fewer infested fruits in plants treated with biorational insecticides. However, treatments did not differ from the untreated control in the mean number of infested fruits.

Infested fallen fruits in the absence of the pheromone in 2019. We repeated the 2018 study in 2019. Mean numbers of infested fruits were collected on all sampling dates in the absence of pheromone traps (Table 5). Mean numbers of infested fruits sharply increased on the fifth, sixth and seventh sampling dates, although treatments did not differ statistically from the untreated control. When means across all sampling dates were considered, mean percentages of pepper weevil infested fruitss decreased by 43.11% and 34.08% in Asana® XL and Xpectro® treated plants, respectively when compared to the untreated control.

Infested fallen fruits in the presence of the pheromone in 2019. When pheromone traps were used, mean numbers of infested fruits decreased in all plants irrespective of treatment

Table 1: Mean numbers of adults/five jalapeño pepper plants chosen randomly per treatment plot on different sampling dates in the field without pheromone Spring 2019.

		Sampling dates							
Treatment	Rate/acre	1 Mar.	8 Mar.	15 Mar.	23 Mar.	29 Mar.	5 Apr.	12 Apr.	Average
Asana	8 oz	0 a[z]	0 a	0 a	0 a	0.1 a	0.25 a	0.55 a	0.13 a
Xpectro	1 qt	0 a	0 a	0 a	0.05 a	0.05 a	0.15 a	1.05 a	0.19 a
Spear	4 lb	0 a	0 a	0 a	0.05 a	0.1 a	0.55 a	1.05 a	0.25 a
Venerate	3 qt	0 a	0 a	0 a	0.05 a	0.1 a	0 a	0.45 a	0.09 a
Control		0 a	0 a	0 a	0.2 a	0.15 a	0.3 a	0.9 a	0.22 a
ANOVA		F = 0; P = 0	F = 0; P = 0	F = 0; P = 0	F = 0.91; P = 0.46	F = 0.27; P = 0.90	F = 3.08; P = 0.02	F = 2.12; P = 0.08	

[z]Means followed by the same letter in each column are not significantly different.

Table 2. Mean numbers of adults/five jalapeño pepper plants chosen randomly per treatment plot on different sampling dates in the field with pheromone Spring 2019.

		Sampling dates							
Treatment	Rate/acre	1 Mar.	8 Mar.	15 Mar.	23 Mar.	29 Mar.	5 Apr.	12 Apr.	Average
Asana	8 oz	0 a[z]	0.1 a	0.2 a	0.05 a	0.45 a	0.7 a	0.6 a	0.3 a
Xpectro	1 qt	0 a	0 a	0.05 a	0.15 a	0 a	0.35 a	0.45 a	0.15 a
Spear	4 lb	0 a	0.1 a	0.2 a	0.15 a	0.15 a	0.2 a	0.55 a	0.19 a
Venerate	3 qt	0 a	0.05 a	0.1 a	0.25 a	0.1 a	0.25 a	0.85 a	0.23 a
Control		0 a	0 a	0.05 a	0.1 a	0 a	0.3 a	0.85 a	0.19 a
ANOVA		F = 0; P = 0	F = 1.04; P = 0.39	F = 0.57; P = 0.68	F = 0.77; P = 0.55	F = 2.08; P = 0.09	F = 1.70; P = 0.16	F = 1.12; P = 0.35	

[z]Means followed by the same letter in each column are not significantly different.

Table 3. Mean numbers of fallen infested fruits/treatment plot on different sampling dates in the absence of pheromone in Spring 2018.

Treatment	Rate/acre	16 Jan.	22 Jan.	31 Jan.	9 Feb.	19 Feb.	25 Feb.	4 Mar.	14. Mar.	Average
Asana	8 oz	0 a[z]	0 a	0.25 a	0 a	1.75 a	10.5 a	18.5 a	66.25 b	12.16 a
Xpectro	1 qt	0 a	0 a	0.25 a	0 a	2.5 a	33 a	50 a	147 a	29.1 a
Spear	4 lb	0 a	0 a	0.25 a	0 a	1.75 a	24.75 a	17 a	110.75 ab	19.31 a
Venerate	3 qt	0 a	0 a	0 a	0 a	2.5 a	20.25 a	40.75 a	100.5 ab	20.5 a
Control		0 a	0 a	0.25 a	0.25 a	1.75 a	18.25 a	33.25 a	98.25 ab	19 a

[z]Means followed by the same letter in each column are not significantly different.

Table 4. Mean numbers of fallen infested fruit/treatment plot on different sampling dates in the presence of pheromone in Spring 2018.

Treatment	Rate/acre	16 Jan.	22 Jan.	31 Jan.	9 Feb.	19 Feb.	25 Feb.	4 Mar.	14. Mar.	Average
Asana	8 oz	0 a[z]	0.6 a	0.4 a	2 a	25.6 a	58 ab	47.4 b	68.4 b	25.3 a
Xpectro	1 qt	0 a	0.2 a	0 a	0.6 a	12.8 a	33.8 b	43 b	116.6 ab	25.88 a
Spear	4 lb	0 a	0.2 a	0.6 a	2 a	35.2 a	108.4 a	122.4 a	162.4 ab	53.9 a
Venerate	3 qt	0 a	0.2 a	1.6 a	1.8 a	36.8 a	106.6 a	119.8 a	174.4 ab	55.15 a
Control		0 a	0.2 a	2 a	2.2 a	34 a	105.4 a	115.6 a	189.4 a	56.1 a

[z]Means followed by the same letter in each column are not significantly different.

Table 5. Mean numbers of fallen infested fruit/treatment plot on different sampling dates in the absence of pheromone in Spring 2019.

		Sampling dates								
Treatment	Rate/acre	15 Mar.	23. Mar	29 Mar.	5 Apr.	12 Apr.	19 Apr.	26 Apr.	6 May	Average
Asana	8 oz	0.50 a[z]	3 a	2.75 a	54 a	67 b	106.5 a	75.25 a	37.75 a	43.34 a
Xpectro	1 qt	2.25 a	2.50 a	5.25 a	55.25 a	76.75 ab	111.75 ab	79.25 a	68.75 a	50.22 a
Spear	4 lb	1.25 a	4.75 a	13.25 a	10.35 a	178.5 a	203.75 a	104.5 a	45 a	81.81 a
Venerate	3 qt	0 a	1.50 a	7.5 a	87 a	131.75 ab	170.5 ab	130.5 a	73 a	75.22 a
Control		0 a	1.25 a	8.5 a	93 a	141 ab	181.5 ab	113.25 a	71 a	76.19 a

[z]Means followed by the same letter in each column are not significantly different.

Table 6. Mean numbers of fallen infested fruit/treatment plot on different sampling dates in the presence of pheromone in Spring 2019.

		Sampling dates								
Treatment	Rate/acre	15 Mar.	23. Mar	29 Mar.	5 Apr.	12 Apr.	19 Apr.	26 Apr.	6 May	Average
Asana	8 oz	1.5 a[z]	4.75 a	1.63 a	11.5 a	5 a	6.88 b	27.75 b	26.88 b	10.74 a
Xpectro	1 qt	1 a	3.13 a	1.13 a	7.13 a	2.75 a	17.75 ab	38.88 ab	58.38 ab	16.27 a
Spear	4 lb	2.75 a	7.5 a	3.5 a	10.88 a	12.75 a	33.25 ab	40.25 ab	52 ab	20.36 a
Venerate	3 qt	0.88 a	6.75 a	3.25 a	16.63 a	15.38 a	44.63 a	57.13 ab	66.25 ab	26.36 a
Control		1 a	3.75 a	1.88 a	14.88 a	11.88 a	37.13 a	74.25 a	90.38 a	29.39 a

[z]Means followed by the same letter in each column are not significantly different.

and sampling dates in 2019 (Table 6). Mean numbers of infested fruits sharply increased on the sixth, seventh and eighth sampling dates in all treated and untreated plants. When means across the sampling dates are considered, numerically fewer numbers of infested fruits were recorded in all treated plants with the lowest number for Asana® XL plots when compared with the untreated control. Mean percentages of decrease in infested fallen fruits in different treatments were 63.45%, 44.64%, 30.72% and 10.30% in plots treated with Asana® XL, Xpectro®, Spear®-T and Venerate™ XC, respectively. However, none of the treatments differed significantly on any of the sampling dates when compared with the untreated control.

Marketable weight at harvest

Mean weight of marketable fruit at harvest did not differ among insecticide treatments and the untreated control in the treatments with the pheromone in either 2018 or 2019 and in the treatments without the pheromone in 2019 (Table 7). In the treatments without the pheromone in 2018, the highest amount of marketable fruit was from Venerate™ XC treated plots and the lowest was from the untreated control. In summary, marketable weight was higher in 2018 than in 2019.

Table 7. Weight of marketable fruit in pounds/acre.

		WTP	WP	WTP	WP
Treatment	Rate/acre	(2018)[y]	(2018)	(2019)	(2019)
Asana	8 oz	7783 ab[z]	2904 a	99.89 a	10.5 a
Xpectro	1 qt	9453 ab	1888 a	58 a	12.99 a
Spear	4 lb	9707 ab	3485 a	194.29 a	18.45 a
Venerate	3 qt	11,522 a	2701 a	151.1 a	10.39 a
Control		5699 b	3136 a	159.51 a	9.45 a

[z]Means followed by the same letter in each column are not significantly different at 0.05%.

[y]WP = with pheromone; WTP = without pheromone.

Adults trapped by the Trece Pherocon® traps

Pepper weevil adults were absent on the Trece Pherocon® traps in the first four weeks and on the first two sampling dates in 2018 and 2019, respectively. Pepper weevil adult numbers increased on the traps and remained high for subsequent weeks (Figs. 1 and 2).

Discussion

The major management tool used for the control of pepper weevil is the use of conventional insecticides. Even with these insecticides, control is difficult especially when population pressure from pepper weevils is high (Seal and Lamberts, 2012; Addesso et al., 2014). Actara® (Thiamethoxam, Syngenta Crop Protection, Inc.) and Vydate® (Oxamyl, DuPont) are standard insecticides used in rotation by growers to maintain low pepper weevil populations and increase yield (Addesso et al., 2014). Susceptibility of pepper weevil populations to these insecticides depends on the history of the type of insecticide used in a given location. It was reported that pepper weevil adults were more susceptible to some groups of insecticides in one location than in another (Servin-Villegas et al., 2008). Alternative management

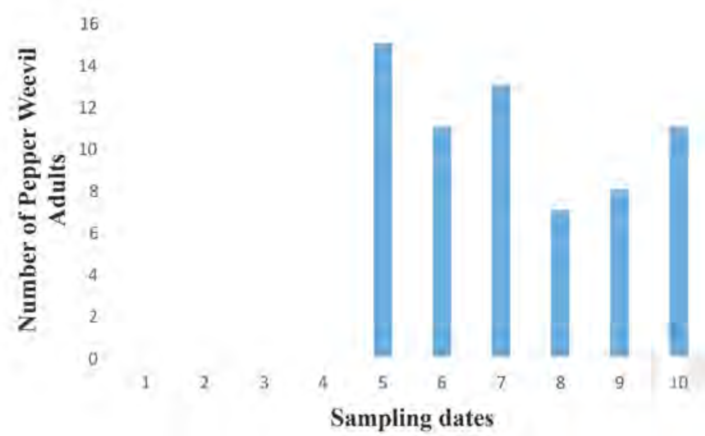

Fig. 1. Number of pepper weevil adults recorded on the Trece Pherocon® trap on various sampling dates, Spring 2018.

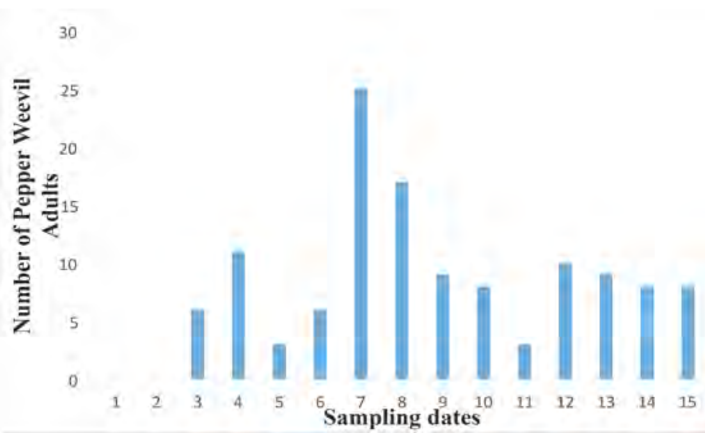

Fig. 2. Number of pepper weevil adults recorded on the Trece Pherocon® trap on various sampling dates, Spring 2019.

strategies are being sought to reduce the dependence on broad spectrum insecticides. Addesso et al. (2014) reported that the standard insecticides used in rotation performed better in providing an increase in marketable yield when compared to the untreated control. Reduced risks product including Requiem® (Mexican tea extract) and diatomaceous earth reduced oviposition and feeding damage in lab trials. Addesso et al. (2014) suggested the integration of the reduced risk products in management programs for pepper weevil control. Rotation of insecticides with different modes of action is a better option to reduce the rate at which pepper weevils develop resistance. Biorational insecticides have been evaluated for the control of various pests including pecan insect pests (Shapiro-Ilan et al., 2013), cereal aphids (Sallam et al., 2009), onion thrips (Mahmoud and Osman, 2007), and other weevil species including boll weevil (Wright and Chandler 1992), although few studies using biorational insecticides have been conducted on pepper weevil (Garcia-Nevarez et al. 2012; Addesso et al., 2014). In one of our studies in the presence of pheromone traps, Xpectro® reduced pepper weevil infested fruits 37.4% by as compared to the untreated control. More studies are needed to evaluate the use of different biorational insecticides in rotation with conventional or other type of biorational insecticides against pepper weevil.

Conclusions

Results from Springs 2018 and Spring 2019 showed that higher number of adults PW were found in plots with pheromone traps while higher marketable yields were observed in plots without pheromone traps in 2018 although statistically there were no significant differences between treatments. The reverse was the case in 2019, though overall marketable yield in both blocks (1 and 3) was low. We speculated that this could be due to changes in the physical environment, plant health at the time of planting, and changes in the pepper agroecosystem. Pepper weevil infestations were higher in the treatments with pheromone traps and as a result, marketable yield was lower with pheromone traps. We will repeat the experiment in Spring 2020 and have three replicates simultaneously to determine if there is a similar trend with the results obtained in 2018 or 2019. This research will help us determine a proper management strategy and deceide whether pheromone traps are having a positive or negative impact on the abundance and infestation of pepper weevils. We recommend the rotation of insecticides as it can help develop a more sustainable approach to pepper weevil management.

Literature Cited

Addesso, K.M., P.A. Stansly, B.C. Kostyk, and H.J. McAuslane. 2014. Organic treatments for control of pepper weevil (Coleoptera: Curculionidae). BioOne Complete. 97:1148–1156.

Aktar, W., D. Sengupta, and A. Chowdhury. 2009. Impact of pesticides use in agriculture: Their benefits and hazards. Interdisciplinary toxicology. 2:1–12.

Andrews, K.L., A. Rueda, G. Gandini, S. Evans, A. Arango, and M. Avedillo. 1986. A supervised control program for the pepper weevil, *Anthonomus eugenii* Cano, in Honduras, Central America. Intl. J. Pest Mgt. 32:1–4.

Biswas, T., Z. Guan and F. Wu. 2018. An overview of the U.S. bell pepper industry. EDIS. Publication #FE1028. University of Florida. Institute of Food and Agricultural Sciences. 21 Sept. 2019 <https://edis.ifas.ufl.edu/fe1028>

Eller, F.J., R.J. Bartelt, B.S. Shasha, D.J. Schuster, D.G. Riley, P.A. Stansly, T.F. Mueller, K.D. Shuler, B. Johnson, J.H. Davis, and C.A. Sutherland. 1994. Aggregation pheromone for the pepper weevil, *Anthonomus eugenii* CANO (Coleoptera: Curculionidae): identification and field activity. J. Chemical Ecol. 20:1537–1555.

Elmore, J.C., A.C. Davis and R.E. Campbell. 1934. The pepper weevil. USDA, Washington DC. Tech. Bull. No. 447. 27 p.

Garcia-Nevarez, G., M. Campos-Figueroa, N. Chavez-Sanchez and F. Quinones-Pando. 2012. Efficacy of Biorational and Conventional insecticides against the pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae) in the South-Central Chihuahua. Southwestern Entomologist. 37:391-401.

Hara A.H. 2000. Finding alternative ways to control alien pests—part 2: New insecticides introduced to fight old pests. Hawaii Landscape. 4:1–5.

Mahmoud, M.F. and M.A.M. Osman. 2007. Relative toxicity of some biorational insecticides to second instar larvae and adults of onion thrips (*Thrips tabaci* Lind.) and their predator *Orius albidipennis* under laboratory and field conditions. J. Plant Protection Res. 47:391–400.

Pedigo L.P. and M.E. Rice. 2009. Entomology and Pest Management, 6th ed. Prentice Hall, Upper Saddle River, New Jersey.

Reddy G.V.P., F.B. Antwi, G. Shrestha, and T. Kuriwada. 2016. Evaluation of toxicity of biorational insecticides against larvae of the alfalfa weevil. Toxicology Reports. 3:473–480.

Riley, D.G. and A.N. Sparks Jr. 1995. The pepper weevil and its management. Texas Agricultural Extension Service, Texas A&M University. College Station. L-5069.

Rodriguez-Leyva, E. 2006. Life history of *Triaspis eugenii* Wharton and Lopez-Martinez (Hymenoptera: Braconidae) and evaluation of its potential for biological control of pepper weevil *Anthonomus eugenii* Cano (Coleoptera: Curculionidae). Doctoral Dissertation, University of Florida.

SAS Institute. 2013. Statistical Analysis Systems for Windows. Cary, NC.

Sallam, A.A., C. Volkmar and N.E. El-Wakeil. 2009. Effectiveness of different biorational insecticides applied on wheat plants to control cereal aphids. J. Plant Diseases and Protection. 116:283–287.

Seal D.R. and M.L. Lamberts. 2012. Pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae), an important pest of pepper. The Vegetarian Newsletter, No. 574, University of Florida, Gainesville, FL.

Servin-Villegas, R., J. Garcia-Hernandez, A. Tejas-Romero, J.L. Martinez-Carrillo and M.A. Toapanta. 2008. Susceptibility of pepper weevil (*Anthonomus eugenii* Cano) (Coleoptera: Curculionidae) to seven insecticides in rural areas of Baja California Sur, Mexico. Acta Zoologica Mexicana. 24:45–54.

Shapiro-Ilan, D.I., T.E. Cottrell, M.A. Jackson, and B.W. Wood. 2013. Control of key pecan insect pests using biorational pesticides. J. Economic Entomol. 106:257–266.

Tewari, S., T.C. Leskey, A.L. Nielsen, J.C. Piñero, and C.R. Rodriguez-Saona. 2014. Use of pheromones in insect pest management, with special attention to weevil pheromones. p. 141–168. In: D.P. Abrol (ed.). Integrated pest management: Current concepts and ecological perspective. ISBN: 9780123985293.

Toapanta, M.A. 2001. Population ecology, life history and biological control of the pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae). PhD. Dissertation, University of Florida, Gainesville, FL.

United States Department of Agriculture, National Agricultural Statistics Service (USDA-NASS). 2017. State agricultural overview. 21 Sept. 2019. <https://www.nass.usda.gov/>

Vasquez E., D. Dean, D. Schuster, and P. Van Etten. 2005. A laboratory method for rearing Catolaccus hunter (Hymenoptera: Pteromalidae), a parasitoid of the pepper weevil (Coleoptera: Curculionidae). Florida Entomologist. 88:191–195.

Witzgall, P., P. Kirsch, and A. Cork. 2010. Sex pheromones and their impact on pest management. J. Chem. Ecol. 36:80–100.

Wright, J.E. and L.D. Chandler. 1992. Development of a biorational mycoinsecticide: *Beauveria bassiana* conidial formulation and its application against boll weevil populations (Coleoptera: Curculionidae). J. Economic Entomol. 85:1130–1135.

Proc. Fla. State Hort. Soc. 132:139–140. 2019.

—Scientific Note—

Genotypic Differences In Specific Biomass of Tomato Grown In Buffered Low-phosphorus Hydroponics

Mary Dixon[1], Eric Simonne[1], Harry Klee[1], Thomas Obreza[2], and Guodong Liu*[1]

[1]*Horticultural Sciences Department, University of Florida/IFAS, P.O. Box 110690, Gainesville, FL 32611-0690*

[2]*Soil and Water Sciences Department, University of Florida/IFAS, P.O. Box 110290, Gainesville, FL 32611-0290*

Additional index words. elite tomato genotypes; phosphorus bioavailability; efficient use of phosphorus; specific biomass

Identifying genotypes efficient in acquiring phosphorus (P) from sparingly soluble phosphates may help reduce fertilizer requirements for tomato production. We measured the biomass of 19 wild genotypes selected from a wide phylogenetic range grown hydroponically with buffered low-P bioavailability.

Phosphate rock reserves are finite world-wide and soil P supplies may become unavailable from soil-P fixation (Schröder et al., 2011). Phosphate may precipitate with aluminum (Al) or ferric iron (Fe) forming aluminum phosphate ($AlPO_4$) or ferric phosphate ($FePO_4$) in acidic soil and with calcium (Ca) forming calcium phosphate [$Ca_3(PO_4)_2$] in alkaline soil (Wang and Nancollas, 2008). Proton exudation into the rhizosphere may enhance P bioavailability. The objectives of this experiment were to (i) explore differences in responses to low-P stress between tomato genotypes and (ii) investigate how low-P bioavailability impacts tomato growth.

Three-week-old tomato seedlings with 1–2 true leaves were grown for 28 d in a modified 25% Hoagland solution. There were two treatments: available-P as sodium dihydrogen phosphate (NaH_2PO_4) and sparingly available-P as tricalcium phosphate [$Ca_3(PO_4)_2$] (with added 15 mM extra $CaCl_2$) each as the only P source. Solution pH and dry root and shoot weight were measured after harvest. Relative and specific biomasses were calculated as:

$$RB = CB/TB \text{ and } SB = CB^2/TB$$

where RB, SB, TB, and CB are relative, specific, treatment, and control biomass, respectively. Biomass and solution pH data were analyzed for treatment and genotypic effects using R version 3.4.3, and means were compared using a two-way analysis of variance (ANOVA) and Tukey HSD test.

Solution pH and biomass varied significantly, particularly between ‘Heatwave II’ and ‘LA1777’ where ‘Heatwave II’ samples tended to be bigger and more flush than ‘LA1777’ samples. ‘Heatwave II’ had a more acidic solution and accumulated more shoot biomass, relative shoot biomass, and specific shoot biomass than ‘LA1777’. Shoot biomass and solution pH were greater with available P than with sparingly available P.

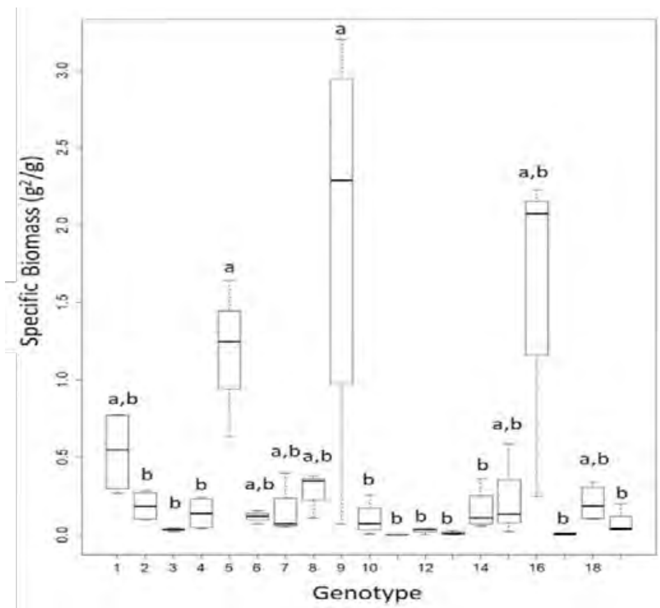

Fig. 1. Specific shoot biomass (g^2/g) of 19 tomato genotypes (corresponding number on X-axis): ‘Ailsa Craig’ (1), ‘Arkansas Traveler’ (2), ‘Bear Creek’ (3), ‘Brandywine Pink’ (4), ‘Cherry Roma’ (5), ‘Farthest North’ (6), ‘Florida 47’ (7), ‘FLA 8601’ (8), ‘Heatwave II’ (9), ‘Heinz 1706’ (10), ‘LA1777’ (11), ‘LA1162’ (12), ‘Money Maker’ (13), ‘Pearson’ (14), ‘Rio Grande’(15), ‘Roma’ (16), ‘Rutgers’ (17), ‘Super Strain B’ (18), ‘VF3’ (19). Means followed by different letters denote significant difference ($P < 0.05$) according to Tukey HSD test.

Solution pH may be genotype-dependent, supporting rhizosphere acidification being a response to low-P stress (Shen et al. 2006). Biomass of genotypes tested was lower with available-P than with sparingly available-P. These results show that decreased P bioavailability resulted in reduced growth. However, plants efficient in mobilizing bound-P grew well in the sparingly soluble P solution. These genotypes included ‘Cherry Roma’, ‘Heatwave II’, and ‘Roma’ (Fig. 1). ‘Heatwave II’ appears to be P-efficient while ‘LA1777’ is P-inefficient. Screening these genotypes helps to identify traits associated with enhanced P use efficiency, which may result in reduced fertilizer dependence.

*Corresponding author. Email: guodong@ufl.edu

P bioavailability may be reduced through adding counterions such as calcium ions or water stress such as PEG addition. P bioavailability can be enhanced through rhizosphere acidification or addition of chelating agents such as EDTA. Plans for future directions include investigating responses of the identified P-efficient and P-inefficient genotypes to P bioavailability as regulated through these additions.

Literature Cited

Schröder, J.J., A.L. Smit, D. Cordell, and A. Rosemarin. 2011. Improved phosphorus use efficiency in agriculture: A key requirement for its sustainable use. Chemosphere 84:822-831.<https://www.ncbi.nlm.nih.gov/pubmed/21349568>.

Shen, H., J. Chen, Z. Wang, C. Yang, T. Sasaki, Y. Yamamoto, H. Matsumoto, and X. Yan. 2006. Root plasma membrane H+-ATPase is involved in the adaptation of soybean to phosphorus starvation. J. of Expt. Bot. 57, 1353-1362, doi: 10.1093/jxb/erj111.

Wang, L. and G.H. Nancollas. 2008. Calcium orthophosphates: crystallization and dissolution. Chemical Reviews 108:4628-4669. doi: 10.1021/cr0782574

Proc. Fla. State Hort. Soc. 132:141. 2019.

—Scientific Note—

Genomic Prediction of Sweet Sorghum Agronomic Performance under Drought and Irrigated Environments in Haiti

Marie D. Dorval*[1], Geoffrey Meru[1], Gael Pressoir[2], John Erickson[3], and Wilfred Vermerris[4]

[1]*Tropical Research and Education Center, University of Florida/IFAS, 18905 SW 280th St., Homestead, FL 33031*

[2]*Research Center on Bio-energy and Sustainable Agriculture, University Quisqueya, Port-au-Prince, Haiti*

[3]*Agronomy Department, University of Florida/IFAS, P.O. Box 110500, Gainesville, FL 32611*

[4]*Microbiology and Cell Science Department, University of Florida/IFAS, P.O. Box 110700 Gainesville, FL 32611*

Additional index words. drought, genomic prediction, Haiti, sorghum breeding

The discovery and application of molecular markers such as single nucleotides polymorphism (SNP) and simple sequence repeats has revolutionized modern plant breeding. Through advancement in high-throughput sequencing platforms, previously expensive marker-genotyping platforms have become affordable for many plant-breeding programs. However, high-throughput phenotyping remains costly and inaccessible to most plant breeding programs. Over the last decade, genomic selection has gained momentum as a tool for predicting genetic gain in plant breeding populations, while lowering costs associated with phenotyping. Different statistical models and approaches have been developed to implement genomic selection (GS) in plant breeding, and strategies that promote accurate and resource-efficient prediction are of increasing interest. Since its establishment in 2010, the sweet sorghum breeding program at CHIBAS, Haiti, has led efforts to develop and release cultivars resilient to abiotic and biotic stress. Among abiotic constraints, drought stress is the most limiting since growers depend on erratic rainfall for sorghum production in Haiti. The goal of the present study was to predict the genomic estimated breeding values (GEBVs) of a sweet sorghum breeding population (n = 250) under contrasting environments in Haiti using four statistical models [Bayes A, B, C, and Bayesian ridge regression (BRR)]. We evaluated twelve sorghum traits across three environments (irrigated, preflowering water stress and vegetative water stress) and performed within and across environments prediction scenarios.

Results

The vegetative water stress was severe and led to substantial reductions in almost all the traits. The genomic heritability estimates varied substantially for all the traits across the three environments. The highest values were obtained for heading (0.82), days to maturity (0.82), soluble solids concentration (0.61), grain yield (0.61), and juice weight (0.60) in the preflowering water stress condition. Phenotypic correlations varied in terms of magnitude across the environments. Grain yield was positively and significantly correlated with all eleven traits in the irrigated environment. For the water stress environments (vegetative and preflowering water stress), no significant correlations were observed between grain yield, heading and maturity. Overall, the values varied from 0.16 to 0.65 for vegetative water stress, 0.29 to 0.82 for the irrigated condition and 0.35 to 0.82 for preflowering water stress. Overall, the four methods showed similar results, however Bayes B and BRR were superior in prediction accuracy and computation time, respectively. Generally, prediction accuracy was higher for within-environment (0.31–0.7) than across-environment (0.06–0.7) involving vegetative water stress scenarios. Prediction accuracy varied substantially for all traits, with total green leaf showing the highest mean value (0.70), and grain yield showing the least (0.49). Accuracies of genomic prediction obtained here are encouraging for implementation of GS in small breeding programs for drought tolerance.

*Corresponding author. Email: dorval.m@ufl.edu

Proc. Fla. State Hort. Soc. 132:142. 2019.

—Scientific Note—

How Does Fertigation Work for Potato Production In Florida

Xiangju Fu[1], Guodong Liu*[1], Lincoln Zotarelli[1], Steven Sargent[1], Crystal Snodgrass[2], and Alan Jones[3]

[1]*Horticultural Sciences, University of Florida/IFAS, P.O. Box 110690, Gainesville, FL 32611-0690*

[2]*Manatee County Extension, University of Florida/IFAS, 1303 17 St. W, Palmetto, FL 34221*

[3]*Jones Potato Farm, 13400 Dickey Rd., Parrish, FL 34219*

Additional index words. nitrogen; overhead irrigation; potato yield

Florida is forecasted to face a water shortage in 2050. Crop production is the largest consumer of fresh water and uses approximately 70% of water sources. Conservative water use is imperative for crop production in Florida. Our previous study showed that the conversion from seepage to center-pivot irrigation saved 58% of irrigation water for potato production in southwest Florida. Once the irrigation method has improved, the fertilizer program should be adjusted accordingly. The objective of this study was to evaluate the effect of fertigation through center-pivot irrigation on potato production in Florida.

As an important state for spring potato production, Florida's traditional field practices for potato production include seepage irrigation and dry granular fertilization. Seepage irrigation is not efficient for water use whereas sprinkler irrigation proved to save irrigation water by 58%, but overhead-irrigated potato crops had smaller tubers and lower yields with dry granular fertilizer programs. To improve potato yield with overhead irrigation, this on-farm study partially replaced dry granular fertilization with fertigation at a private potato farm in Manatee County, FL for 3 years. There were two treatments: 1) fertigation and 2) dry granular fertilization. The fertigation treatment used 75% of the nitrogen as a granular fertilizer, applied 25% pre-plant and 50% at emergence; the remaining 25% nitrogen was applied as a liquid fertilizer through center pivot irrigation in tuber initiation stage with 3–5 fertigation events. The dry granular fertilization treatment applied at 100% of the nitrogen as a granular fertilizer: 25% pre-plant, 50% at emergence, and 25% at the beginning of tuber initiation. The two treatments were side by side and used the same amount of fertilizers; there were four replications. In the 2015–16 growing season, chipping potato, 'Atlantic', was used. In the 2016–17 growing season, tablestock potato, 'Red LaSoda', was used. The results showed that the fertigation treatment had 24% and 25% greater marketable tuber yield than the dry granular fertilization treatment in the two growing seasons, respectively (Table 1). As the fertigation fertilizer program increased marketable tuber yield by more than 24% on average with the same amount of nitrogen, the cooperative growers reduced nitrogen by 30% for the fertigation treatment in the 2017–18 growing season with the tablestock 'Algeria'. Tuber yield for the fertigation treatment with only 70% of nitrogen still had 6% higher tuber yield than the dry granular fertilization treatment with 100% nitrogen (Table 1). Fertigation may be a more productive field practice for potato production in Florida. This is because fertigation supplies nutrients to the crop at the right time and hence minimizes nitrogen loss through leaching or run-off.

This fertigation field practice can help save both irrigation water and fertilizers. Fertigation can minimize water pollution and enhance the economic and environmental sustainability of Florida's crops. In the future, the State government may need to develop more cast-share programs for center-pivots for growers. University of Florida/IFAS faculty will educate them to use fertigation not only for potato but also for other vegetables in order to save water and nutrients.

Table 1. Potato tuber yield (lbs/acre) of two fertilizer programs with the same amount of fertilizers in three growing seasons.

Growing season	2015–16			2016–17			2017–18		
Potato type	Chipping potato			Tablestock potato			Tablestock potato		
Yield separation	M	N	T	M	N	T	M	N	T
Fertigation[z]	39960	4460	44420	40540	3200	43740	42640	2780	45420
Dry granular fertilization	32340	6000	38340	32480	2840	35320	41000	1980	42980
Increment (%)	23.6	25.7	15.9	24.8	12.7	23.8	4.0	40.4	5.7

[z]The fertigation treatment used only 70% nitrogen of that the dry granular fertilization treatment used in the 2017–18 growing season.
M = marketable yield, N = nonmarketable yield, and T = total yield.

This study was financially supported by SWFWMD (Contract ID: B406).

*Corresponding author. Email: guodong@ufl.edu

Proc. Fla. State Hort. Soc. 132:143. 2019.

—Scientific Note—

Lettuce Performance In Two Types of Soil In Florida

Gustavo F. Kreutz, Jesse J. Murray, and Germán V. Sandoya*

Everglades Research and Education Center, 3200 East Palm Beach Rd., University of Florida/IFAS, Belle Glade, FL 33430

Additional index words. breeding, lettuce, muck soils, sandy soils, yield

In Florida, lettuce is planted primarily in rich organic soils of the Everglades Agricultural Area (EAA). This research seeks alternative production locations such as the profuse sandy soils in the state. The EAA, located south of Lake Okeechobee in Florida, is well-known for its highly fertile soils, often referred to as "muck" (Histosols). These soils present an organic matter content of 65% to 85% and high concentrations of plant essential nutrients, making the area highly suitable for production of sugarcane, sod, and winter vegetables, including lettuce (*Lactuca sativa* L.). Annually, lettuce is planted on 15,000 acres as a rotational crop between sugarcane cycles and generates a revenue of nearly $80 million to local farmers. The crop often faces limiting factors such as soil-borne pests and diseases, competition with other winter vegetables, high weed pressure, and soil subsidence. Therefore, it is imperative to identify alternative planting slots for lettuce cultivation to the abundant sandy soils, which could benefit small stakeholders of the leafy vegetable industry. Such benefits may include increasing productivity and competitiveness in the national market, and reducing fertilizer, pesticides, and irrigation applications because of the use of plastic mulch and fertigation. Other benefits could be decreasing the period between harvest cycles by using transplants grown in nurseries and providing Florida lettuce to markets earlier and later than the current production window. The purpose of this study was to evaluate the performance of 24 lettuce genotypes of crisphead (10), romaine (10), and butterhead (4) in muck and sandy soils.

Four experiments with three replicates each were conducted between 2017 and 2019. Two trials were direct-seeded in muck soils in Belle Glade, FL and two trials were conducted using 4- to 6-week-old seedlings grown in germination trays and transplanted onto plastic mulched beds in sandy soils in Loxahatchee, FL. Head weight (HW, g/head) was estimated for each genotype at market maturity by weighing 10 heads/plot. Data were analyzed separately by lettuce type using SAS® software, Version 9.4 (SAS, Cary, NC). Analysis of variance was conducted to investigate the effects of soil, genotype, and the soil × genotype interaction. The soil factor had a statistically significant ($P > 0.05$) effect on HW of crisphead and butterhead. The effect of genotype on HW was found to be significant ($P > 0.05$) among romaine and butterhead lettuces. The soil × genotype interaction was significant ($P > 0.05$) among all lettuce types, suggesting differential responses of genotypes to different soil types. HW in crisphead decreased between 9.3% and 45.6% when planted in sandy soils, except for '60180'. 'Flagler', 'Belle Glade', and 'Chosen' performed poorly in sandy soils with 37%, 42%, and 46% less yield, respectively, compared to muck soils. Six out of ten romaine genotypes produced higher yields in sand than in muck, with HW increases ranging from 5.25% ('Sawgrass') to 88.4% ('Floricos 83'). Yield of four romaine genotypes suffered a small reduction when grown in sand, ranging from 1.1% (70096) to 18.3% (60166). Butterhead genotypes '60176' and 'Floribibb' presented nearly twice the biomass in sandy soils compared to organic soils (increases of 91.5% and 95.8%, respectively); in contrast, 'Palmetto' and '60178' had slight reductions in HW (0.3% and 5.2%, respectively). Most genotypes experienced a decrease in fresh biomass when cultivated in mineral soils compared to muck. This fact could be attributed to a better adaptability of current lettuce cultivars to organic soils because breeding of lettuce has been primarily focused on developing cultivars adapted to the organic soils of the EAA. Nevertheless, the superior performance of some genotypes in sandy soils in these experiments seem to indicate genetic variability in lettuce adapted to these conditions. Genotypes with such characteristics could be introduced into breeding programs as sources of genetic diversity to develop new cultivars adapted to sand. Additional research is necessary to assess the performance of lettuce cultivars throughout different sites and growing periods. More importantly, a detailed assessment of the nutritional requirements and the economic feasibility is required prior the adoption of lettuce production on sandy soils by farmers.

*Corresponding author. Email: gsandoyamiranda@ufl.edu

Proc. Fla. State Hort. Soc. 132:144. 2019.

—Scientific Note—

Building a Successful Hop Yard in Florida: Plant Materials, Trellis Designs, and Management Practices

Aleyda Acosta Rangel[1], Zhanao Deng[1], Tiare Silvasy[1], Jack Rechcigl[1], Simon Bollin[2], and Shinsuke Agehara*[1]

[1]*Gulf Coast Research and Education Center, University of Florida/IFAS, 14625 CR 672, Wimauma, FL 33598*

[2]*Agriculture Industry Development, Hillsborough County Economic Development Department, Tampa, FL*

Additional index words. alternative crop, *Humulus luplus*, supplemental lighting

Hop (*Humulus luplus* L.) is a dioecious, herbaceous perennial in the Cannabaceae family. It has recently gained interest as an alternative crop in Florida because of the dramatic increase in the popularity of craft beer. Hops are typically grown in temperate climates between latitudes 35° to 55° north. The major challenge in growing hops in Florida's subtropical climate is inadequate day length, which induces premature and unsynchronized blooming. This article focuses on important considerations in building a hop yard for successful hop production in Florida.

Step 1. Site selection. Soil testing is strongly recommended to determine soil pH and basic nutrient needs. Nematode testing is also recommended, as plant-parasitic nematodes are common in Florida soils. Sites with high root-knot nematode populations or flood-prone areas should be avoided. Soils with good water holding capacity and high organic matter are ideal. Because hops are susceptible to wind damage, windbreaks are desirable to prevent bine damage and subsequent disease risks. A north-south field orientation maximizes light interception.

Step 2. Field preparation. Soil pH should be adjusted to between 6.0 and 6.8. A slight downslope toward the furrow is important for proper drainage. Row spacing can be between 10 and 15 ft, depending on one's farm equipment. Before bedding, we recommend incorporating compost (up to 80 t/acre or 2-inch thickness) in the soil to improve soil organic matter and water holding capacity. Beds should be covered with ground covers for weed control. High water and nutrient demand of hops can be managed by using two lines of drip tubing per bed. For nematode control, K-Pam (metam-potassium) can be injected at 20 gal/acre at least 3 weeks before planting.

Step 3. Trellis construction. Trellis construction involves the installation of poles, wires, and supplemental lighting. We recommend using pressure-treated 6- and 5-inch poles for the perimeter and inside the hop yard, respectively. The pole length depends on the desired trellis height. If installing an 18–20 ft high trellis, we recommend using 25-ft poles with 5 ft in the ground. The number of poles and amount of wires will depend on the trellis design. Compared to a straight-design trellis (one wire per row), a V-design trellis (two wires per row) requires approximately 1.8 and 2.6 times more poles and wires, respectively. We highly recommend installing LED lights (no far red light) in the trellis to control the timing of flowering via photoperiod extension. The photoperiod should be extended to 16 hours until plants produce a sufficient amount of bines and laterals. Artificial control of flowering enables two seasons per year (spring and fall) and once-over harvests.

Step 4. Planting. Certified tissue culture seedlings are highly recommended. Rhizomes should not be used as they can be infected with pathogens. Cultivar selection is particularly important because many hop cultivars are not suitable for subtropical climates. In our cultivar trials, we identified 'Cascade' and 'Galena' as high-yielding cultivars in the spring and fall seasons, respectively.

Building a successful hop yard in Florida must be determined based on economic considerations. The estimated total material cost for hop yard establishment is $15,780 per acre for a straight trellis and $18,687 per acre for a V-trellis. We are currently evaluating the productivity and production costs of different trellis designs. We are also developing crop management practices and pest management recommendations optimized for hops grown in Florida's subtropical climate. The complete information from these projects will be available in extension articles in the near future.

*Corresponding author. Email: sagehara@ufl.edu

Proc. Fla. State Hort. Soc. 132:145. 2019.

—Scientific Note—

Effect of Calcium Sulphate and Ammonium Sulphate on Soil pH, Electrical Conductivity and Exchangeable Sodium

Gustavo Franco de Castro[1,2], Guilherme Bossi Buck[1], Edson Marcio Mattiello[2], and Lincoln Zotarelli*[1]

[1]*Horticultural Sciences Department, University of Florida/IFAS, P.O. Box 110690, Gainesville, FL 32611*

[2]*Department of Soils, Universidade Federal de Viçosa, Peter Henry Rolfs Avenue, s/n - Campus Universitário, Viçosa, MG 36570, Brazil*

Additional index words. cations, sandy soil, soil salinity, sodium, *Solanum tuberosum*

The use of calcium sulfate (CS) in agricultural fields has become popular among growers. In Florida, applications of CS are primarily used to minimize some potato tuber disorders associated with calcium (Ca) deficiency. CS is commonly used for the removal of soluble salts such as sodium (Na). However, the effects of large applications of CS on soil pH, electrical conductivity (EC), and nutrient availability to plants are not negligible. The objective of this study was to investigate the effects of CS and ammonium sulfate (AS) rates on soil pH, EC, and exchangeable Na.

Samples from the 0–20 cm soil depth layer were collected from a commercial potato field in Hastings, FL. The soil chemical properties were: pH-H_2O 6.2; Na 127.9 mg/dm^3; EC 1.27 dS/m; Ca 5.18 $cmol_c/dm^3$; and cation exchange capacity 7.1 $cmol_c/dm^3$. A subsample of 200 g of air-dried soil was incubated with 12 rates of CS (0, 100, 200, 400, 600, 1000, 1400, 1800, 2200, 2800, 3400, and 4000 kg/ha) and 12 rates of AS (0, 25, 50, 75, 100, 125, 150, 175, 200, 300, 400, 500, and 600 kg/ha). The experimental design was completely randomized, with four replications. The soil was incubated for 60 d at 25 °C and moisture was maintained at 60% of soil field capacity. After incubation, the soil was analyzed for pH, EC, and Na. Data were analyzed using the PROC-GLM and REG procedures of SAS.

Soil pH decreased from 6.2 to 5.9 during incubation for the treatments without any CS or AS. There was a linear decrease in soil pH from 5.9 to 5.6 with the increasing rates of CS (soil pH = $5.87 - 6.33*10^{-5}$CS, $R^2 = 0.63$, $P < 0.001$). There was also a linear increase in soil EC with the increasing rates of CS (soil EC = $1.31 + 4.78*10^{-4}$CS, $R^2 = 0.84$, $P < 0.001$). The Ca^{2+} in the soil solution can replace the Na^+ in the exchange complex, forming salts with high solubility such as Na_2SO_4 and $MgSO_4$, which would be prone to leach if conditions permitted. The AS has a high acidifying potential, which would invariably reduce soil pH. In contrast to CS, the application of AS significantly reduced soil pH from 5.9 to near 4.6, resulting in a significant negative linear response (soil pH = 5.52 – 0.0015*AS, $R^2 = 0.45$, $P < 0.001$). Under the soil moisture conditions of the incubation, the AS was dissociated, and ammonium became present in the soil solution. There was a quadratic increase in the soil EC with increasing application rates of AS (soil EC = $1.53 + 0.01*AS - 9.5292*10^{-6}AS$, $R^2 = 0.89$, $P < 0.001$). Raising the soil EC was due to the release of NH_4^+ into the solution as a result of the dissolution of AS, raising the salt content in the soil and lowering the soil pH. The initial soil EC of 1.27 dS/m linearly increased to 3.2 dS/m with CS and quadratically increased peaking at 4.1 dS/m with AS. Soil pH dropped somewhat from 6.2 to 5.5 with the increasing rates of CS. In contrast, the application of AS further decreased soil pH to 4.6. As a result of the acidification of the soil, there was a quadratic increase of the concentration of extractable Na in the soil (Na = $-658.322 + 379.475*pH - 40.774*pH^2$, $R^2 = 0.67$, $P < 0.001$). The displacement Na was due to the release or increase in H^+ in response to the nitrification process, which also causes bases with a lower valence to be exchanged for H^+ in the soil solution.

The results of this study indicate that the application of high rates of CS or AS can increase soil EC in the rootzone and potentially impacting the crop. Selection of low-saline fertilizer and split fertilizer applications are strategies that may mitigate the impact of fertilizer in areas high soil EC or elevated Na content.

*Corresponding author. Email: lzota@ufl.edu

Proc. Fla. State Hort. Soc. 132:146. 2019.

—Scientific Note—

Economic Feasibility of Nitrogen Fertilizer Rates For Chip Potato Production In Florida

Rodrick Z. Mwatuwa[1], Lincoln Zotarelli*[1], Tara Wade[2], Kelly T. Morgan[2], and Balasubramani Rathinasabapathi[1]

[1]*Horticultural Sciences Department, University of Florida/IFAS, P.O. Box 110690, Gainesville, FL 32611*

[2]*Southwest Florida Research and Education Center, University of Florid/IFAS, 2685 State Rd. 29, North, Immokalee FL 34142*

Additional index words. enterprise budget, gross revenue, marketable tuber yield, net revenue

Chip potato (*Solanum tuberosum*) production in Florida faces challenges related to the efficient use of nitrogen (N) fertilizer. Several agronomic studies have suggested total N fertilizer rates that improve N fertilizer use efficiency and maximize potato yields. However, the economic benefits or losses associated with the N fertilizer rates that improve N fertilizer use efficiency have yet to be quantified. The overall goal of this study was to determine the economic feasibility of N fertilizer rates for chip potato in Florida. The study used yield data from studies that evaluated the effect of total N fertilizer rate on yield for chip potato in Florida. The studies were conducted on commercial farms in northeast Florida from 2010 to 2012. Economic analyses included determination of production costs and creation of enterprise budgets where net returns, profit margins and marginal returns from N fertilizer were assessed. The enterprise budget allowed for comparisons of the economic effects emanating from various N fertilizer rates.

The objective of the first study was to develop an enterprise budget and update production costs for chip potato cultivars 'Atlantic' and 'FL1867' cultivated under seepage irrigation in northeast Florida. The production cost of chip potato cultivated with a total N fertilizer rate of 200 lb/acre and seepage irrigation was $2694 per acre with $2484 in operating costs and $210 in fixed costs. Comparing the 2008–09 budget with the 2018–19 budget, operating costs in 2018–19 increased because growers adopted more improved production strategies such as fumigation and banding fertilizer application to improve efficiency and compliance with BMP practices. Adoption of new cultural practices required by the BMPs resulted in a reduction of the total production costs by $363 and an increase in operating costs by $348, partly due to eliminating outdated cultural practices and refining practices designed to be implemented when needed. In 2018–19, N–P–K fertilizer costs decreased by 53%, operating costs increased by 14% and total production costs decreased by 13%.

The objective of the second study was to examine the effect of increasing the N fertilizer rate (total rate applied of 50, 100, 150, 200, 250, and 300 lb/acre, using 50 lb/acre applied preplant at soil fumigation) on profitability. Yield data were obtained from multiple field trials conducted on three commercial farms from 2010 to 2012. The production costs per acre was $2,543, $2,611, $2,668, $2,694, $2,719, and $2,745 with mean ± standard deviations for marketable yield of 229 ± 37, 281 ± 53, 296 ± 57, 314 ± 50, 304 ± 47, and 293 ± 51 cwt/acre for 50, 100, 150, 200, 250, and 300 lb/acre of N fertilizer, respectively. With average price of $14.50 per cwt, 200 lb/acre of N had the highest net return and profit margin. The mean net return ± standard deviation was $137 ± 537, $779 ± 769, $904 ± 827, $1134 ± 725, $991 ± 682, and $609 ± 740 per acre, with mean profit margins of 5%, 23%, 25%, 30%, 27%, and 18% per acre when 50, 100, 150, 200, 250, and 300 lb/acre of N fertilizer, respectively, were applied.

N fertilizer accounts for a small percentage of the production cost (11% to 14%), however increasing N-fertilizer cost and higher N-fertilizer rates reduces the marginal return and profit margin. A comparison between N-fertilizer rates indicated that applying 200 lb/acre of N resulted in the lowest production cost per cwt with maximum net returns expected from 178 lb/acre of N. Increasing N-fertilizer rates above 200 lb/acre resulted in increasing losses per dollar invested in N fertilizer.

Regular annual reviews of chip potato production practices and associated costs as well as commodity price changes is necessary to make chip potato budgets a tool that would help growers adopt economically and agronomically feasible cultural practices and N fertilizer rates. An automated web-based enterprise budget can be easily updated for use by growers and Extension agents.

*Corresponding author. Email: lzota@ufl.edu

Proc. Fla. State Hort. Soc. 132:147. 2019.

—Scientific Note—

Effects of Gypsum Application on Potato Production in Northeast Florida

Muhammad Shahid[1], Guodong Liu[1]*, Benjamin Hogue[1], Moshe Doron[1], David Dinkins[2], and Danny Johns[3]

[1]*Horticultural Sciences Department, University of Florida/IFAS, P.O. Box 110690, Gainesville, FL 32611-0690*

[2]*Putnam County Extension, University of Florida/IFAS, 111 Yelvington Rd., Ste. 1, East Palatk , FL 32131*

[3]*Blue Sky Farms, 4805 County Rd. 13 S, Elkton, FL 32033*

Additional index words. calcium level, potato, tuber quality; tuber yield

Gypsum application is a routine field management practice for potato production in Florida no matter how much calcium is contained in the soil of the potato field. One of the reasons for yearly application of gypsum is that gypsum is a byproduct of the phosphate industry and hence is inexpensive in Florida. Known as the "Potato Capital of Florida," the Hastings area has been growing potatoes since the 1890s. The soil in Hastings has accumulated approximately 1600 lb/acre calcium (Ca) on average, based on the soil test report. This amount of calcium is equal to 6800 lb/acre gypsum. Gypsum application may be unnecessary for potato production in the area. The objective of this study was to evaluate how long-term gypsum application impacts tuber yield.

Calcium is essential for improving soil structure and producing high quality potato tubers. The potato growers in the Hastings area currently apply an annual gypsum rate of 750–1000 lb/acre. Soil analysis confirmed high levels of Ca in the Hastings soils. Therefore, our hypothesis is that there is no need for gypsum application in potato production. This study was conducted on a private farm with 'Red LaSoda' to evaluate the effect of gypsum rates on tuber yield from 2013 through 2018. This study used the same layout at the same field in north Hastings except in 2013 when the trial was conducted in south Hastings. There were four gypsum application rates: 0, 500, 1000, and 2000 lb/acre. A randomized complete block design was used with four replications. The results showed that there were no significant differences in plant biomass, leaf greenness, or marketable tuber yield as shown in Table 1. After this on-farm study for six years, we found that yearly gypsum applications did not contribute to potato yield. Therefore, it is concluded that the field practice of gypsum application can be omitted for potato production without sacrificing potato tuber yield in the Hastings area. As a result of this omittance, growers' profitability may be improved due to reduction of labor and input costs. These results suggest that soil testing is important and worth following for potato production.

Suggestions for future direction are that gypsum application is not needed for fertilization reasons for potato production in northeast Florida if soil testing shows high levels of calcium.

Table 1. Marketable and nonmarketable tuber yields of tablestock potato, 'Red LaSoda' with four gypsum rates from 2013 through 2018.

Gypsum rate	2013		2014		2015		2016		2017		2018	
(lb/acre)	M[z]	N[y]	M	N	M	N	M	N	M	N	M	N
0	30902	3000	39451	3534	38672	3109	36721	983	26431	2113	25671	2844
500	30621	2000	41894	2800	39124	4211	41003	2355	29721	2811	24566	1821
1000	32500	1500	42213	1500	40304	1934	38411	2641	26931	1956	26832	973
2000	33211	800	41893	2612	36401	1892	39541	3865	28732	1304	27531	1132

[z]M = Marketable.

[y]N = Nonmarketable.

*Corresponding author. Email: guodong@ufl.edu

Proc. Fla. State Hort. Soc. 132:148–149. 2019.

—Scientific Note—

Dynamics of Soil Phosphorus and pH in Large-scale Potato Trials in Northeast Florida

Thioro Fall[1], Fernando Bortolozo[1], Wendy Mussoline[2], Timothy Wilson[3], Kelly Morgan[4], David Dinkins[5], Gary England[6], Mark Clark[7], James H. Fletcher[8], and Guodong Liu*[1]

[1]*Horticultural Sciences Department, University of Florida/IFAS, P.O. Box 110690, Gainesville, FL 32611-0690*

[2]*Flagler County Extension, University of Florida/IFAS, 150 Sawgrass Rd., Bunnell, FL 32110*

[3]*St. Johns County Extension, University of Florida/IFAS, 3125 Agriculture Center Dr., St. Augustine, FL 32092*

[4]*Southwest Florida Research and Education Center, University of Florida/IFAS, 2685 State Rd. 29 North, Immokalee FL 34142*

[5]*Putnam County Extension, University of Florida/IFAS,111 Yelvington Rd., Ste. 1, East Palatka FL 32131*

[6]*Hastings Agricultural Extension Center, University of Florida/IFAS, P.O. Box 728 Hastings, FL 32145*

[7]*Soil and Water Science, University of Florida/IFAS, P.O. Box 110290-0290, Gainesville, FL 32611-0290*

[8]*Mid-Florida Research and Education Center, University of Florida/IFAS, 2725 S. Binion Rd., Apopka, FL 32703*

Additional index words. nutrient dynamics, phosphate management, potato production, soil pH

The University of Florida/IFAS' (UF/IFAS) recommendation for zero application of phosphorus (P) for potato production is 45 ppm Mehlich 3 extractable soil P. The soils in Hastings contain 100–600 ppm extractable P. Potato growers need to apply 100 lb/acre P_2O_5 every growing season to reach targeted yield. To optimize phosphate management for potato production, this large-scale study for P management has collaborated with four commercial farms to evaluate potato yield at three rates: 0, 50, and100 lb/acre P_2O_5 except for one farm which receives an additional rate of 150 lb/acre P_2O_5. Two of the four farms planted tablestock potato, 'Red LaSoda' and the other two grew chipping potato, 'Atlantic'. A randomized complete block design was used with four replications. Biweekly soil samples starting from preplant, were analyzed for pH and P, potassium (K), calcium (Ca), and magnesium (Mg) with Mehlich 3 extractant.

The soil nutrients tested were not significantly different among the P rates or among the six samplings in 2019. The extractable nutrients were significantly different between the farms (Table 1). The average extractable P was 426 ppm, which was 8.5-fold greater than the zero P application of the UF/IFAS recommendation for potato production. This discrepancy resulted from high soil aluminum and iron contents and yearly soil fumigation, which kills all beneficial soil microbes. Soil pH varied throughout the growing season. It reached its lowest in the mid-season (~5.4) for all farms but increased at harvest (Table 2). P uptake may be more critical for potato plants in the middle of the growing season because soil aluminum/iron oxides are

Table 1. Average content of soil nutrients.

Cultivars	Farms	Soil Nutrients (ppm) P	K	Mg	Ca
Red LaSoda	Farm 1	412.73 b[z]	183.55 a	76.88 b	999.55 b
	Farm 2	513.22 a	116.39 b	67.70 b	770.10 c
Atlantic	Farm 3	548.25 a	190.18 a	134.81 a	1244.02 a
	Farm 4	228.69 c	63.09 b	66.84 b	744.23 c

[z]Different letters within same column denote significant differences ($P < 0.05$).

*Corresponding author. Email: guodong@ufl.edu

Table 2. Soil pH trend during potato growing season in 2019 from the six sampling periods (pre-plant to harvest).

Farm	Soil pH fluctuation during potato growing season 2019						
Farm 1	24 Jan. 2019	25 Feb, 2019	11 Mar.2019	25 Mar. 2019	8 Apr. 2019	22 Apr. 2019	6 May 2019
pH	6.4	6.4	6.0	5.7	5.9	5.8	6.4
Farm 2	21 Dec. 2018	11 Feb. 2019	25 Feb. 2019	11 Mar. 2019	25 Mar. 2019	8 Apr. 2019	24 Apr. 2019
pH	6.1	5.7	5.5	5.5	5.5	5.5	5.4
Farm 3	21 Dec. 2018	30 Jan. 2019	13 Feb. 2019	27 Feb. /2019	13 Mar. 2019	27 Mar. 2019	25 Apr. 2019
pH	6.6	6.3	5.9	5.7	5.8	5.9	6.8
Farm 4	6 Dec. 2018	28 Jan. 2019	11 Feb. 2019	25 Feb. 2019	11 Mar. 2019	25 Mar. 2019	22 Apr. 2019
pH	6.3	6.4	5.9	5.8	5.6	5.5	5.8

more soluble at low pH and may tie up most P in the soil solution. Potato production on acidic and P-fixing soils has many challenges because P is an immobile nutrient in soil and potato plants' shallow root system is ineffective for exploring large soil volumes for water and nutrient uptake (Joshi et al., 2016).

Future research directions include crop rotation and diversifying cover crops to improve soil health. As the acreage of potato production in the Hastings declined to approximately 50%, 14,000 acres, other crops, especially Asian vegetables, are expanding rapidly. This crop diversity provides potato growers great opportunities to rotate other crops with potato plus introducing new cover crops. This cropping system improvement may minimize the need for soil fumigation and increase P use efficiency for potato production.

Literature Cited

Joshi, M., E. Fogelman, E. Belausov, and I. Ginzberg. 2016. Potato root system development and factors that determine its architecture. J. Plant Physiol. 205:113–123.

Proc. Fla. State Hort. Soc. 132:150. 2019.

—Scientific Note—

A Scanner-based Rhizotron System and Simple Image Analysis Techniques to Evaluate Root Development in Vegetable Seedlings

Atsushi Sanada[1] and Shinsuke Agehara[2]*

[1]*Tokyo University of Agriculture, Tokyo, Japan*

[2]*University of Florida, Gulf Coast Research and Education Center, 14625 County Rd. 672, Wimauma, FL 33598*

Additional index words. ImageJ, imaging, rhizosphere

Root architecture and growth dynamics are important traits that determine crop productivity. However, observing and measuring these root traits are difficult in the field. Traditional techniques involve coring, trenching, excavating, and root washing, which are tedious and time-consuming, and the accuracy of their results is generally low. Rhizotrons are tools for making nondestructive, repeated observations and measurements of root systems. Since the use of rhizotrons was first reported in the 1950s, various sizes and shapes of rhizotrons have been developed. Advanced imaging and image analysis techniques are often used to improve the precision and efficiency of quantitative measurements.

The main objective of this study was to develop a quick and inexpensive method to examine root and shoot traits of vegetable seedlings using a scanner-based rhizotron system and ImageJ, an open source image processing program. The rhizotron was composed of a wood frame and transparent acrylic sheets attached on both sides, all of which were inexpensive materials. The soil volume was 2655 cm^3 and the scanning window was 22.9 × 30.5 cm. Four test crops used in this study were soybean (*Glycine max*), tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.), and strawberry (*Fragaria* ×*ananassa* Duch.). The soil collected from the field was packed in the rhizotrons using the field bulk density. Seeds were sown directly in rhizotrons for soybean, whereas seedlings were used for other crops. After planting, rhizotrons were kept at 30 degree inclination in the greenhouse to induce root growth along the acrylic sheet facing downward. The experiment duration was six weeks for strawberry because of the relatively slow root development, whereas it was 14–18 d for other crops. Overhead canopy images and root images were obtained periodically using a digital camera and a flat-bed scanner, respectively. Seedlings were sampled at the end of each experiment for additional growth measurements. Root images were analyzed using ImageJ, as well as two commercial programs (WinRHIZO Tron for rhizotron images and WinRHIZO for washed root images). To measure root projected area using ImageJ, original color images were converted to binary images after separating roots from the soil using the color threshold tool. This process took about 2–3 min per image, regardless of tested crops and their root density. For all tested crops, root projected area measured by ImageJ showed high positive correlations with that by WinRHIZO Tron (rhizotron images) and WinRHIZO (washed root images). Although WinRHIZO Tron can provide additional root measurements simultaneously, it was extremely laborious and took up to 60 min per image. Furthermore, overhead canopy images were analyzed by ImageJ to measure leaf area non-destructively, which showed high linear positive correlations with destructive measurements performed by a leaf area meter. These results demonstrate that our rhizotron combined with image analysis techniques using ImageJ allows non-destructive, quick, and inexpensive root and shoot growth evaluation for seedlings of various vegetable crops.

*Corresponding author. Email: sagehara@ufl.edu

Proc. Fla. State Hort. Soc. 132:151. 2019.

—Scientific Note—

Strawberry Yield Prediction Using Ground-based Imagery and Automated Fruit and Flower Identification

Weining Wang, Amr Abd-Elrahman, Katie Britt, Feng Wu, and Shinsuke Agehara*

Gulf Coast Research and Education Center, University of Florida /IFAS, 14625 County Rd. 672, Wimauma, FL 33598

Additional index words. *Fragaria* ×*ananassa*, fruit detection, yield forecasting

Florida is the primary supplier of winter strawberries (*Fragaria* ×*ananassa* Duch.) in the United States, with a farm gate value of approximately $300 million. In recent years, however, the industry has been facing many challenges, including rising production costs and labor shortages. Rapid increases in imports from Mexico and consequent falling prices have also significantly reduced growers' profit margin. Because harvesting is the most labor-intensive operation in strawberry production, accurate yield prediction could help make cost-effective hiring and marketing decisions. To predict weekly yields of 'Festival' strawberries, Mackenzie and Chandler (2009) built a prediction model using flower count and weather data. Although the model had good accuracy ($r^2 = 0.93$), the major drawback was that it requires flower counts with manual data collection, which is extremely laborious.

In this study, we examined the accuracy of using high resolution imagery to assist strawberry flower and fruit counting to accelerate the yield prediction process. The field trial was conducted at the Gulf Coast Research and Education Center in Balm during the 2017–2018 season. Two cultivars, Florida Radiance and Florida Beauty, were arranged in a randomized complete-block design with 6 replicated plots for each cultivar. Ground-based images were collected twice a week from November to January. Manual flower and fruit counts were performed in the field on the same data collection dates. Percent match (PM) between image derived counts and field counts (image-derived counts/field counts) is used as the parameter to measure the imagery accuracy. On average across the two cultivars, the PM for flowers was 82% to 197% in November, 77% to 123% in December, and 52% to 114% in January, indicating the tendency of overestimation during the early season and underestimation during the late season. The PM for fruits was 17% to 68% in November, 41% to 58% in December, and 29% to 51% in January, indicating the tendency of underestimation throughout the season. When the sum of flower and fruit counts was used as one parameter, the PM was improved: 61% to 98% in November, 50% to 74% in December, and 43% to 64% in January. Overall, an estimation using the sum of flower and fruit counts was more reliable for 'Florida Beauty' than for 'Florida Radiance', probably because of its compact canopy structure that reduced hidden flowers and fruits. Our next step is to improve the imagery accuracy by having better trained personnel and having multi-view images, and by taking advantage of deep learning to automate the flower and fruit counting process.

Literature Cited

MacKenzie, S.J., and C.K. Chandler. 2009. A method to predict weekly strawberry fruit yields from extended season production systems. Agron. J. 101:278-287.

*Corresponding author. Email: sagehara@ufl.edu

Proc. Fla. State Hort. Soc. 132:152. 2019.

—Scientific Note—

Development of Management Practices for Winter Artichoke Production in Florida

Shinsuke Agehara* and Tiare Silvasy

University of Florida, Gulf Coast Research and Education Center
14625 County Road 672, Wimauma, FL 33598

Additional index words. alternative crop, *Cynara cardunculus,* vernalization

Globe artichoke (*Cynara cardunculus* L.) belongs to a genus of thistle-like plants in the Asteraceae family and is cultivated for its flower buds. It is a high-value crop, whose production value is higher than most major vegetable crops in Florida. Nearly 100% of artichokes in the United States are currently produced in California. Artichoke prices are seasonal and typically peak during winter months when the supply is low. Although Florida's subtropical climate does not provide adequate chill hours, we have previously demonstrated that artichokes can be produced in Florida during winter and early spring by using gibberellic acid (GA_3) as a bud induction method.

The objective of this study is to develop production guidelines for artichoke production in Florida. Field experiments were performed in the 2017–2018 and 2018–2019 seasons at Gulf Coast Research and Education Center in Balm, Florida. All plants were treated with GA_3 at 49 g·ha^{-1} three times during the vegetative growth stage to induce bud formation. Among six cultivars evaluated, 'Imperial Star' had the highest marketable yield (6.41 t·ha^{-1}), followed by 'Opal' (3.80 t·ha^{-1}), 'Green Globe Improved' (3.41 t·ha^{-1}), 'Colorado Red Star' (0.87 t·ha^{-1}), 'Purple Romagna' (0.28 t·ha^{-1}), and Madrigal' (0 t·ha^{-1}). The marketable yield of 'Imperial Star' was significantly higher than that of three lowest-yielding cultivars. There was a significant positive correlation between yield per hectare and bud number per plant. By staggering planting dates (4 Sept. 2018 to 13 Nov. 2018), harvests started in late Dec. and lasted over 107 days (26 Dec. 2018 to 12. Apr. 2019). The marketable yield of 'Imperial Star' increased nearly 3-fold by increasing nitrogen fertilization rates from 119 to 288 kg·ha^{-1}, whereas it was unaffected by in-row plant spacing (76, 91, and 107 cm). 'Imperial Star' appears to be the most suitable cultivar, mostly because of its relatively low chilling requirement. Staggering planting dates is a good strategy to extend the harvest period and increase the early yield in winter. Our results suggest that, using 'Imperial Star', GA_3 and, optimized crop management strategies, it is feasible to establish winter artichoke production in Florida.

*Corresponding author. Email: sagehara@ufl.edu

Proc. Fla. State Hort. Soc. 132:153. 2019.

—Scientific Note—

Agronomic Performance and Genetic Diversity Among Common Bean Varieties In Haiti

Riphine Mainviel*[1,2], Geoffrey Meru[1,2], Edouardo C. Vallejos[2], and Raphael Colbert[3]

[1]*Tropical Research and Education Center, University of Florida/IFAS, 18905 SW 280th St., Homestead, FL 33031*

[2]*Horticultural Sciences Department, University of Florida/IFAS, P.O. Box 110690, Gainesville, FL 32611-0690*

[3]*Panamerican Agricultural University, Zamorano, Valley of the Yeguare River, Honduras*

Additional index words. common bean, genetic diversity

Common bean (*Phaseolus vulgaris* L.) is one of the most essential food legumes in the world and plays an important role in providing nutritional security and revenue for smallholders in Haiti. Despite its economic importance, bean production in Haiti is constrained by many biotic and abiotic factors that limit yield. The objectives of the current study were to evaluate the agronomic performance of 13 elite common bean-breeding lines currently under development by the University of Florida—Appui à la Recherche et au Développment Agricole (UF-AREA) and the Legume Innovation Lab programs and determine the genetic diversity among 92 common bean accessions in Haiti using genotyping by sequencing.

In the study we evaluated the agronomic performance of 13 elite common bean-breeding lines along with 10 cultivar checks. The experiment was conducted at a highland location in the West region in Haiti. The bean genotypes were planted in three replicated plots in a randomized complete-block design. Phenotypic assessment of bean lines was done by measuring eleven agronomic traits, according to Gomez et al. (2004). Besides, the diseases that were more prevalent during the experiment including Powdery Mildew, Bean Common Mosaic Virus (BCMV) and Bean Golden Mosaic Virus (BGMV) were evaluated using a scale of 1–9 according to the International Center for Tropical Agriculture (CIAT), with 1–3 = "resistant", 4–6 = "intermediately resistant" and 7–9 = "susceptible". Analysis of variance and trait mean separation were done in R statistical package. Significant differences were found among the genotypes for most of the traits measured. PR1627-8 (white bean) genotype outperformed the other breeding lines in yield and disease resistance; therefore, it is a candidate for release to growers. Furthermore, the cultivar checks, including 'LORE 234' local, and 'Local 3' red could be used as a source of earliness for breeders. Based on phenotypic relationships, the number of pods per plant and number of seeds per pod may be useful traits for indirect selection for seed yield in common bean.

The genetic diversity was also assessed among a collection of 92 common bean lines from Haiti. The DNA was extracted using a modified nuclear fraction protocol. Genotyping by sequencing was conducted according to Schröder et al. (2016), at Georgia Genomics and Bioinformatics Core at the University of Georgia, using double digestion with MseI and TaqαI restriction enzymes. Sequencing was done in an Illumina NextSeq 150x High output Flow Cell. The sequencing reads were aligned to the *P. vulgaris* reference genome using Burrow Wheelers Alignment tools. The reference-based pipeline in Stacks was used for the extraction of single nucleotide polymorphism (SNP). The population structure analysis was conducted with Structure v2.3.4, and the optimum subpopulations was calculated according to Evanno et al. (2005). Cluster and principal component analysis (PCA) were conducted using Darwin 6.0.021. The GBS revealed 27,823 SNPs, among which 1115 were used for the diversity analysis after filtering for missing data and minor allele frequency. Structure, cluster, and PCA revealed the presence of two gene pools in Haitian bean collection; Andean and Mesoamerican. From these two subpopulations, the Mesoamerican gene pool was predominant with about 87% of the accessions collected, thus reflect the preference for this bean type among Haiti consumers. However, 12 lines showed some level of introgression between the two main gene pools.

Literature Cited

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology. 14(8):2611–2620.

Gomez, O.J., M.W. Blair, B.E. Frankow-Lindberg, and U. Gullberg. 2004. Molecular and phenotypic diversity of common bean landraces from Nicaragua. Crop Science. 44(4):1412–1418.

Schröder, S., S. Mamidi, R. Lee, M.R. McKain, P.E. McClean, and J.M. Osorno. 2016. Optimization of genotyping by sequencing (GBS) data in common bean (*Phaseolus vulgaris* L.). Molecular Breeding. 36(1):6.

*Corresponding author. Email: riphinemainviel@ufl.edu

Proc. Fla. State Hort. Soc. 132:154. 2019.

—Scientific Note—

Genetic Loci Associated with Resistance to *Phytophthora* Crown Rot in Squash

Geoffrey Meru*[1,2], Alexis Ramos[1], Yuqing Fu[1], and Vincent Michael[1,2]

[1]*Tropical Research and Education Center, University of Florida/IFAS, 18905 SW 280th St., Homestead, FL 33031*

[2]*Horticultural Sciences Department, University of Florida/IFAS, P.O. Box 110690, Gainesville, FL 32611-0690*

Additional index words. disease resistance, *Cucurbita moschata*, QTL

Breeding cultivars resistant to *Phytophthora* crown rot (*P. capsici*) is an important goal for squash (*Cucurbita* spp.) breeders worldwide. The disease is particularly severe in south Florida where frequent flooding results in rapid establishment and distribution of *P. capsici* spores across grower fields, leading to significant crop losses. Currently, there are no commercial cultivars resistant to this pathogen (Babadoost and Islam, 2003). Resistance to *Phytophthora* crown rot in University of Florida breeding line #394-1-27-12 (*C. moschata*) is conferred by three independent dominant genes (R1R2R3) (Padley et al., 2009). Molecular breeding targeting R1R2R3 would improve selection efficiency for resistance, thus expedite the development and release of commercial cultivars carrying these genes. In the current study, the QTL-seq method was used to identify quantitative trait loci (QTL) associated with resistance to *Phytophthora* crown rot in an F_2 population generated from a cross between 394-1-27-12 (resistant) and 'Butter Bush' (susceptible). Disease severity was determined for the parents (n = 16) and F_2 (n = 168) by burying a 0.5 cm^2 agar plug around the crown of each plant, followed by a second inoculation with another agar plug 7 days later. DNA was extracted from the parents and twenty most (DS = 0) and twenty least (DS ≥ 4) resistant F_2 progeny using a commercial kit. DNA was pooled for each bulk (R vs. S) and sequenced together with the parental DNA on the Illumina HiSeq X. After removal of adapters and reads containing more than 50% low quality bases (quality value ≤ 12), best practices for variant calling were employed for mapping the sequences to a reference genome and calling variants using Genome Analysis Toolkit (GATK) (McKenna et al. 2010). The QTLseqr R package was used to detect QTL. For each bulk, the SNP-index across all loci was calculated as the proportion of reads that were different from the parental reference allele. Delta (Δ) SNP-index was calculated by subtracting the SNP-indices of the bulks at each loci. Candidate QTL regions were identified using a 1mb sliding window in R (10,000 simulations). Three QTL significantly ($P \leq 0.05$) associated with resistance to *Phytophthora* crown rot were detected on chromosome 4 (QtlPC-C04), 11 (QtlPC-C11) and 14 (QtlPC-C14). Molecular markers linked to these QTL will be useful for marker-assisted selection for *Phytophthora* crown rot resistance in squash. Forty-six candidate resistant genes were found within the confidence interval of the three QTL. Functional analysis of these genes will reveal the molecular mechanisms underlying *Phytophthora* crown rot resistance in squash.

Literature Cited

Babadoost, M. and S.Z. Islam. 2003. Fungicide seed treatment effects on seedling damping-off of pumpkin caused by *Phytophthora capsici*. Plant Dis. 87:63–68.

McKenna, A., M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis, A. Kernytsky, K. Garimella, D. Altshuler, S. Gabriel, M. Daley, and M.A. DePristo. 2010. The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 20:1297–1303.

Padley, L.D., E.A Kabelka, and P.D. Roberts. 2010 Inheritance of resistance to crown rot caused by *Phytophthora capsici* in *Cucurbita*. HortScience 44:211–213.

*Corresponding author. Email: gmeru@ufl.edu

Proc. Fla. State Hort. Soc. 132:155–157. 2019.

Inheritance of Resistance to *Phytophthora* Crown Rot in *Cucurbita pepo*

Vincent Njung'e Michael, Yuqing Fu, and Geoffrey Meru[1]
Horticultural Sciences Department and Tropical Research and Education Center, University of Florida, 18905 SW 280th Street, Homestead, FL 33031

Additional index words. Phytophthora capsici, crown rot, breeding, dominant gene, PI 181761

***Abstract. Phytophthora* crown rot, caused by *Phytophthora capsici* Leonian, is a devastating disease in commercial squash (*Cucurbita pepo* L.) production across the United States. Current management practices rely heavily on the use of chemical fungicides, but existence of fungicide-resistant pathogen populations has rendered many chemicals ineffective. Host resistance is the best strategy for managing this disease; however, no commercial cultivars resistant to the pathogen are currently available. Resistance to *Phytophthora* crown rot in PI 181761 (*C. pepo*) is an important genetic resource for squash breeders worldwide; however, the underlying genetic basis of resistance in PI 186761 that would allow designing of sound breeding strategies is currently unknown. The goal of the current study was to determine the inheritance of resistance in breeding line #186761-36P, a resistant selection of PI 181761, using phenotypic data from F_1, F_2, and backcross populations derived from a cross between #181761-36P and a susceptible acorn-type cultivar, Table Queen. The results indicated that resistance in #181761-36P is controlled by three dominant genes (R4, R5, and R6). Introgression of these genes into susceptible cultivar groups of *C. pepo* will provide an important tool in the integrated management of *Phytophthora* crown rot.**

Phytophthora capsici Leonian is a major pathogen with a wide host range, including vegetable crops belonging to Solanaceae, Cucurbitaceae, Leguminosae, and Brassicaceae families (Krasnow and Hausbeck, 2015; Lamour et al., 2012). It is an oomycete that overwinters in the soil as sexually produced oospores (Babadoost and Pavon, 2013). These may infect host plants directly, or, in presence of water, develop sporangia that release swimming zoospores to cause infection. *P. capsici* is the causal agent of foliar blight, root rot, fruit rot, and crown rot disease syndromes in cucurbits (Babadoost, 2016). *Phytophthora* crown rot is particularly prevalent in fields prone to flooding, often resulting in total crop loss. Consequently, current integrated management strategies rely heavily on soil water management and chemical treatment of seed, soil, and plants (Sanogo and Ji, 2012); however, *P. capsici* isolates from major *Cucurbita* growing regions, such as Michigan, New York, and Florida, exhibit insensitivity to recommended chemical pesticides (Lamour and Hausbeck, 2000, 2003; Ploetz et al., 2002). Therefore, host resistance in *Cucurbita* crops is important to augment *P. capsici* management practices (Babadoost, 2016; Hausbeck and Lamour, 2004; Sanogo and Ji, 2012).

Received for publication 6 Mar. 2019. Accepted for publication 11 Apr. 2019.
This research was partially funded by the National Institute of Food and Agriculture (Hatch project FLA-TRC-005564).
[1]Corresponding author. E-mail: gmeru@ufl.edu

Cucurbita moschata Duchesne, *Cucurbita maxima* Duchesne, and *Cucurbita pepo L.* are the three main squash species cultivated in the United States, with the latter being the most economically important (Paris, 2008). All commercial *Cucurbita* cultivars are susceptible to *Phytophthora* crown rot (Babadoost and Islam, 2003); however, resistance to *Phytophthora* crown rot was identified in a wild accession of *Cucurbita lundeliana* Bailey, and subsequently transferred into *C. moschata* background (breeding line #394-1-27-12) (Padley et al., 2009). Resistance in #394-1-27-12 is conferred by three independent dominant genes (Padley et al., 2009). Resistance in *C. pepo* was identified in a set of 16 PIs (Padley et al., 2008), which were later found to be genetically similar (genetic distance = 0.31) (Michael et al., 2019). Among these, PIs 181761 and 615132 were the most resistant (disease severity = 1.3 of 5.0) (Padley et al., 2008), but the former was considered ideal for breeding due to close genetic similarity to cultivar groups of subspecies *pepo* and *texana* (Michael et al., 2019). A highly resistant selection of PI 181761 (designated #181761-36P) was developed through several generations of selection. Knowledge of the underlying genetic basis of *Phytophthora* crown rot resistance in breeding line #181761-36P is essential to design sound breeding strategies for introgressing resistance into susceptible, elite cultivars of *C. pepo*, but this information is currently lacking. The objective of this study was to determine the mode of inheritance of *Phytophthora* crown rot resistance in breeding line #181761-36P.

Materials and Methods

Plant material. Breeding line #181761-36P, which is highly resistant to *Phytophthora* crown rot, was crossed with 'Table Queen' (TQ), a susceptible elite acorn-type winter squash. Controlled pollinations were carried out in the greenhouse to generate F_1, F_2, and backcross populations.

Inoculum preparation. Inoculum for the experiment was prepared from a virulent *P. capsici* isolate #121 (provided by Dr. Pamela Roberts, University of Florida) following the protocol described by Krasnow et al. (2017), with minor modifications. Briefly, a 5-mm cornmeal agar mycelial plug was transferred to 14% V8 agar plates (140 mL V8 juice, 3 g $CaCO_3$, 16 g agar per liter) and grown under constant fluorescent light at 28 °C. After 7 d, the plates were flooded with cold sterile distilled water (4 °C), and chilled at 4 °C for 30 min before incubation at 21 °C for 1 h to allow synchronous release of zoospores. Zoospores were quantified with a hemocytometer and diluted to 2.0×10^4 zoospores per milliliter.

Phenotyping and statistical analysis. Seeds of parents (n = 12, each), reciprocal F_1 (n = 100), F_2 (n = 200), and reciprocal backcross (n = 60–142) progenies were sown in 4-inch pots containing sterilized Proline C/B growing mix (Jolly Gardener, Quakertown PA) amended with 14N–4.2P–11.6K controlled-release fertilizer (Osmocote; Scotts, Marysville, OH). At the second to third true-leaf stage, the seedlings were inoculated by delivering 5 mL of 2.0×10^4 zoospores per milliliter at the crown of each plant using a pipette. Disease severity was recorded visually every 3 days from 8 d postinoculation (dpi) to 28 dpi on a scale of 0 to 5 in which, 0 = no symptoms, 1 = small brown lesion at base of stem, 2 = lesion has progressed up to the cotyledons causing constriction at the base, 3 = plant has partially collapsed with apparent wilting of leaves, 4 = plant has completely collapsed with severe wilting present, and 5 = plant death (Padley et al., 2008) (Fig. 1). Plants having a score of 1 or less at 28 dpi were classified as resistant, whereas those having a score ≥2 were classified as susceptible (Padley et al., 2009). A χ^2 test (McHugh, 2013) was used to compare segregation ratios for each population with hypothetical segregation patterns to determine possible number of resistant genes.

Results and Discussion

Breeding line #181761-36P exhibited high resistance to *Phytophthora* crown rot characterized by only a small water-soaked lesion at the crown that dried out within a few days forming a brown scar, indicating inability of the pathogen to colonize crown tissues (Fig. 2). All #181761-36P plants grew vigorously throughout the duration of the experiment. In contrast, water-soaked lesions on susceptible TQ plants quickly expanded around the crown and progressed

Fig. 1. A 0 to 5 rating scale was used in the experiment, in which (**A**) indicates a score of 0 for an asymptomatic plant, (**B**) a score of 1 for a plant with a small brown lesion at the base of the crown, (**C**) a score of 2 for a plant with the lesion progressed to the cotyledons, (**D**) a score of 3 for a plant that has partially collapsed with apparent wilting of leaves, (**E**) a score of 4 for a plant with all leaves wilted and stem collapsed, and (**F**) a score of 5 for a dead plant.

Fig. 2. Response of resistant breeding line #181761-36P and susceptible acorn-type cultivar, Table Queen (TQ), to infection by a virulent isolate of *Phytophthora capsici* (isolate #121).

Table 1. Segregation patterns of reciprocal F_1, F_2, and reciprocal backcross (BC) progenies derived from a cross between resistant (R) breeding line #181761-36P and susceptible (S) acorn-type cultivar, Table Queen (TQ), at 28 d post inoculation with a virulent isolate of *Phytophthora capsici* (isolate #121).

	Total plants		Expected	
Genotype	R	S	ratios (R:S)	χ^2
181761-36P	12	0	1:0	—
TQ	1	11	0:1	—
F_1: (#181761-36P × TQ)	98	2	1:0	0.04 (NS)
F_1: (TQ × #181761-36P)	97	3	1:0	0.09 (NS)
F_2	179	21	57:7	0.04 (NS)
BC: (#181761-36P × F_1)	58	2	1:0	0.06 (NS)
BC: (TQ × F_1)	99	43	5:3	3.15 (NS)

NS = χ^2 value not significant ($P \leq 0.05$).

to the cotyledons, followed by wilting, collapsing, and plant death (Fig. 2). All TQ plants, except one, died within 6 dpi. The surviving plant may have escaped infection due to human error, such as skipped inoculation or pipetting error. Such errors are common in disease screening experiments involving manual inoculation of large plant populations (Sy et al., 2005).

The resistance reaction in reciprocal F_1 progenies (#181761-36P × TQ and TQ × #181761-36P) resembled that of #181761-36P except for a few plants that succumbed to the pathogen (Table 1). The F_2 population segregated in a 57: 7 [resistant (R): susceptible (S)] ratio, whereas that of backcross to susceptible parent (TQ × F_1) segregated in 5:3 (R:S) ratio (Table 1). On the other hand, progeny from backcross to resistant parent (#181761-36P × F_1) exhibited similar reaction to that in #181761-36P, except for two plants that succumbed to the pathogen (Table 1).

Taken together, the segregation patterns support a genetic model in which resistance to *Phytophthora* crown rot in breeding line #181761-36P is conferred by three dominant genes. These genes are designated R4, R5, and R6 to distinguish them from those (R1, R2, and R3) conferring resistance in *C. moschata* (Padley et al., 2009). R4 gene can confer resistance to *Phytophthora* crown rot in homozygous or heterozygous state (R4), independent of R5 and R6. However, R5 and R6 both must be present in the homozygous or heterozygous state (R5_R6_) to confer resistance to the pathogen. Occurrence of a few susceptible individuals in the F_1 and backcross (#181761-36P × F_1) progenies did not alter segregation ratios significantly ($P \leq 0.05$) (Table 1), and may have resulted from a higher zoospore concentration due to non-homogeneity of pipetted inoculum. A high inoculum density often leads to higher disease incidence (Hammond-Kosack and Jones, 1997; Nelson et al., 2017), even for resistant genotypes (Martyn and McLaughlin, 1983).

A similar inheritance model was reported for *Phytophthora* crown rot resistance in *C. moschata* line #394-1-27-12 (Padley et al., 2009), in which three independent dominant genes (R1R2R3) are required for expression of resistance. Further genetic studies will determine whether R1R2R3 and R4R5R6 in *C. moschata* and *C. pepo*, respectively, are syntenic. Deployment of R4R5R6 (from #181761-36P) in susceptible cultivar groups of *C. pepo* will provide oligogenic resistance against *Phytophthora* crown rot (Bowers and Mitchell, 1991; Raftoyannis and Dick, 2002), thus providing a critical tool in the integrated management of the disease. Further experiments are required to confirm field resistance to *Phytophthora* crown rot in breeding line #181761-36P.

To circumvent phenotyping challenges associated with traditional breeding for *Phytophthora* crown rot resistance, it is important to identify molecular markers linked to R4R5R6 in breeding line #181761-36P. This will allow efficient discrimination of resistant and susceptible progenies through marker-assisted selection, thus saving breeding resources. Linkage analysis of F_2 and $F_{2:3}$ populations segregating for resistance to *Phytophthora* crown rot is currently under way to identify DNA markers linked to R4R5R6 in breeding line #181761-36P.

Literature Cited

Babadoost, M. 2016. Oomycete diseases of cucurbits: History, significance, and management, p. 279–314. In: J. Jules (ed.). Horticultural reviews. John Wiley & Sons, Inc., Hoboken, NJ.

Babadoost, M. and C. Pavon. 2013. Survival of oospores of *Phytophthora capsici* in soil. Plant Dis. 97(11):1478–1483.

Babadoost, M. and S.Z. Islam. 2003. Fungicide seed treatment effects on seedling damping-off of pumpkin caused by *Phytophthora capsici*. Plant Dis. 87:63–68.

Bowers, J.H. and D.J. Mitchell. 1991. Relationship between inoculum level of *Phytophthora capsici* and mortality of pepper. Phytopathology 81(2):178–184.

Hammond-Kosack, K.E. and J.D.G. Jones. 1997. Plant disease resistant genes. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48(1):575–607.

Hausbeck, M.K. and K.H. Lamour. 2004. *Phytophthora capsici* on vegetable crops: Research progress and management challenges. Plant Dis. 88(12):1292–1303.

Krasnow, C.S., R. Hammerschmidt, and M.K. Hausbeck. 2017. Characteristics of resistance to *Phytophthora* root and crown rot in *Cucurbita pepo*. Plant Dis. 101(5):659–665.

Krasnow, C.S. and M.K. Hausbeck. 2015. Pathogenicity of *Phytophthora capsici* to brassica vegetable crops and biofumigation cover crops (*Brassica* spp.). Plant Dis. 99(12):1721–1726.

Lamour, K.H. and M.K. Hausbeck. 2000. Mefenoxam insensitivity and the sexual stage of *Phytophthora capsici* in Michigan cucurbit fields. Phytopathology 90(4):396–400.

Lamour, K.H. and M.K. Hausbeck. 2003. Susceptibility of mefenoxam-treated cucurbits to isolates of *Phytophthora capsici* sensitive and insensitive to mefenoxam. Plant Dis. 87(8):920–922.

Lamour, K.H., R. Stam, J. Jupe, and E. Huteima. 2012. The oomycete broad-host-range pathogen *Phytophthora capsici*. Mol. Plant Pathol. 13(4):329–337.

Martyn, R.D. and R.J. McLaughlin. 1983. Effects of inoculum concentration on the apparent resistance of watermelons to *Fusarium oxysporum* f. sp. *niveum*. Plant Dis. 67:493–495.

McHugh, M.L. 2013. The chi-square test of independence. Biochem. Med. (Zagreb) 23:143–149.

Michael, V.N., P. Moon, Y. Fu, and G. Meru. 2019. Genetic diversity among accessions of *Cucurbita pepo* resistant to *Phytophthora* crown rot. HortScience 54:17–22.

Nelson, R., T. Wiesner-Hanks, R. Wisser, and P. Balint-Kurti. 2017. Navigating complexity to breed disease-resistant crops. Nat. Rev. Genet. 19(1):21–33.

Padley, L.D., E.A. Kabelka, and P.D. Roberts. 2009. Inheritance of resistance to crown rot caused by *Phytophthora capsici* in *Cucurbita*. HortScience 44:211–213.

Padley, L.D., E.A. Kabelka, P.D. Roberts, and R. French. 2008. Evaluation of *Curcurbita pepo* accessions for crown rot resistance to isolates of *Phytophthora capsici*. HortScience 43:1996–1999.

Paris, H.S. 2008. Summer squash, p. 351–379. In: J. Prohens and F. Nuez (eds.). Handbook of plant breeding, Vegetables I. Springer, New York.

Ploetz, R., G. Heine, J. Haynes, and M. Watson. 2002. An investigation of biological attributes that may contribute to the importance of *Phytophthora capsici* as a vegetable pathogen in Florida. Ann. Appl. Biol. 140(1):61–67.

Raftoyannis, Y. and M.W. Dick. 2002. Effects of inoculum density, plant age and temperature on disease severity caused by pythiaceous fungi on several plants. Phytoparasitica 30(1):67–76.

Sanogo, S. and P. Ji. 2012. Integrated management of *Phytophthora capsici* on solanaceous and cucurbitaceous crops: Current status, gaps in knowledge and research needs. Can. J. Plant Pathol. 34(4):479–492.

Sy, O., P.W. Bosland, and R. Steiner. 2005. Inheritance of *Phytophthora* stem blight resistance as compared to *Phytophthora* root rot and *Phytophthora* foliar blight resistance in *Capsicum annuum* L. J. Amer. Soc. Hort. Sci. 130:75–78.

This paper was presented during the 2019 FSHS Annual Meeting and originally published in *HortScience* [HORTSCIENCE 54(7):1156–1158. 2019. https://doi.org/10.21273/HORTSCI14021-19]. The paper is included in this Proceedings as a reprint (with permission).

Proc. Fla. State Hort. Soc. 132:158–165. 2019.

Reducing Preharvest Bolting in Open-field-grown Cilantro (*Coriandrum sativum* L. cv. Santo) through Use of Growth Regulators

Bo Meyering, Adam Hoeffner, and Ute Albrecht
Southwest Florida Research and Education Center, University of Florida/IFAS, 2685 State Road 29 North, Immokalee, FL 34142

Additional index words. coriander, gibberellin, gibberellin biosynthesis inhibitors, plant growth regulators

***Abstract*. Cilantro (*Coriandrum sativum* L.), also called coriander, is an herbaceous, annual plant that is cultivated worldwide for its leaves and seeds. Cilantro has a strong propensity to bolt quickly in hot weather and under long-day (LD) conditions, which affects the flavor and renders the crop unmarketable. High incidence of preharvest bolting in open-field production can cause significant economic loss. The phytohormone gibberellic acid (GA) regulates stem elongation and floral initiation in many LD rosette plants. In pilot experiments, we found that GA induced bolting in greenhouse-grown cilantro and that plant growth regulators (PGRs) with anti-GA activity can delay this process. We then explored the effects of different GA inhibitors on reducing the incidence of bolting in cilantro grown in a commercial open-field environment. Four field trials were conducted on a commercial farm near Clewiston in Florida between Fall 2016 and Spring 2018. Different growth regulators were applied at different times, ranging from 5 to 8 weeks after seeding (WAS), and plants were harvested 2 to 3 weeks thereafter. Applications of GA inhibitors significantly reduced the incidence of bolting in three of the four trials, but the extent depended on the type of inhibitor used. The results from one trial were inconclusive due to changes in weather that prevented bolting in the entire field. Overall, plots treated with prohexadione calcium and paclobutrazol were most effective and reduced bolting by up to 78%. Applying the PGRs at 5 and 6 WAS was more effective than at 7 or 8 WAS.**

Coriander (*Coriandrum sativum* L.) or cilantro, as it is commonly called in the United States, is an herbaceous, annual plant with a basal rosette growth habit that belongs to the family *Apiaceae* and is widely cultivated throughout the world (Morales-Payan, 2011). Both the seeds and vegetative parts of the plant contain high levels of essential oils and organic compounds with broad medicinal properties (Laribi et al., 2015). Cilantro is grown mostly for its dried fruits, although its highly aromatic leaves, which are used to flavor a variety of dishes from salsas to curries, are the main part of the plant consumed in the United States. Its origin has not been conclusively determined, but most accessions can be placed into one of two broad categories: 1) those that originated in the Caucasus and Central Asia and are characterized by a prolonged vegetative growth state with many basal rosette leaves and 2) those that originated in the Near East and the Indian subcontinent where cultural selection favored quick flowering and large fruits over leaf production (Diederichsen, 1996).

Commercially grown cilantro is commonly cultivated in open field systems and suffers from relatively few production problems, although bacterial leaf spot, *Phytophthora* root rot, and *Fusarium* wilt can sometimes cause economic damage (Dugan et al., 2017; Pernezny et al., 1997). Several studies have explored various treatments and storage methods to increase its postharvest shelf life (Hassan and Mahfouz, 2012; Luo et al., 2004), and the effects of fertilization and water use have also been investigated extensively (Angeli et al., 2016). However, one persistent physiological trait of cilantro that has received proportionately little attention is its propensity to "bolt" or initiate rapid stem elongation from the rosette and flower under certain environmental conditions, causing the leaves to assume an unpleasant flavor (Bashtanova and Flowers, 2011). This averse flavor, which is most likely due to changes in the essential oil composition in the leaves (Potter, 1996), renders the crop unmarketable. During the winter in south Florida, it is possible to harvest a crop twice before bolting commences, but this becomes increasingly difficult as temperatures start to rise (Chuck Obern, personal communication, 2016). The relationship of cilantro bolting to increases in temperature and LD environmental conditions has been suggested in several extension publications; however, these provide little more than general observations on yield and early flowering (Blade et al., 2016; Smith et al., 2011).

Putievsky (1983) showed that LD conditions accelerate cilantro flowering but reduce overall yield, and Nawata et al. (1995), in their survey of cilantro landraces in southeast Asia, gathered evidence suggesting that cilantro is a facultative LD plant. Tomitaka et al. (2001) later provided support for these results by examining the stages of floral development in relation to changes in daylength through a series of controlled greenhouse experiments. Their results showed a strong, negative linear relationship between photoperiod and the elapsed time until flowering, even though flowering eventually occurred in all photoperiods tested. In their study, flower bud pre-differentiation occurred at an average of 49 d after seeding under daylength neutral conditions. This is in agreement with values published elsewhere (Diederichsen and Hammer, 2003).

Plant growth, development, and organ ontogenesis are complex processes, which are highly regulated by endogenous plant hormones and genetic and epigenetic control of regulatory pathways (Bishopp et al., 2006; Johnson and Lenhard, 2011; Pikaard and Scheid, 2014). Floral development and regulation in several other Apiaceous species are well understood and were shown to be regulated mainly through photoperiod and vernalization. Dill (*Anethum graveolens*), an LD plant, can be induced to flower once exposed to a single 11-hour daylength cycle, and early flowering is accompanied by lower vegetative yields (Hälvä et al., 1993; Hamner and Naylor, 1939). Similarly, fennel (*Foeniculum vulgare*) will bolt after being exposed to several photoperiod cycles for a minimum of 13.5 h (Peterson et al., 1993). Several other studies reported that celery (*Apium graveolens*) is a day-neutral species that is induced to flower by vernalization (Jenni et al., 2005), although LD conditions after vernalization treatments greatly reduced the time to bolting (Ramin and Atherton, 1994).

Floral induction in *Arabidopsis thaliana*, also a facultative LD plant with basal rosette growth, has been studied extensively and is highly regulated by several integrated pathways that monitor both environmental and plant internal conditions (Simpson and Dean, 2002). Bioactive gibberellins (GA) are a class

Received for publication 2 Oct. 2019. Accepted for publication 31 Oct. 2019.
Published online 16 December 2019.
We thank Thang Kim, Gustavo Piccin, Indu Tripathi, and Gabriel Pugina for their support in the field and with laboratory measurements. We also thank Chuck Obern (C&B Farms) for his support and helpful discussions and for allowing us to conduct the field trials at his farm.
This paper is associated with a presentation titled "Using Plant Growth Regulators to Reduce Bolting in Cilantro (*Coriandrum sativum*)" given during the 2019 Annual Meeting of the Florida State Horticultural Society, which was held 9–11 June in Orlando, FL.
U.A. is the corresponding author. E-mail: ualbrecht@ufl.edu.

of endogenous phytohormones that positively direct seed germination, stem elongation, and floral initiation and significantly interact with the auxin–cytokinin pathways for root and shoot meristem development (Vanstraelen and Benková, 2012). *Arabidopsis* mutants deficient in GA synthesis genes show extreme dwarfing phenotypes with tight rosette patterns and are nonresponsive to LD inductive conditions, supporting the role that GA plays in integrating these two pathways (Reeves and Coupland, 2001).

Several studies have investigated the effects of exogenously applied GA in cilantro to increase yield. Das et al. (2018) found that a 20 ppm GA application at 25 and 30 d after seeding increased leaf yield by 47%, but they did not comment on the incidence of preharvest flowering. Morales-Payan and Stall (2004) also observed a significant yield increase with GA applications. GA applied at rates of 10 and 20 $g \cdot ha^{-1}$ to plots of cilantro at 7 WAS had no effect on overall yield but significantly increased preharvest bolting and plant height (Kahn and Maness, 2010). When applied to plots at rates of 50 to 75 ppm, GA increased the number of flowering umbels, plant height, seed yield, and size and decreased the days to flowering and seed maturity (Yugandhar et al., 2016). Although early flowering and increased seed yield are beneficial traits for cultivation of coriander fruit, this is not the case when leaf production is the objective. Applying auxin or cytokinin to the foliage to prevent early bolting has been proposed, but no evidence was provided for its efficacy (Morales-Payan, 2011), and to date, no studies have specifically examined any methods to reduce the incidence of preharvest bolting in the field.

PGRs are synthetically produced compounds which alter growth and development and can attenuate the effects of environmental conditions and of endogenous phytohormones such as GA (Rademacher, 2015). Some compounds such as the diethanolamine salt of mefluidide (MFL) are cell division inhibitors and are used strictly to inhibit seedhead formation in turfgrass (Haguewood et al., 2013). Other PGRs, such as chlormequat chloride (CQC), paclobutrazol (PBZ), flurprimidol, and prohexadione calcium (PCa), directly inhibit GA biosynthesis (Rademacher, 2016). Currently, PCa has widespread use in controlling fire blight in apples (McGrath et al., 2009), reducing vegetative growth in peanuts (Beam et al., 2002), and decreasing shoot growth in apples and cherries (Cline, 2017). Although PBZ is mainly used in the ornamental plant industry, it has some efficacy in reducing shoot growth in tropical and subtropical tree crops (Rademacher, 2016).

It is possible to control certain environmental conditions within a greenhouse setting; however, it is currently not practical to do so in open production systems. Given our assumptions that cilantro is induced under LD conditions and that GA at least partially controls stem elongation and floral initiation in cilantro, we hypothesized that PGRs that inhibit GA biosynthesis would decrease the incidence of early bolting in the field. The objective of our study was therefore to determine the efficacy of several commercially available GA inhibitors to prolong the vegetative stages of cilantro in the field and to identify the appropriate time at which to apply these products.

Materials and Methods

Greenhouse experiments

Plant material. Two pilot experiments were conducted in an enclosed greenhouse to screen prospective antigibberellin compounds and identify appropriate concentrations for spray application. One-gallon nursery containers (Pro-Cal, South Gate, CA) were surface sterilized with a 1% NaClO solution and air-dried. Cilantro seeds (cv. Santo) were sown into sterilized pots filled with Fafard #2 potting medium (Sun Gro Horticulture, Agawam, MA). Both the seeds and potting medium were pretreated with Oxidate 2.0 (BioSafe Systems LLC, East Hartford, CT) according to the manufacturer's directions. Seeds were germinated and then thinned to six seedlings per pot. Once the first true leaf was fully expanded, pots were fertilized three times per week with 150 mL of a water-soluble fertilizer at 100 ppm N (Peter's Professional, $20N–10P_2O_5–20K_2O$, Israel Chemicals Ltd., Tel Aviv, Israel). The fertilizer dosage was increased to 300 mL starting at 6 WAS as the plants began to mature.

Experimental design. Plants were arranged on the greenhouse benches in a completely randomized design (CRD) with 6 replicates per treatment. Each replicate consisted of one pot containing 6 plants.

Treatments. PGRs applied to plants in Expt. 1 were PBZ (paclobutrazol, Bonzi, 0.4%, Syngenta, Greensboro, NC) at 3 $mg \cdot L^{-1}$ a.i., CQC (chlormequat chloride, Cycocel 11.8%, OHP, Bluffton, SC) at 2000 $mg \cdot L^{-1}$ a.i., and GA (gibberellic acid, N-large 4% GA_3, Stoller) at 25 $mg \cdot L^{-1}$ a.i. as a positive control. PGRs applied to plants from Expt. 2 included PBZ and GA at the same rates used in Expt. 1. In addition, PCa1 (prohexadione calcium, Apogee, 27.5% (w/w), BASF Corporation, Florham, NJ) at 119 $mg \cdot L^{-1}$ a.i. and MFL (mefluidide, Embark 3.2%, PBI/Gordon Corporation, Kansas City, MO) at 45 $mg \cdot L^{-1}$ a.i. were used. CQC was used in Expt. 1 as a proof of concept but later omitted because of its restricted use in an open-field environment. PBZ was applied as a 200-mL drench; all other materials were applied foliar with a handheld sprayer and included Tween 20 (Sigma-Aldrich, St. Louis, MO) at 0.1% (v/v) as a surfactant. The spray volume per pot was ≈50 mL, which was enough to cover the leaves until runoff. An untreated control (UTC) using water and surfactant only was included in each experiment. In Expt. 1, plants were sprayed at 8 WAS and evaluated 10 WAS. In Expt. 2 plants were sprayed at 5 WAS and evaluated at 8 WAS. The average daylength during the experiments was 11.25 and 11.5 h for Expts. 1 and 2, respectively. Treatments and supporting information are summarized in Table 1.

Field trials

Field location and management. Four field trials were conducted on a commercial vegetable farm in Clewiston, FL (lat. 26°27′18″ N, long. 81°1′52″ W). Trial 1 was conducted in Winter 2016, trials 2 and 3 in Spring 2017, and trial 4 in Spring 2018. The prominent soil type at the farm site is siliceous Basinger fine sand, which is typically poorly drained but rapidly permeable (Soil Survey Staff, Natural Resources Conservation Service, U.S. Department of Agriculture, Official Soil Series Descriptions, 2014). Cilantro seeds (cv. Santo) were sown in 1.27-m-wide × 30.0-m-long raised beds with five rows per bed. Fields were broadcast fertilized with a granular fertilizer containing N, P, K ($7N–9P_2O_5–17K_2O$) at a rate of 672.1 $kg \cdot ha^{-1}$ and then supplemented with three side dress applications of liquid fertilizer containing $10N–0P–8K_2O$ at a rate of 375 $L \cdot ha^{-1}$ for a total of 134.5 $kg \cdot ha^{-1}$ N. Liquid fertilizer applications were performed at 2, 4, and 6 WAS. Irrigation was by seepage, and pest and disease management was performed according to the grower's standard practice. Average daylength ranged from 10.5 h during trial 1 to 11.9 h during trial 4, and the average temperatures ranged from 19.6 °C during trial 3 to 21.4 °C during trial 4 (Table 1).

Experimental design. The experimental design was a randomized complete block design (RCBD) using four replicates (trials 1–3) or six replicates (trial 4) per treatment. Each treatment plot was 1.27 m (the width of one bed) × 3.66 m (4.65 m^2) in size with a 0.2-m buffer area between plots to reduce the chance of spray carryover from adjacent plots. The treatments were applied to the entire plot, and sampling was conducted on a smaller subplot, which excluded 0.6 m on either end of the plot as well as the outer two rows of cilantro within a bed to minimize any edge effect. Plants from field trials 1, 2, and 3 were harvested and evaluated at 8 WAS, and plants from trial 4 were harvested at 9 WAS, coinciding with the grower's harvest of the remainder of the field.

Treatments. Treatments were applied using a motorized backpack sprayer equipped with a flat fan nozzle wand (Solo 417, Solo Inc., Newport News, VA) and were sprayed at 40 psi. All materials were applied together with a liquid soap (Dawn, Proctor & Gamble, Cincinnati, OH) as adjuvant favored by the grower at 0.1% (v/v). The UTC plants were sprayed with the adjuvant only. The total spray volume for each plot was 3.785 L for field trials 1 through 3 and 325 mL for field trial 4. Treatments for field trial 1 were applied in Dec. 2016 at 5 WAS. The time of application was 10:00 HR. The PGRs used were PBZ applied as a sprench ("sprayed drench" directed at the soil surface near the stem) at 3 $mg \cdot L^{-1}$ a.i. and PCa1 applied foliar at 42.5 $mg \cdot L^{-1}$ a.i. Field trials 2 and 3 were sown 1 week apart in neighboring fields. Treatments were applied at 10:00 HR on the same day in Feb. 2017 at 6 WAS (trial 2) and at 5 WAS (trial 3). The plant growth regulators used were PBZ applied as a sprench at

Table 1. Treatments, treatment rates, planting dates, application times, and other supporting information of cilantro (cv. Santo) greenhouse experiments and field trials conducted in this study.

	Greenhouse experiments		Field trials			
	1	2	1	2	3	4
Treatments and treatment rates						
GA	25 mg·L^{-1}	25 mg·L^{-1}	—	—	—	—
PBZ	3 mg·L^{-1}	3 mg·L^{-1}	3 mg·L^{-1}	6 mg·L^{-1}	6 mg·L^{-1}	—
PCa1	—	119 mg·L^{-1}	42.5 mg·L^{-1}	42.5 mg·L^{-1}	42.5 mg·L^{-1}	42.5 mg·L^{-1}
PCa2	—	—	—	—	—	42.5 mg·L^{-1}
CQC	2000 mg·L^{-1}	—	—	—	—	—
MFL	—	45 mg·L^{-1}	—	45 mg·L^{-1}	45 mg·L^{-1}	—
Supporting information						
Planting date	21 Sept. 2016	23 Jan. 2017	11 Sept. 2016	12 Jan. 2017	21 Jan. 2017	2 Apr. 2018
Application time	8 WAS	5 WAS	5 WAS	6 WAS	5 WAS	6, 7, 8 WAS
Evaluation time	10 WAS	8 WAS	8 WAS	8 WAS	8 WAS	9 WAS
Avg daylength (h)	11.25	11.5	10.5	11.25	11.5	11.9
Avg high temp (°C)	29.9	27.5	28.0	27.9	27.6	29.2
Avg low temp (°C)	18.8	13.1	15.5	14.0	13.1	15.0
Avg temp (°C)	23.3	19.5	20.8	20.0	19.6	21.4

Treatment rates listed are mg of a.i. per liter. GA = gibberellic acid; PBZ = paclobutrazol; PCa1 and PCa2 = prohexadione calcium; CQC = chlormequat chloride; MFL = mefluidide; WAS = weeks after seeding.

6 mg·L^{-1} a.i., PCa1 applied as a foliar spray at 42.5 mg·L^{-1} a.i., and MFL applied as a foliar spray at 45 mg·L^{-1} a.i. Plants from field trials 1 through 3 were evaluated 8 WAS.

In field trial 4, we explored the interaction of treatment and application time. In addition to PCa1, a second prohexadione calcium material, PCa2 [Kudos, 27.5% (w/w), Fine Americas Inc., Walnut Creek, CA] was used. Both materials were applied as foliar sprays at 42.5 mg·L^{-1} a.i. with spray grade AMS (21–0–0) at 476 mg·L^{-1} to facilitate uptake into the leaves and a nonionic surfactant (Activator 90, Loveland Products Inc., Loveland, CO) at 1.25 mL·L^{-1} according to the manufacturer's recommendations. The UTC consisted of AMS and nonionic surfactant only. The total spray volume per plot was reduced from 3.785 L in field trials 1 through 3 to 325 mL to more accurately represent volumes used in commercial production. Applications were conducted 6, 7, and 8 WAS, and plants were evaluated 9 WAS. Treatments and supporting information are summarized in Table 1.

Plant evaluations

Plant collection. For the greenhouse experiments, the six plants in each pot were cut at the stem below the base of the rosette leaves and immediately analyzed for bolting and biometric parameters. For the field trials, a sampling quadrat was used to randomly collect 10 (field trial 1) and 20 (field trial 2) plants from a sample subplot (2.44 m × 0.76 m, 1.85 m^2) in the center of each treatment plot. Plants were cut at the stem below the base of the rosette leaves, pooled by plot, bagged in macroperforated clear polyethylene bags, and transported to the laboratory for assessment of bolting and other biometric parameters.

Biometric measurements. Plants were defined as bolting when internodes measured at least 1 cm in length. Plant length was determined from the base of the first rosette leaf to the length of the distal end of the leaves when laid on end, excluding any aberrant leaves. The stem diameter was measured with a digital caliper directly beneath the first true leaf of each plant. If a plant was scored as bolting, then the longest internode on the caulis (in rosette plants, the main stem that elongates before anthesis) or the total length of the caulis (field trial 4) was measured. Finally, the fresh weight of each plant was determined (not measured for field trial 4).

Leaf morphology changes. In the field trials, the percentage of plants displaying leaf morphology changes (inflorescent stems with leaves of highly pinnate to filiform morphology) was assessed in whole plots before harvest. In field trials 2 and 3, the percentage of plants with leaf morphology changes was additionally assessed 1 and 2 weeks after harvest (WAH) in regrown plants; we were unable to collect these data for field trials 1 and 4 because fields were accidentally plowed shortly after harvest.

Yield. The yield for field trial 1 was determined as the total biomass of 20 random plants from the sample subplot. The yield for field trials 2 through 4 was determined as the total biomass of all plants in the sample subplot and expressed as kg/m^2 standardized to the average plant density per plot. Yields were determined on location immediately after harvest using a commercial scale (CWP-150, CAS-USA, East Rutherford, NJ).

Statistical analysis

Measurements were averaged for the six plants grown in one pot (greenhouse experiments) or collected from each plot (field trials) before calculating treatment means, and 95% confidence intervals for each variable mean were constructed. Data were analyzed separately for each greenhouse experiment and each field trial. Analysis of variance (ANOVA) was conducted using R in the RStudio environment (R Core Team, 2018; RStudio Team, 2016). The means from significant tests were separated with Tukey's honestly significant difference (HSD) test using the "Agricolae" package (de Mendiburu, 2017). One-way ANOVA was employed for greenhouse experiments. RCBD ANOVA was employed for field trials with treatment as a main factor and block as a random factor (trials 1–3) or treatment and application time as the main factors and block as a random factor (trial 4). Means were separated by Tukey's HSD test. Differences were defined as statistically significant when the P value was <0.05. Graphs were constructed using the "ggplot2" package (Wickham, 2016).

Results

Greenhouse experiments

In both greenhouse experiments, GA treatment significantly increased plant length and induced bolting in 100% of the plants (Table 2) with many plants displaying leaves with pinnate to filiform morphology typical of cilantro plants that have initiated flowering (Fig. 1). In Expt. 1, plants treated with both PBZ and CQC were significantly shorter (30.5 cm and 31.8 cm) and bolted less frequently (11.7% and 24.2%) than the UTC plants, which were 39.3 cm long and bolted with a frequency of 82.8%. In Expt. 2, only PBZ significantly reduced bolting (30.6%) compared with the UTC (71.1%). Plant fresh weight was not significantly affected by any of the treatments in Expt. 1. In Expt. 2, plants treated with MFL were significantly reduced in weight and in length compared with the UTC and displayed severe apical growth aberrations. No growth aberrations were observed for the other treatments. The internodes of plants from Expt. 2 were shortest in MFL treated plants (2.0 cm) and longest in GA-treated plants (8.8 cm), but no significant differences were found among plants having received PCa1 or PBZ compared with the UTC.

Field trials

Bolting. In field trial 1, plants treated with PCa1 bolted at a significantly lower percentage (25.0%) compared with PBZ-treated plants and the UTC, which bolted at a percentage of 67.5% and 97.5%, respectively (Table 3). Plot-to-plot variability for percent bolting of PBZ-treated plants was high,

Table 2. Plant length, percent bolting, fresh weight, and internode length of cilantro (cv. Santo) plants from greenhouse Expts. 1 and 2 after treatment with different plant growth regulators.

Treatment	Plant length (cm) Expt. 1	Plant length (cm) Expt. 2	Bolting (%) Expt. 1	Bolting (%) Expt. 2	Fresh wt (g) Expt. 1	Fresh wt (g) Expt. 2	Internode length (cm) Expt. 1	Internode length (cm) Expt. 2
UTC	39.3 ± 3.4 b	27.8 ± 4.3 b	82.8 ± 15.7 a	71.1 ± 21.8 a	31.1 ± 6.6	22.9 ± 7.9 a	—	4.7 ± 1.9 bc
GA	57.3 ± 4.0 a	36.9 ± 10.2 a	100.0 ± 0.0 a	100.0 ± 0.0 a	34.8 ± 9.4	18.1 ± 8.3 ab	—	8.8 ± 1.7 a
PBZ	30.5 ± 1.8 c	21.0 ± 5.1 bc	11.7 ± 14.6 b	30.6 ± 23.2 b	25.6 ± 6.4	19.3 ± 4.5 ab	—	6.1 ± 2.7 ab
PCa1	—	20.5 ± 1.6 bc	—	66.9 ± 25.2 a	—	14.7 ± 3.2 ab	—	4.4 ± 2.0 bc
CQC	31.8 ± 4.1 c	—	24.2 ± 22.4 b	—	24.3 ± 12.5	—	—	—
MFL	—	15.4 ± 2.4 c	—	71.1 ± 25.8 a	—	12.9 ± 5.5 b	—	2.0 ± 1.0 c
Avg	39.7 ± 4.8	24.3 ± 3.4	54.7 ± 17.2	67.9 ± 11.0	28.9 ± 3.9	17.6 ± 2.4	—	5.1 ± 1.1
Treatment	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P = 0.0001$	$P = 0.1570$	$P = 0.0554$	—	$P < 0.0001$

Values presented are the mean ± the 95% confidence interval (n = 4). Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. Internode length was not measured in Expt. 1. UTC = untreated control; GA = gibberellic acid; PBZ = paclobutrazol; PCa1 = prohexadione calcium; CQC = chlormequat chloride; MFL = mefluidide.

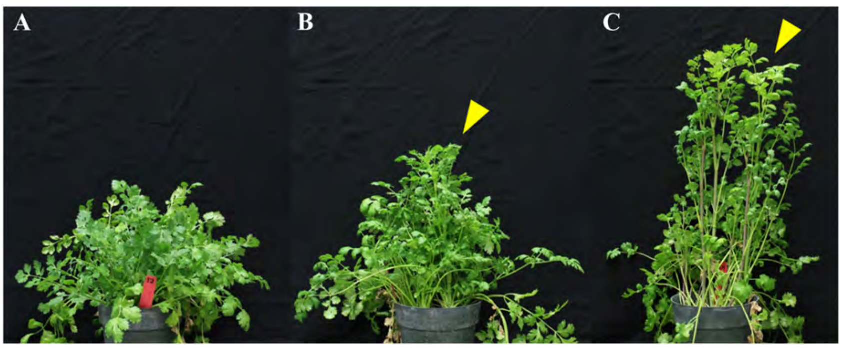

Fig. 1. Cilantro (cv. Santo) plants from greenhouse Expt. 1 treated with (**A**) paclobutrazol (PBZ), (**B**) no growth regulator [untreated control (UTC)], and (**C**) gibberellic acid (GA). Photos were taken 10 weeks after harvest. The yellow arrows point to leaves with pinnate to filiform morphology typical of cilantro plants that have initiated flowering. Note the more compact growth habit in the PBZ-treated plants and the elongated stems in GA-treated plant compared with the UTC.

ranging from 30% to 90%. In trial 2, plants treated with both PCa1 and PBZ bolted significantly less frequently (8.8% and 13.8%) than MFL-treated plants and the UTC (41.3% and 40.0%). In trial 3, the percentage of bolting ranged from 1.3% to 11.3%, and no significant differences were measured among differently treated plants.

In trial 4, a significant effect was found for both treatment and application time, but the interaction between the two factors was not significant (Fig. 2A). The average percentage of bolting in the UTC plots was 95%. Both PCa1 and PCa2 reduced the incidence of bolting to 72.5% and 73.5%, respectively. Plants bolted less frequently when they were sprayed at 6 and 7 WAS than when they were sprayed at 8 WAS. Block was significant at a level of 0.4%, but no interaction with main effects was observed.

Leaf morphology changes. The percentage of plants with leaf morphology changes was significantly different among treatments in three of the four field trials (Table 3, Fig. 2B). In field trial 1, leaf morphology changes occurred least frequently in PCa1 treated plants (1.7%), followed by PBZ treated plants (17.9%), and most frequently in the UTC (39.6%). In field trial 2, the average percentage of plants with leaf morphology changes was low (1.1%), with the lowest percentage observed for PCa1- and PBZ-treated plants (0.4%) and the highest percentage observed for plants treated with MFL (2.9%). In trial 3, none of the plants displayed leaf morphology changes at the time of harvest. In field trial 4, both treatment and application time were significant factors and significantly interacted; leaf morphology changes occurred least frequently in plants treated with PCa1 and PCa2 at 6 and 7 WAS (7.3% to 11.4%) and most frequently in untreated plants (33.3% to 36.5%), regardless of the application time (Fig. 2B). Block was not a significant factor.

Leaf morphology changes after harvest. The percentage of plants with changed leaf morphology was assessed 1 and 2 WAH in field trials 2 and 3. Significant differences were found in field trial 2 at 1 WAH (Table 3) with the highest percentage measured for the UTC (32.6%) and the lowest percentage measured for PCa1-treated plants (14.5%). The average percentage of plants with changed leaf morphology at 2 WAH in field trial 2 was 45.4.5% with no significant difference among treatments. In field trial 3, the average percentage of plants with changed leaf morphology after harvest was 28.6% (1 WAH) and 61.9% (2 WAH) but did not differ significantly among treatments.

Plant biometrics. Application of growth regulators did not significantly influence plant length in trials 1 and 3, which averaged 44.3 cm and 46.1 cm, respectively (Table 4). Plant length in trial 2 varied significantly among treatments. Plants treated with MFL were shortest (39.5 cm), while the UTC plants were longest (46.9 cm). Neither PCa1- nor PBZ-treated plants were different in length from the UTC plants. Block was a significant factor for plant length in field trial 2.

In trial 4, both treatment and application time significantly affected plant length, but there was no significant interaction (Fig. 2C). PCa1- and PCa2-treated plants were significantly shorter (53.6 cm and 54.7 cm) compared with the UTC (58.2 cm). Application of treatments at 6 and 7 WAS resulted in shorter plants (54.6 cm and 54.8 cm) than applications at 8 WAS (57.1 cm). Block was a significant factor ($P < 0.0001$), as was the interaction of block with treatment ($P = 0.0463$) and application time ($P = 0.0313$).

The average stem diameters of plants ranged from 8.4 mm in field trial 4 to 9.8 mm in field trial 1 and no significant differences were found among treatments in any of the trials (data not shown).

The length of the longest internode of bolting plants was measured in field trials 1 through 3 (Table 4). Internode length was a significant trait in trial 1; plants treated with PCa1 had the shortest internodes (3.2 cm), and UTC plants had the longest (6.7 cm). No significant differences for internode length were measured in trials 2 and 3. Block was not a significant factor in any of the three trials.

Caulis length was measured for bolting plants in trial 4 (Fig. 2D). Treatment was a significant factor; caules of plants treated with PCa1 and PCa 2 were significantly shorter (28.6 and 29.7 cm) than caules of untreated plants (41.1 cm). Caulis length was not significantly affected by application time, and no interaction between main effects was observed. Block was a significant factor ($P < 0.0001$) and interacted significantly with application time ($P = 0.0011$). A significant interaction was also found for block, application time, and treatment ($P = 0.0045$). There was a moderate positive correlation ($R = 0.5214$, $P < 0.0001$) between caulis length and the incidence of bolting across all treatments.

The average fresh weight of plants from trials 1 through 3 ranged from 50.0 g in trial 1 to 89.0 g in trial 2 (Table 5). Significant

Table 3. Percent bolting and leaf morphology changes of cilantro (cv. Santo) plants from field trials 1 through 3 treated with different plant growth regulators.

Treatment	Bolting (%)			Leaf morphology changes (%)			Leaf morphology changes (%) 1 WAH		Leaf morphology changes (%) 2 WAH	
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 2	Trial 3	Trial 2	Trial 3
UTC	97.5 ± 8.0 a	40.0 ± 17.2 a	5.0 ± 0.0	39.6 ± 8.3 a	0.8 ± 1.1 ab	0	32.6 ± 5.6 a	24.7 ± 10.1	44.7 ± 10.4	63.4 ± 11.4
PCa1	25.0 ± 9.2 b	8.8 ± 13.6 b	5.0 ± 6.5	1.7 ± 2.2 c	0.4 ± 0.8 b	0	14.5 ± 6.9 b	27.8 ± 7.1	47.5 ± 21.3	55.4 ± 20.2
PBZ	67.5 ± 45.7 a	13.8 ± 17.6 b	1.3 ± 4.0	17.9 ± 6.2 b	0.4 ± 1.3 b	0	18.2 ± 7.7 ab	26.2 ± 8.4	45.5 ± 27.2	66.2 ± 6.3
MFL	—	41.3 ± 7.6 a	11.3 ± 20.9	—	2.9 ± 4.0 a	0	24.3 ± 15.7 ab	35.6 ± 33.3	44.2 ± 13.7	62.7 ± 23.1
Avg	63.3 ± 22.1	25.9 ± 9.2	5.6 ± 3.9	19.7 ± 10.5	1.1 ± 0.9	0	22.4 ± 4.8	28.6 ± 5.9	45.4 ± 5.8	61.9 ± 5.5
Treatment	*P* = 0.0033	*P* = 0.0018	*P* = 0.2960	*P* < 0.0001	*P* = 0.0304	—	*P* = 0.0219	*P* = 0.6060	*P* = 0.9380	*P* = 0.5890
Block	*P* = 0.5089	*P* = 0.7595	*P* = 0.4030	*P* = 0.0305	*P* = 0.0565	—	*P* = 0.8576	*P* = 0.6210	*P* = 0.0140	*P* = 0.7010

Values presented are the mean ± the 95% confidence interval (n = 4). Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. WAH = weeks after harvest; UTC = untreated control; PCa1 = prohexadione calcium; PBZ = paclobutrazol; MFL = mefluidide. MFL was not used in trial 1.

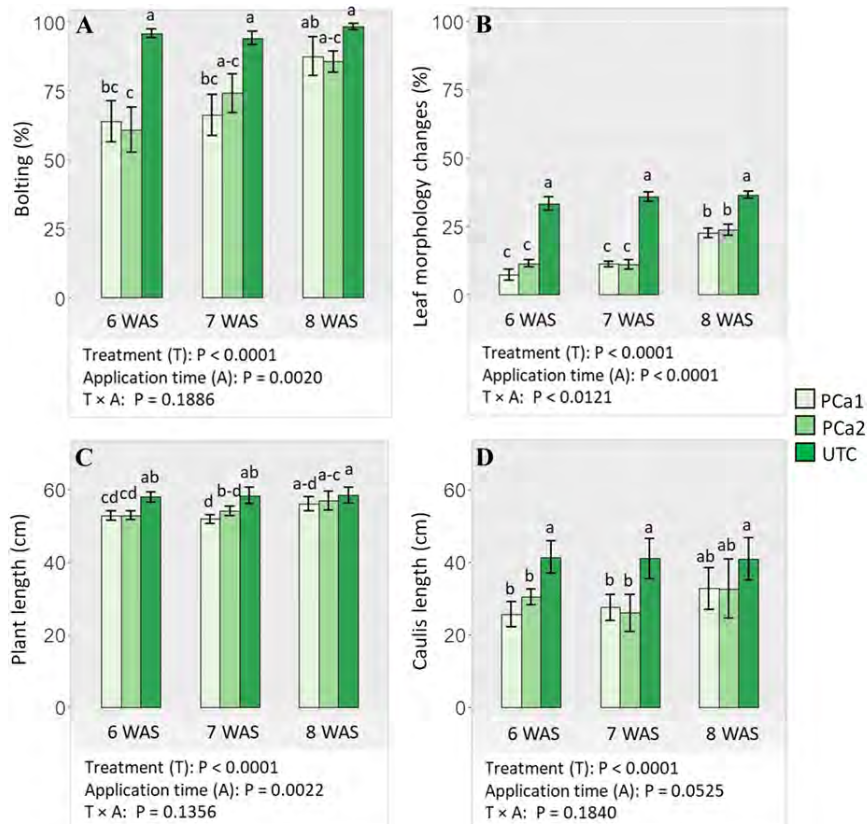

Fig. 2. Percent bolting (**A**), percent leaf morphology changes (**B**), plant length (**C**), and caulis length (**D**) of cilantro (cv. Santo) plants from field trial 4 after application of prohexadione calcium (PCa1 and PCa2) at 6, 7, and 8 weeks after seeding (WAS). Plants were assessed at 9 WAS. Values presented are the mean ± the SE (n = 6). Different letters above bars indicate significant differences according to Tukey's honestly significant difference test. UTC = untreated control.

differences among plants from the different treatments were only found in trial 2 where MFL-treated plants weighed significantly less (79.4 g) than UTC plants (101.7 g). Fresh weight per plant was not measured in trial 4.

The yield for trial 1, expressed as the combined weight of 20 plants per plot, was 1.0 kg on average but did not differ significantly among treatments (Table 5). The yield for trials 2 and 3, expressed as plant weight per square meter, was 4.3 kg/m^2 in trial 2 and 5.1 kg/m^2 in trial 3, but no significant differences were found among plants from the different treatments. Block was a significant factor in field trial 2.

The yield for field trial 4 ranged from 4.5 kg/m^2 when plants were treated with PCa1 to 4.9 kg/m^2 when plants were untreated, but differences were not significant (data not shown). Yields of plants treated at 6, 7, or 8 WAS ranged from 4.7 to 4.8 kg/m^2 with no significant differences among application times. No interaction of yield and application time was found. Block was not a significant factor.

Discussion

Preharvest bolting in rosette plants entails rapid stem elongation and floral initiation and is an undesirable trait in many leafy crops such as lettuce, cabbage, and spinach. In field-grown cilantro, it can cause significant reductions in leaf yield and therefore profitability. High temperatures and increased daylength are factors thought to exacerbate bolting (Morales-Payan, 2011); however, it is not practical to control these in large open-field production systems. GA is known to have repressive effects on flowering and bud formation in perennial, woody plants, but promotes bolting in most herbaceous plant species (Li et al., 2018; Vanstraelen and Benková, 2012). Previous research that focused on increasing leaf yield in cilantro (cv. Santo) reported increased incidences of bolting in response to exogenous applications of GA (Kahn and Maness, 2010). GA was also found to be effective in decreasing the time to flowering in other cilantro cultivars (Kurmi et al.,2019; Yugandhar et al., 2014). Our pilot experiments conducted in the greenhouse and using the cilantro cultivar 'Santo' confirmed these findings. These pilot experiments also demonstrated that plant growth regulators that inhibit GA biosynthesis such as PBZ could effectively reduce bolting. GA biosynthesis inhibitors were previously examined regarding their influence on growth and anatomical characteristics of greenhouse-grown cilantro (Kofidis et al., 2008). To assess whether these PGRs are also effective

Table 4. Plant length and internode length of cilantro (cv. Santo) plants from field trials 1 through 3 treated with different plant growth regulators.

	Plant length (cm)			Internode length (cm)		
Treatment	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
UTC	44.4 ± 7.7	46.9 ± 6.1 a	47.5 ± 11.6	6.7 ± 2.6 a	3.1 ± 0.9	2.8 ± 3.0
PCa1	46.0 ± 3.9	44.2 ± 8.8 a	47.8 ± 5.5	3.2 ± 2.3 b	2.6 ± 2.5	1.7 ± 1.8
PBZ	42.5 ± 4.9	45.3 ± 5.3 a	45.7 ± 4.4	5.6 ± 2.8 ab	2.8 ± 0.8	2.0 ± 0.0
MFL	—	39.5 ± 6.9 b	43.5 ± 9.1	—	3.2 ± 0.7	2.4 ± 1.4
Avg	44.3 ± 2.3	44.0 ± 2.6	46.1 ± 2.6	5.1 ± 1.4	3.0 ± 0.4	2.3 ± 0.8
Treatment	$P = 0.5060$	$P = 0.0042$	$P = 0.6900$	$P = 0.0235$	$P = 0.7620$	$P = 0.6540$
Block	$P = 0.7600$	$P = 0.0009$	$P = 0.7780$	$P = 0.1557$	$P = 0.3050$	$P = 0.1750$

Values presented are the mean ± the 95% confidence interval (n = 4). Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. UTC = untreated control; PCa1 = prohexadione calcium; PBZ = paclobutrazol; MFL = mefluidide. MFL was not used in trial 1.

Table 5. Fresh weight and yield of cilantro (cv. Santo) plants from field trials 1 through 3 treated with different plant growth regulators.

	Fresh wt (g/plant)			Yield (kg/20 plants)	Yield (kg/m²)	
Treatment	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
UTC	49.0 ± 21.1	101.7 ± 17.9 a	59.9 ± 14.5	1.05 ± 0.4	4.1 ± 1.0	5.0 ± 2.4
PCa1	46.8 ± 10.9	85.8 ± 10.1 ab	65.5 ± 22.6	0.93 ± 0.2	4.6 ± 1.1	6.1 ± 2.3
PBZ	54.1 ± 21.1	89.1 ± 21.1 ab	64.9 ± 13.9	1.08 ± 0.1	4.6 ± 0.5	4.6 ± 2.7
MFL	–	79.4 ± 7.1 b	60.3 ± 20.4	—	4.0 ± 0.4	5.3 ± 3.6
Avg	50.0 ± 6.9	89.0 ± 6.4	62.6 ± 5.6	1.0 ± 0.1	4.3 ± 0.5	5.1 ± 0.8
Treatment	$P = 0.6380$	$P = 0.0477$	$P = 0.8630$	$P = 0.3300$	$P = 0.3023$	$P = 0.3390$
Block	$P = 0.3300$	$P = 0.4054$	$P = 0.6000$	$P = 0.2230$	$P = 0.0085$	$P = 0.2470$

Values presented are the mean ± the 95% confidence interval (n = 4). UTC = untreated control; PCa1 = prohexadione calcium; PBZ = paclobutrazol; MFL = mefluidide. MFL was not used in trial 1.

in a commercial open-field environment, we conducted four field trials between Nov. 2016 and April 2018. GA biosynthesis inhibitors have been successfully used to curtail bolting in Chinese cabbage, spinach, and lettuce (Abed, 2018; Fukuda et al., 2005; Pressman and Aviram, 1986). In cilantro, applications of indole-3-acetic acid and kinetin delayed the onset of flowering (Hernández-Dávila et al., 2004), but GA biosynthesis inhibitors were not examined in that study.

Although PBZ was effective in reducing the frequency of bolting in the greenhouse experiments, it was less effective in the field trials. In field trial 2, bolting was reduced by 65% compared with the UTC, but in trial 1, any effects that may have been present were masked by high variation among PBZ-treated plots. The differences between greenhouse and field results may have been caused by the stronger retention of PBZ in the peat-based potting medium compared with the sandy permeable soils present at the farm site (Environmental Protection Agency, 2007). In addition, a larger volume per plant was used in the greenhouse experiments than in the field trials. In contrast to PBZ, PCa was highly effective in field trials and reduced bolting by 74% to 78% in trials 1 and 2 and by 21% to 36% in trial 4. The lower efficacy of PCa in field trial 4 could have been a consequence of the reduced spray volume used in that trial. Although the reduced spray volume more accurately reflects spray volumes used by growers, it may not have provided enough coverage.

The average percentage of bolting in field trial 3 was low with no differences among treatments, despite the longer average daylength during this trial compared with trials 1 and 2. Fluctuations in temperature, as discussed later in this section, may have contributed to these findings.

MFL, a plant growth regulator used to inhibit vegetative growth and seed head formation in turfgrass, reduced plant length and caused apical growth aberrations but had no effect on bolting in greenhouse experiments or field trials. MFL is a fast-acting PGR that rapidly accumulates in the apical meristem where it inhibits mitosis within 48 h of application (March et al., 2013; Tautvydas, 1983); however, time of application and concentration must be precisely optimized to be effective (Christians, 2001). Therefore, this compound may be more effective in suppressing bolting in cilantro when applied at lower concentrations and several days before bolting instead of several weeks as explored in our study.

CQC, which, like PBZ and PCa, inhibits GA biosynthesis and reduced bolting incidence by 70% in greenhouse Expt. 1, was not used in the open-field trials because of the potential environmental hazards listed on the label. In addition to effectively reduce bolting, greenhouse-grown plants treated with CQC were shorter compared with untreated plants, which is similar to results obtained in field-grown lettuce (Passam et al., 2008). PBZ reduced plant length in greenhouse Expt. 1 but had no effect on plant length in the field trials. PCa reduced plant length only in field trial 4 but reduced neither plant length nor percentage of bolting in greenhouse Expt. 2. A strong effect of PCa on plant length was observed in a study by Kofidis et al. (2008); however, PCa concentrations were higher, and plants were sprayed multiple times and were measured after fruit ripening.

Applications of PGRs were performed at different time points (weeks) after seeding. The greatest reductions in the incidence of bolting under open-field conditions were observed when plants received applications at 5 to 6 WAS and were harvested at 8 WAS, although applications of PCa at 7 WAS were also effective when plants were harvested 2 weeks later (9 WAS). When plants received PCa at 8 WAS and were harvested at 9 WAS, effects were diminished. This suggests that for the effects of GA biosynthesis inhibiting growth regulators to become manifest in cilantro, applications should commence at a minimum of 2 weeks before the anticipated time of bolting. This period may not be appropriate for PGRs with a different mode of action, such as MFL.

Field trials 2 and 3 were sown in adjacent fields and 1 week apart from each other. The percentage of bolting in the untreated plots was 40% in trial 2 compared with 5% in trial 3. Detailed review of the climatic data during the time of the trials revealed a drop in the minimum temperature to 4 °C for a period of 12 h, which occurred 10 d after seeding trial 3. It is possible that the sudden drop in temperature occurred at a developmental stage that is critical for floral induction, and that floral induction already occurred in plants of trial 2 where minimum temperatures were higher (10–15 °C) during the same time frame after seeding. PCa is absorbed quickly by plants and does not persist in the plant tissue, and thus applications made before induction of floral pathways may not effectively curtail bolting (Evans et al., 1999).

Because of the apparent strong influence of environmental conditions on cilantro development, a morphology marker indicative of the developmental stage may be more useful for determining the most effective time of application rather than the time after seeding and the estimated time of harvest. Stem diameter at the base of the rosette leaves was measured as one possible indicator of bolting in our field studies. However, in none of the trials did we observe any significant differences of stem diameter that was associated with bolting.

Cilantro plants were further assessed to determine whether PGRs influence the internode length of the stem (caulis) during the later stage of bolting. Except for field trial 1, no internode length reductions were observed. In contrast, internode length was reduced in greenhouse-grown cilantro treated with PCa (Kofidis et al., 2008) and in field-grown tef treated with PBZ (Tesfahun, 2017). Different application rates and different environmental conditions are possible reasons for these variations.

In field trial 4, instead of internode length, the total length of the caules was assessed. Plants treated with PCa had significantly shorter caules than untreated plants. The average caulis length per plot was also moderately correlated with the percentage of bolting in each plot, suggesting that in addition to curtailing bolting, PCa also delayed caulis development in plants that had initiated bolting. Caulis length may therefore be a useful indicator of the developmental stage of cilantro in relation to flowering.

The biometric assessment of individual plants to determine the developmental stage is time consuming. To determine the percentage of bolting in open-field-grown cilantro more quickly, we measured the percentage of plants with leaf morphology changes typical for cilantro plants at a later stage of bolting. Although there were significant differences among PGRs that mirrored the results of bolting and caulis length, the visual assessments underestimated the percentage of bolting considerably in all field trials. However, this method was useful when assessing plants regrown after harvest because leaf morphology changes occurred immediately. In trial 2, PCa reduced the percentage of plants with leaf morphology changes by more than 50% at 1 WAH but was ineffective 2 WAH. This suggests that if a second harvest is desired, a second application before the first harvest may be necessary to prevent postharvest bolting beyond the first week of regrowth.

Neither the fresh weight of individual cilantro plants nor the yield per area was affected by PGR applications in both greenhouse and field trials. These results are different from results for okra and potatoes, where applications of PCa and PBZ reduced plant height and aboveground biomass (Ilias et al., 2007; Njiti et al., 2013). Reductions in yield resulting from PBZ applications have also been linked to reduced internode length in tef forage grass (Tesfahun, 2017). However, in contrast to these plants, cilantro is a rosette plant without a visible stem during the vegetative growth phase, precluding a direct comparison with nonrosette plants. In carrots, PBZ also decreased the shoot weight, but increased the weight and diameter of the roots (Wang et al., 2015). In that study, multiple PBZ applications were performed contrary to our study in which single applications were performed. Although we included both bolting and nonbolting plants to assess the yield in our study, in commercial cilantro production, plants displaying clear signs of bolting are usually not harvested. Any reduction in bolting due to the activity of PGRs would therefore offset losses in marketable yield.

Although PBZ has found widespread use in mango cultivation to mitigate the effects of alternate bearing, research indicates that the compound can persist in field soils up to 6 months and negatively affect soil microbial and fungal diversity (Rademacher, 2016; Silva et al., 2003). PBZ residues in the soil also inhibited growth and development in potatoes and taro (Jiang et al., 2019) and reduced seed germination and plant growth in cucumber and tomato (Magnitskiy et al., 2006; Still and Pill, 2003). When used in closed capture irrigation systems, PBZ residues can accumulate and reduce growth of subsequent crops, which necessitates remediation (Grant et al., 2018). Therefore, routine use of PBZ may not be an option for growers cultivating in-field sown crops or rotating PBZ-sensitive crops. In our study, PBZ was not effective in reducing bolting in plants regrown after harvest, suggesting that it may not persist in the sandy soils typical for Florida's agricultural production systems. Conversely, PCa is fast acting, effective, and has low persistence in the environment. It is also one of the few GA inhibitors currently on the market that is labeled for use on some agricultural crops in the United States.

Conclusion

Our study suggests that GA is at least partially responsible for bolting in cilantro and, when applied exogenously, induces bolting under greenhouse conditions. PGRs that inhibit GA biosynthesis curtailed the incidence of bolting effectively when applied at 5 to 6 WAS both in the greenhouse and in a commercial open-field environment when plants were harvested 2 to 3 weeks later. PCa and PBZ were the most effective of the PGRs tested and reduced the incidence of bolting by up to 78%. However, they had little to no residual effect on the bolting incidence of plants regrown for a second harvest. Applications of PCa and PBZ caused no reductions in plant fresh weights and yield compared with untreated plots. The results from our study suggest that use of PCa or PBZ may be an effective strategy to delay bolting in rosette plants like cilantro and reduce crop losses during growth conditions conducive to preharvest bolting.

Literature Cited

Abed, M. 2018. Effect of paclobutrazol on growth and delaying bolting of lettuce (*Lactuca sativa* L.) cv. Balady. Asian J. Agr. Hort. Res. 1:1–9.

Angeli, K.P., F.T. Delazari, C. Nick, M.G. Ferreria, and D.J.H. da Silva. 2016. Yield components and water use efficiency in coriander under irrigation and nitrogen fertilization. R. Bras. Eng. Agríc. Ambiental 20:415–420.

Bashtanova, U. and T.J. Flowers. 2011. Diversity and physiological plasticity of vegetable genotypes of coriander improves herb yield, habit and harvesting window in any season. Euphytica 180:369–384.

Beam, J.B., D. Jordan, A. York, T. Isleib, J.E. Bailey, T.E. McKemie, J.F. Spears, and P.D. Johnson. 2002. Influence of prohexadione calcium on pod yield and pod loss of peanut. Agron. J. 94:331–336.

Bishopp, A., A.P. Mahonen, and Y. Helariutta. 2006. Signs of change: Hormone receptors that regulate plant development. Development 133:1857–1869.

Blade, S., M. Bandara, and S. Hu. 2016. Agri-Facts: Coriander (Agdex 147/20-2). 18 Nov. 2019. <https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex121/$file/147_20-2.pdf?OpenElement>.

Christians, N. 2001. Creative uses for plant growth regulators. USGA Green Sec. Rec. 39:11–13.

Cline, J. 2017. Prohexadione-Ca and ethephon suppress shoot growth of sweet cherry [*Prunus avium* (L.) L.]. Can. J. Plant Sci. 97:601–609.

Das, D., A.K. Bhadra, and M. Moniruzzaman. 2018. Foliar spray of gibberellic acid influences morphology attributes and foliage yield of coriander (*Coriandrum sativum* L.). Res. Agr. Livest. Fish. 5:1–9.

de Mendiburu, F. 2017. Agricolae: Statistical Procedures for Agricultural Research. R package version 1.2-8. <https://cran.r-project.org/package=agricolae>.

Diederichsen, A. 1996. Promoting the conservation and use of underutilized and neglected crops: Coriander (*Coriandrum sativum* L.). Intern. Plant Genet. Res. Inst. (IPGRI), Rome.

Diederichsen, A. and K. Hammer. 2003. The infraspecific taxa of coriander (*Coriandrum sativum* L.). Genet. Resources Crop Evol. 50:33–63.

Dugan, F.M., S.L. Lupien, and J. Hu. 2017. Fungal plant pathogens associated with emerging crops in North America: A challenge for plant health professionals. Plant Health Prog. 18: 221–229.

Environmental Protection Agency. 2007. Paclobutrazol summary document registration review. Initial docket. Available in docket number EPA-HQ-OPP-2006-0109 at <http://www.regulations.gov>.

Evans, J., R. Evans, C. Regusci, and W. Rademacher. 1999. Mode of action, metabolism, and uptake of BAS 125W, prohexadione-calcium. HortScience 34:1200–1201.

Fukuda, N., M. Kondo, S. Nishimura, M. Koshioka, S. Tanakadate, A. Ito, and L.N. Mander. 2005. The role of phytohormones in flowering and bolting of Spinach (*Spinacia oleracea* L.) under mid-night lighting. Acta Hort. 711: 247–254.

Grant, G.A., P.R. Fisher, J.E. Barrett, and P.C. Wilson. 2018. Removal of paclobutrazol from irrigation water using granular-activated carbon. Irrig. Sci. 36:159–166.

Haguewood, J.B., E. Song, R.J. Smeda, J.Q. Moss, and X. Xiong. 2013. Suppression of annual bluegrass seedheads with mefluidide, ethephon, and ethephon plus trinexapac-ethyl on creeping bentgrass greens. Agron. J. 105:1832–1838.

Hälvä, S., L. Craker, J. Simon, and D. Charles. 1993. Growth and essential oil in dill, *Anethum graveolens* L., in response to temperature and photoperiod. J. Herbs Spices Med. Plants 1:47–56.

Hamner, K.C. and A.W. Naylor. 1939. Photoperiodic responses of dill, a very sensitive long day plant. Bot. Gaz. 100:853–861.

Hassan, F. and S. Mahfouz. 2012. Effect of 1-methylcyclopropene (1-MCP) on the postharvest senescence of coriander leaves during storage and its relation to antioxidant enzyme activity. Scientia Hort. 141:69–75.

Hernández-Dávila, J., F. Zavala-García, C.G.S. Valdés-Lozano, G. Salinas-García, E. Cárdenas-Cerda, F. Montes-Cavazos, and H. Gámez-González. 2004. Retraso de la floración en cilantro (*Coriandrum sativum* L.) con sustancias reguladoras del crecimiento. Rev. Chapingo Ser. Hort. 10:51–56.

Ilias, I., G. Ouzounidou, A. Giannakoula, and P. Papadopoulou. 2007. Effects of gibberellic acid and prohexadione-calcium on growth, chlorophyll fluorescence and quality of okra plant. Biol. Plant. 51:575.

Jenni, S., I. Gamache, J.C. Côté, and K.A. Stewart. 2005. Early field detection of bolting in celery. HortTechnology 15:843–845.

Jiang, X., Y. Wang, H. Xie, R. Li, J. Wei, and Y. Liu. 2019. Environmental behavior of paclobutrazol in soil and its toxicity on potato and taro plants. Environ. Sci. Pollut. Res. Intl. 26: 27385–27395.

Johnson, K. and M. Lenhard. 2011. Genetic control of plant organ growth. New Phytol. 191:319–333.

Kahn, B.A. and N.O. Maness. 2010. Row arrangements, seeding rates, and gibberellic acid treatments to improve yield of machine-harvested cilantro. HortScience 45:1049–1051.

Kofidis, G., A. Giannakoula, and I. Ilias. 2008. Growth, anatomy and chlorophyll fluorescence of coriander plants (*Coriandrum staivum* L.) treated with prohexadione-calcium and daminozide. Acta Biol. Cracov. Ser. Bot. 50(2):55–62.

Kurmi, A.K., I.S. Naruka, S.S. Kushwah, and G.S. Chouhan. 2019. Effect of PGRs on growth, yield and quality of coriander (*Coriandrum sativum* L.) cv. NRCSS-Acr-1. Intl. J. Curr. Microbiol. Appl. Sci. 8:2162–2167.

Laribi, B., K. Kouki, M. M'Hamdi, and T. Bettaieb. 2015. Coriander (*Coriandrum sativum* L.) and its bioactive constituents. Fitoterapia 103:9–26.

Li, J., B.-Z. Pan, L. Niu, M.-S. Chen, M. Tang, and Z.-F. Xu. 2018. Gibberellin inhibits floral initiation in the perennial woody plant *Jatropha curcas*. J. Plant Growth Regul. 37:999–1006.

Luo, Y., J.L. McEvoy, M.R. Wachtel, J.G. Kim, and Y. Huang. 2004. Package atmosphere affects postharvest biology and quality of fresh-cut cilantro leaves. HortScience 39:567–570.

Magnitskiy, S.V., C.C. Pasian, M.A. Bennett, and J.D. Metzger. 2006. Effects of soaking cucumber and tomato seeds in paclobutrazol solutions on fruit weight, fruit size, and paclobutrazol level in fruits. HortScience 41:1446–1448.

March, S., D. Martins, and J. McElroy. 2013. Growth inhibitors in turfgrass. Planta Daninha 31:733–747.

McGrath, M.J., J.M. Koczan, M.M. Kennelly, and G.W. Sundin. 2009. Evidence that prohexadione-calcium induces structural resistance to fire blight infection. Phytopathology 99:591–596.

Morales-Payan, J.P. 2011. Herbs and leaf crops: Cilantro, broadleaf cilantro and vegetable amaranth, p. 1–28. In: W.H. Verheye (ed.). Soils, plant growth and crop production. Encyclopedia of Life Support Systems (EOLSS), Eolss Publishers, Paris, France.

Morales-Payan, J.P. and W.M. Stall. 2004. Effect of selected growth stimulators on the yield of cilantro. HortScience 39:859D–859.

Nawata, E., J. Itanai, and Y. Masanaga. 1995. The distribution and dissemination pathway of coriander in Asia. Acta Hort. 390:167–174.

Njiti, V.N., Q. Xia, L.S. Tyler, L.D. Stewart, A.T. Tenner, C. Zhang, D. Alipoe, F. Chukwuma, and M. Gao. 2013. Influence of prohexadione calcium on sweetpotato growth and storage root yield. HortScience 48:73–76.

Passam, H., A. Koutri, and I. Karapanos. 2008. The effect of chlormequat chloride (CCC) application at the bolting stage on the flowering and seed production of lettuce plants previously treated with water or gibberellic acid (GA3). Scientia Hort. 116:117–121.

Pernezny, K., R. Raid, and J. Jones. 1997. Bacterial leaf spot of cilantro in Florida. Plant Dis. 81:232.

Peterson, L., R. Clark, and R. Menary. 1993. Umbel initiation and stem elongation in fennel (*Foeniculum vulgare*) initiated by photoperiod. J. Essent. Oil Res. 5:37–43.

Pikaard, C.S. and O.M. Scheid. 2014. Epigenetic regulation in plants. Cold Spring Harb. Perspect. Biol. 6:a019315.

Potter, T.L. 1996. Essential oil composition of cilantro. J. Agr. Food Chem. 44:1824–1826.

Pressman, E. and H. Aviram. 1986. Inhibition of flowering in Chinese cabbage by applying heat and growth retardants to transplants. Plant Growth Regulat. 4:87–94.

Putievsky, E. 1983. Effects of daylength and temperature on growth and yield components of three seed spices. J. Hort. Sci. 58:271–275.

Rademacher, W. 2015. Plant growth regulators: Backgrounds and uses in plant production. J. Plant Growth Regul. 34:845–872.

Rademacher, W. 2016. Chemical regulators of gibberellin status and their application in plant production, p. 359–403. In: P. Hedden and S.G. Thomas (eds.). Ann. Plant Rev., Vol. 49, The gibberellins. John Wiley & Sons, Chichester, UK.

Ramin, A. and J. Atherton. 1994. Manipulation of bolting and flowering in celery (*Apium graveolens* L. var. dulce). III. Effects of photoperiod and irradiance. J. Hort. Sci. 69:861–868.

Reeves, P.H. and G. Coupland. 2001. Analysis of flowering time control in Arabidopsis by comparison of double and triple mutants. Plant Physiol. 126:1085–1091.

R Core Team. 2018. R: A Language and Environment for Statistical Computing. Vienna, Austria.

RStudio Team. 2016. RStudio: Integrated Development Environment for R. Boston, MA.

Silva, C.M.M.S., R.F. Vieira, and G. Nicolella. 2003. Paclobutrazol effects on soil microorganisms. Appl. Soil Ecol. 22:79–86.

Simpson, G.G. and C. Dean. 2002. Arabidopsis, the Rosetta stone of flowering time? Science 296: 285–289.

Smith, R., J. Bi, M. Cahn, M. Cantwell, O. Daugovish, S. Koike, E. Natwick, and E. Taklee. 2011. Cilantro Production in California, p. 4. University of California: Agriculture and Natural Resources.

Soil Survey Staff, Natural Resources Conservation Service, U.S. Department of Agriculture, Official Soil Series Descriptions. 2014. 18 Nov. 2019. <https://soilseries.sc.egov.usda.gov/OSD_Docs/B/BASINGER.html>.

Still, J.R. and W.G. Pill. 2003. Germination, emergence, and seedling growth of tomato and impatiens in response to seed treatment with paclobutrazol. HortScience 38:1201–1204.

Tautvydas, K.J. 1983. Synergistic growth retardation of grasses with mefluidide/PGR combinations. Proc. Plant Growth Regulator Soc. Amer. 10:51–56.

Tesfahun, W. 2017. Effect of rates and time of paclobutrazol application on growth, lodging, and yield and yield components of tef [*Eragrostis Tef* (Zucc.) Trotter] in Adadistrict, East Shewa, Ethiopia. J. Biol. Agr. Healthcare 7.

Tomitaka, Y., A. Karimata, and A. Noguchi. 2001. Effect of daylength on the flower bud differentiation and development in coriander (*Coriandrum sativum* L.). J. Agr. Sci., Tokyo Univ. of Agr. 46:196–200.

Vanstraelen, M. and E. Benková. 2012. Hormonal interactions in the regulation of plant development. Annu. Rev. Cell Dev. Biol. 28:463–487.

Wang, G.L., F. Que, Z.S. Xu, F. Wang, and A.S. Xiong. 2015. Exogenous gibberellin altered morphology, anatomic and transcriptional regulatory networks of hormones in carrot root and shoot. BMC Plant Biol. 15:290.

Wickham, H. 2016. ggplot2: Elegant graphics for data analysis. Springer-Verlag New York, NY.

Yugandhar, V., P.S.S. Reddy, G. Thanuja Sivaram, and D.S. Reddy. 2014. Effect of growth regulators on growth, seed yield and quality of coriander (*Coriandrum sativum* L.) cv. Sudha. Plant Arch. 14:1083–1086.

Yugandhar, V., P.S.S. Reddy, G. Thanuja Sivaram, and D.S. Reddy. 2016. Influence of plant growth regulators on growth, seed yield, quality and economics of coriander (*Coriandrum sativum* L.) cv. Sudha. J. Spices Aromatic Crops 25:13–17.

This paper was presented during the 2019 FSHS Annual Meeting and originally published in *HortScience* [HORTSCIENCE 55(1):63–70. 2020. https://doi.org/10.21273/HORTSCI14614-19]. The paper is included in this Proceedings as a reprint (with permission).

Proc. Fla. State Hort. Soc. 132:166–171. 2019.

Comparative Evaluation of the Effects of Gibberellic Acid Concentrations on Dormancy Break in Tubers of *Solanum chacoense*

Christian T. Christensen[1], Lincoln Zotarelli[1], Kathleen G. Haynes[2], and Charles Ethan Kelly[1]

ADDITIONAL INDEX WORDS. potato, sprout number, tuber size

SUMMARY. *Solanum chacoense* is a wild relative of potato (*Solanum tuberosum*) that is of interest because of its many desirable traits, but it exhibits variations in tuber dormancy across accessions. The objective of this study was to determine an appropriate gibberellic acid (GA_3) concentration and soak time treatment to encourage sprout development across four accessions of *S. chacoense* (A, B, C, and D) from the 174 accessions of the U.S. Department of Agriculture Potato Genebank. Twelve treatments were created by using four concentrations of GA_3 (0, 50, 100, and 150 $\mu g \cdot mL^{-1}$) across three soak periods (5, 45, and 90 minutes). Small (average weight, 1.4 g), medium (2.6 g), and large (5.6 g) tubers were distributed among all treatments. Percentage of tubers sprouted, time to sprouting, sprout length, and sprout number per tuber were analyzed to determine the effectiveness of GA_3 treatments on dormancy breaking. GA_3 concentrations of 50, 100, and 150 $\mu g \cdot mL^{-1}$ partially broke dormancy within accessions B and C. None of the tested treatments were effective for breaking dormancy in accession D within 46 days after treatment. Accession A showed weaker dormancy, thus producing a similar percentage of sprouted tubers across all GA_3 treatments. Soak time had no significant effect on all parameters measured. Larger tubers produced greater sprout number per tuber and percentages of sprouted tubers. Soaking tubers in 50 $\mu g \cdot mL^{-1}$ of GA_3 may be an effective treatment for *S. chacoense* accessions with mild dormancy, but alternative methods to break dormancy may be required for *S. chacoense* accessions with stronger dormancy.

Solanum chacoense ($2n = 2x = 24$) is a wild potato species that is native to South America. It has been evaluated for several traits of interest for future incorporation in commercially produced potato, such as greater root biomass linked to higher nitrogen uptake efficiency (Errebhi et al., 1999, 1998), late blight resistance (Colon and Budding, 1988), salinity tolerance (Bilski et al., 1988), resistance to verticillium wilt [*Verticillium dahlia* (Lynch et al., 1997; Uribe et al., 2014)], resistance to potato virus Y (PVY) [*Potyvirus* (Sato et al., 2006)], and potato leafroll virus (PLRV) [*Polerovirus* (Brown and Thomas, 1993)]. This species also accumulates a number of glycoalkaloid compounds that work as natural deterrents of colorado potato beetle [*Leptinotarsa decemlineata* (Bamberg et al., 1996; Mweetwa et al., 2011; Sinden et al., 1980)]. *S. chacoense* naturally produces unreduced gametes (Capo et al., 2002; Leue and Peloquin, 1980), which allows for the transmission of these valuable genes from diploid *S. chacoense* to tetraploid *S. tuberosum* via 4x-2x crosses. However, newly harvested material of *S. chacoense* across populations, also referred to as accessions, has shown uneven dormancy and plant emergence.

Below the surface of the soil, a potato plant produces both roots and stem organs called stolons. Flowering in potato plants generally coincides with the swelling of stolon tips in which a majority of the tuber is formed by randomly oriented cell division and expansion (Jackson, 1999). Deposited in these cells are storage carbohydrates and proteins, including starch and patatin, respectively (Shewry, 2003), making tubers strong storage sink organs (Fernie and Willmitzer, 2001). Coinciding with tuber formation is the onset of dormancy. Dormancy can be described as the halting of all meristematic activity in the stolon apex and nodes (Sonnewald, 2001; Xu et al., 1998), and it serves physiological adaptation by allowing survival during periods of unfavorable conditions (Sonnewald, 2001). According to Suttle (2011), there are three stages of dormancy in potato tubers. The first stage of dormancy is referred to as endodormancy, during which endogenous factors restrict the formation of sprouts even under ideal conditions. The second stage of dormancy is referred to as paradormancy, during which sprouting is restricted by external physiological factors. The third stage of dormancy is referred to as ecodormancy, during which meristematic activity is halted by external environmental factors. Immediately after harvest, tubers enter endodormancy and will not produce sprouts when stored at temperatures of 3 °C or below (Suttle, 2011). Aside from the effects of temperature during the development and storage of tubers (Davidson, 1958), time to sprouting in potato tubers can vary among varieties between years (Kim et al., 1999; Van Ittersum, 1992), by tuber size (Claassens and Vreugdenhil, 2000, Krijthe, 1962; Van Ittersum, 1992), and between species (Hermundstad and Peloquin, 1985; Thompson et al., 1980). Among wild species of tuber-bearing

Received for publication 1 July 2019. Accepted for publication 28 Oct. 2019.

Published online 5 December 2019.

[1]University of Florida, Horticultural Sciences Department, Institute of Food and Agricultural Sciences, 1241 Fifield Hall, Gainesville, FL, 32611

[2]U.S. Department of Agriculture, Agricultural Research Service, Genetic Improvement of Fruits and Vegetables Laboratory, Beltsville, MD 20705

This manuscript was orally presented at the 2019 Annual Meeting of the Florida State Horticultural Society in Maitland, FL.

L.Z. is the corresponding author. E-mail: lzota@ufl.edu.

https://doi.org/10.21273/HORTTECH04448-19

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
0.0929	ft²	m²	10.7639
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
28.3495	oz	g	0.0353
1	ppm	$\mu g \cdot mL^{-1}$	1
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

Solanum species, the tuber dormancy length can range from a short period such as 20 d (Thompson et al., 1980) to up to 8 years (Bamberg, 2010).

At the time of sprouting, the tuber becomes repurposed from a storage organ to a source organ for newly developing sprouts (Sonnewald, 2001). Premature potato tuber sprouting during storage can lead to decreased crop values and loss of quality due to remobilization of starch and proteins (Börnke et al., 2007), whereas delayed sprouting after planting can lead to reduced yields (Gandarillas and Nylund, 1949). To simultaneously evaluate populations of tuber-bearing *Solanum* species for rooting or tuber traits, uniform breaking of tuber dormancy is required. Literature discussing breaking physiological dormancy in tubers suggested using gibberellic acid (GA_3) (Brian et al., 1955; Galun, 2010; Jansky and Hamernik, 2014; Rappaport, 1956; Rappaport et al., 1957; Sasani et al., 2009) or a combination of ethylene gases (ethylene chlorohydrin, 1,2-dichloroethane, and carbon tetrachloride at the 7:3:1 proportion), often referred to as rindite (Bryan, 1989; Kim et al., 1999; McDonald and Coleman, 1988). The major issue with rindite is the toxicity of the three components. When they are mixed together, rindite creates high toxicity risks for the workers handling the chemicals and the environment (Hansen et al., 2002). Limited information regarding breaking dormancy exists for *S. chacoense*. The objective of this study was to determine an appropriate GA_3 concentration and soak time treatment to encourage sprout development across four accessions of *S. chacoense*.

Materials and methods

Plant material and growth conditions. Eleven *S. chacoense* clones across four accessions were selected for a seed nursery at the U.S. Department of Agriculture (USDA) Agricultural Research Station in Beltsville, MD. The accessions were previously obtained from the USDA Potato Genebank in Sturgeon Bay, WI. Accessions A (PI 275136; clones A-3, A-5, and A-6) and B (PI 320288; clones B-3, B-5, and B-10) originated from Argentina. Accessions C (PI 537025; clones C-6 and C-8) and D (PI 566738; clones D-6, D-7, and D-8) originated from Bolivia and Paraguay, respectively. These clones were selected based on tuber availability and the ability to tuberize in pots. Tubers were cultivated from tuberlings in 6-inch-diameter containers using peatmoss potting mixture (ProMix Flex; Premier Tech Horticulture, Delson, QC, Canada) under a 12-h photoperiod and fertilized using 24N–3.5P–13.3K water-soluble fertilizer (MiracleGro All-Purpose Fertilizer; Scotts, Marysville, OH). Tubers were harvested on 1 Oct. 2013 at the USDA facility in Beltsville, MD, and shipped overnight to the University of Florida in paper bags.

Experimental treatments. Immediately after arrival, tubers were maintained in complete darkness at 25 °C for 5 d after harvest before treatment. A total of 72 tubers of each of the 11 clones were divided evenly into three tuber size classes (small, medium, and large) and exposed to four GA_3 concentrations for each of the three desired soak times (12 treatments total). The average fresh weights (±SD) were 1.4 ± 0.03, 2.6 ± 0.06, and 5.6 ± 0.14 g for small, medium, and large classes, respectively. The factors were four GA_3 concentrations (0, 50, 100, and 150 $\mu g \cdot mL^{-1}$), three soak periods (5, 45, and 90 min), tuber size class (small, medium, and large), and accession (A, B, C, and D). The concentrated GA_3 (Fisher Scientific, Toronto, ON, Canada) was dissolved in distilled deionized (DDI) water at 20 °C and homogenized for 2 h. Treatments were applied at a rate of 24 tubers in 200 mL of GA_3 solution. Treated tubers were then removed and allowed to air-dry for 3 h before placement inside two calibrated incubators (MIR-153; Sanyo Electric Co., Moriguchi, Japan) on 6 Oct. 2013, and maintained at 23 °C. Tubers were periodically removed from the incubators into a lighted room to collect data. To ensure that all tubers were exposed to light for the same length of time, a 12-h photoperiod was supplied by a fluorescent lamp (15 W, 6500 °K, 198 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

Data collection. The experiment was conducted for 46 d after treatments (DAT). The number and length of sprouts were determined every 2 d and summed on a per-tuber basis at the end of the experiment. The number of sprouts per tuber was determined by counting viable sprouts, which was determined as a sprout at least 2 mm long that showed no signs of desiccation. Time to sprouting was determined as DAT required for the average sprout number (with a minimum length of 2 mm) in a single replicate to equal or exceed one. The percentage of sprouted tubers was calculated as the number of tubers sprouted divided by the total number of tubers in that treatment 46 DAT. After 46 DAT, nonsprouted tubers started to shrivel and become desiccated.

Statistical analysis. The experiment was a factorial arrangement of treatments in a completely randomized design with three replicates. An analysis of variance (ANOVA) for each measured variable was conducted using the PROC GLM procedure. Treatments were compared using Tukey-Kramer of the SAS statistical package (version 9.4 for Windows; SAS Institute, Cary, NC).

Results and discussion

Number of sprouted tubers. The ANOVA showed that the GA_3 concentration, accession, and tuber size class significantly affected the percentage of sprouted tubers of *S. chacoense*. Application of GA_3 significantly increased the number of sprouted tubers. Treatments including 50, 100, and 150 $\mu g \cdot mL^{-1}$ GA_3 resulted in 48%, 40%, and 43% of sprouted tubers, respectively, whereas the non-GA_3 treatment resulted in only 29% of sprouted tubers at 46 DAT (Fig. 1A). Application of GA_3 accelerated sprout formation compared with non-GA_3 treatment. The 50 $\mu g \cdot mL^{-1}$ GA_3 resulted in the earliest formation of sprouts at 9 DAT (Fig. 2A). The 100 and 150 $\mu g \cdot mL^{-1}$ GA_3 treatments and 50 $\mu g \cdot mL^{-1}$ GA_3 treatment resulted in similar percentage of tubers sprouted at 19 and 46 DAT. The effect of exogenous GA_3 application on sprouting promotion has been previously reported for potato (Alexopoulus et al., 2008), but it has not yet been reported for *S. chacoense* tubers.

There was a significant variation in the percentage of sprouted tubers across the four accessions. The overall percentages of sprouted tubers were 60%, 63%, 34%, and 3% for accessions A, B, C, and D, respectively (Fig. 1B). Accession A was the most effective for producing sprouts, with 69% of tubers sprouted in the absence of GA_3, followed by accession B with 38%

(Table 1). Only 2% of tubers from accession C and 6% of tubers from accession D produced sprouts in the absence of GA_3, indicating a relatively high level of endodormancy. Accessions A and B were more effective for producing sprouts earlier in the study, resulting in a greater percentage of tubers sprouted at 19 DAT and for the remainder of the study (Fig. 2B). Accession C was less effective for producing sprouts, followed by accession D, which produced minimal sprouts 46 DAT (Fig. 2B).

The interaction of GA_3 concentration × accession on the percentage of sprouted tubers at 46 DAT was significant (Table 1). The percentage of sprouted tubers was significantly higher with the application of GA_3, regardless of concentration, for accessions B and C.

Application of 50, 100, and 150 $\mu g \cdot mL^{-1}$ of GA_3 significantly increased tuber sprouting in 61% to 77% and 43% to 50% in accessions B and C, respectively, compared with non-GA_3 treatment. There were no significant differences among the 50, 100, and 150 $\mu g \cdot mL^{-1}$ GA_3 treatments on sprouted tubers within accessions, indicating that 50 $\mu g \cdot mL^{-1}$ of GA_3 was a sufficient concentration to break dormancy of three of the four tested accessions of *S. chacoense*. The use of GA_3, regardless of concentration, had no effect on the percentage of sprouted tubers for accessions A and D due to opposite reasons. Accession D showed a stronger endodormancy mechanism (Table 1) and no sensitivity to GA_3 treatments, whereas accession A showed very weak dormancy, producing a similar percentage of sprouted tubers when they were treated or not treated with GA_3. The variability of dormancy among potato species and varieties is expected (Bisognin et al., 2018; Brandt et al., 2003; Kim et al., 1999) and, in most of the cases, some variation in the dormancy of tubers can be attributed to the genetic makeup of individuals. Acceleration of breaking dormancy with GA_3 has been extensively studied in several potato varieties (Alexopoulos et al., 2008; Hartmann et al., 2011; Van Ittersum, 1992). The present study represents a small proportion of the genetic variability of *S. chacoenses* because only four of the 174 available accessions of *S. chacoenses* from the USDA Potato Genebank were evaluated. The accessions of the present study were selected based on their ability to tuberize in pots and the availability of tubers at harvest. Therefore, much greater variations in

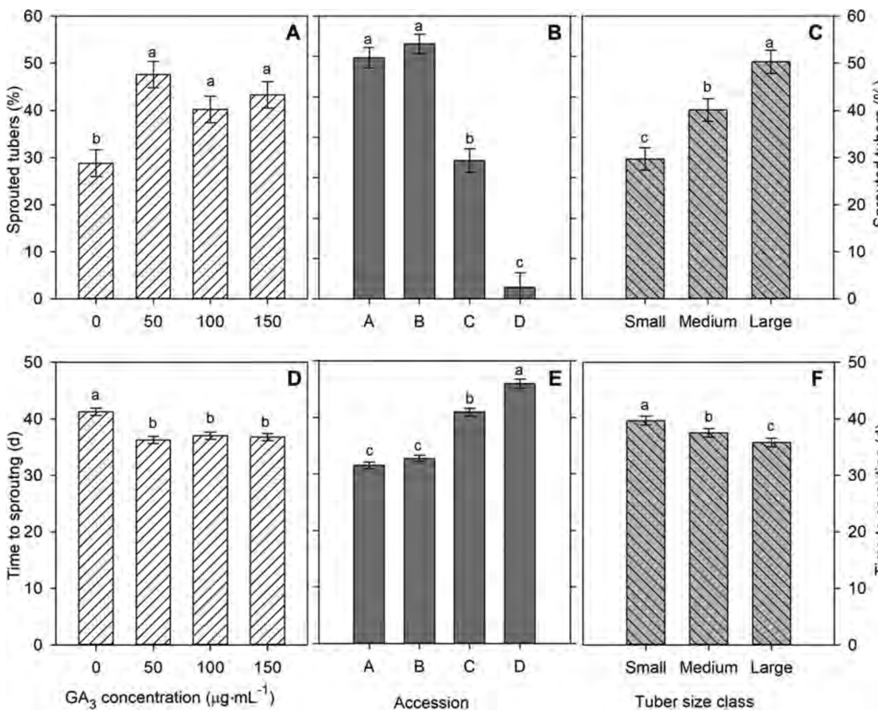

Fig. 1. Main effects of gibberellic acid (GA_3) concentrations (A, D), accession (B, E), and tuber size class (C, F) on the percentage of sprouted tubers 46 d after treatment and time to sprouting of *Solanum chacoense*. Tuber size classes were classified according to the average tuber weight as small (1.5 g), medium (2.4 g), and large (5.8 g). Values followed by the same lowercase letter indicate that means are not significantly different at $P < 0.05$ according to the Tukey-Kramer test within each graph. Error bars indicate standard error of the mean; 1 g = 0.0353 oz; 1 $\mu g \cdot mL^{-1}$ = 1 ppm.

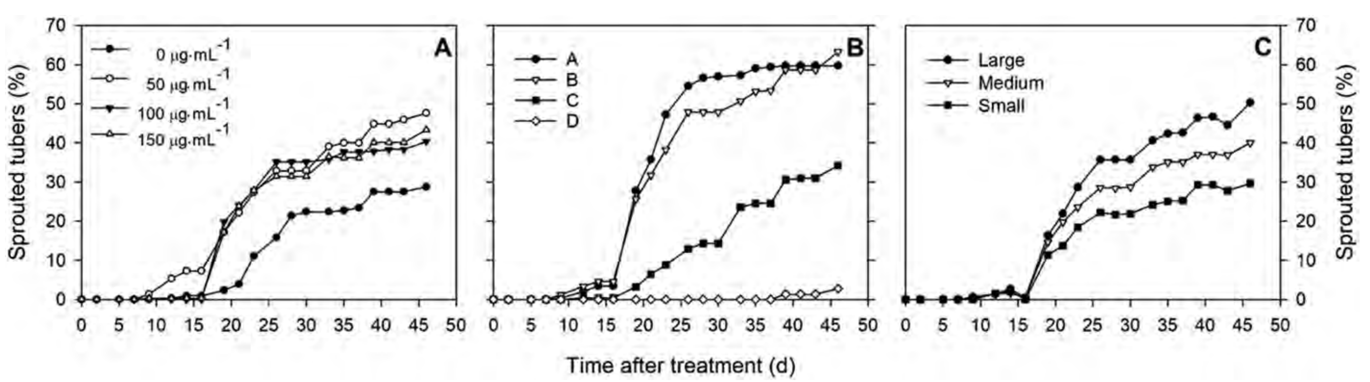

Fig. 2. Main effects of gibberellic acid (GA_3) concentrations (A), *Solanum chacoense* accession (B), and tuber size class (C) on the percentage of sprouted tubers of *S. chacoense*. Tuber size classes were classified according to the average tuber weight as small (1.5 g), medium (2.4 g), and large (5.8 g); 1 g = 0.0353 oz; 1 $\mu g \cdot mL^{-1}$ = 1 ppm.

Table 1. Interaction between gibberellic acid (GA_3) concentration treatments and accession of *Solanum chacoense* on the percentage of sprouted tubers and time to sprouting at 46 d after treatments.

GA_3 ($\mu g \cdot mL^{-1}$)[z]	*S. chacoense* accessions A	B	C	D
	Sprouted tubers [mean ± SE (%)]			
0	69.4 ± 5.1 a[y] A[x]	38.3 ± 5.4 b B	1.9 ± 1.0 b C	5.6 ± 2.8 a C
50	58.3 ± 5.1a AB	76.5 ± 4.8 a A	50.0 ± 6.9 a B	5.6 ± 3.9 a C
100	56.9 ± 5.9a A	61.2 ± 5.4 a A	42.6 ± 6.8 a A	0.0 ± 0.0 a B
150	54.2 ± 5.9a B	76.5 ± 4.7 a A	42.6 ± 6.8 a B	0.0 ± 0.0 a C
	Time to sprouting [mean ± SE (d)]			
0	32 ± 1.3 a B	41 ± 0.9 a A	>46 ± 0.4 a A	>46 ± 0.2 a A
50	31 ± 1.3 a B	29 ± 1.2 b B	39 ± 1.5 b A	>46 ± 1.9 a A
100	31 ± 1.6 a B	31 ± 1.4 b B	40 ± 1.2 b A	>46 ± 0.0 a A
150	32 ± 1.6 a B	30 ± 1.3 b B	39 ± 1.3 b A	>46 ± 0.0 a A

[z]1 $\mu g \cdot mL^{-1}$ = 1 ppm.
[y]Values within columns followed by the same lowercase letter indicate that means are not significantly different at $P < 0.05$ according to the Tukey-Kramer test between GA_3 concentrations within each *S. chacoense* accession.
[x]Values within rows followed by the same uppercase letter indicate that means are not significantly different at $P < 0.05$ according to the Tukey-Kramer test between the *S. chacoense* accession within each GA_3 concentration.

Table 2. Interaction between tuber size class and accession of *Solanum chacoense* on sprout length and time to sprouting 46 d after treatment. Tuber size classes were classified according to the average tuber weight in small (1.5 g), medium (2.4 g), and large (5.8 g).[z]

Tuber size class	*S. chacoense* accessions A	B	C	D
	Sprout length [mean ± SE (mm)]			
Small	7.4 ± 0.9 a[z] A[y]	3.3 ± 0.5 b B	3.6 ± 0.5 a B	0.0 ± 0.0 b C
Medium	7.1 ± 0.7 a A	3.7 ± 0.3 ab B	3.5 ± 0.5 a B	0.0 ± 0.0 b C
Large	6.4 ± 0.5 a A	5.6 ± 0.4 a A	3.5 ± 0.7 a B	3.0 ± 0.4 a C
	Time to sprouting [mean ± SE (d)]			
Small	36 ± 0.9 a B	35 ± 0.9 a B	42 ± 1.1 a A	>46 ± 1.3 a A
Medium	30 ± 1.1 b B	32 ± 1.0 ab B	42 ± 1.3 a A	>46 ± 1.6 a A
Large	27 ± 0.9 b D	31 ± 0.9 b C	39 ± 1.1 a B	>46 ± 1.3 a A

[z]1 g = 0.0353 oz; 1 mm = 0.0394 inch.
[y]Values within columns followed by the same lowercase letter indicate that means are not significantly different at $P < 0.05$ according to the Tukey-Kramer test between tuber size class within each *S. chacoense* accession.
[x]Values within rows followed by the same uppercase letter indicate that means are not significantly different at $P < 0.05$ according to the Tukey-Kramer test among *S. chacoense* accessions within each tuber size class.

tuberization and tuber dormancy across different accessions of *S. chacoense* should be expected across all accessions of *S. chacoense*.

The initial separation of tubers into size classes (small, medium, large) significantly affected the percentage of sprouted tubers of *S. chacoense* (Fig. 1C). In the smaller class, 29% of the tubers had sprouted at 46 DAT; however, in the medium and large tuber classes, 40% and 50% of the tubers had sprouted at 46 DAT. Large tubers produced sprouts much earlier than the medium and small classes. Approximately 39% of the large tubers sprouted at 25 DAT; however, at the same DAT, the percentages of sprouted tubers were 31% and 24% for medium and small tuber classes, respectively. The difference in sprouting among tuber size classes was maintained until the end of the study (Fig. 2C). These results corroborate previous research evaluating potato (Claassens and Vreugdenhil, 2000; Van Ittersum, 1992) that indicated that larger tubers exhibited a higher percentage of sprouted tubers earlier in the study.

Soak time had no significant effect on the percentage sprouted tubers of *S. chacoense*. Soak time has been shown to have no effect on the rate or nature of sprouting on potato tubers (Rappaport et al., 1958).

Time to sprouting. GA_3 concentration, accession, and tuber size class had effects on time to sprouting of *S. chacoense*. Again, the minutes of soak time had no effect on time to sprouting. Overall, treatments with GA_3 shortened dormancy by 4 d (Fig. 1D). Under 0 $\mu g \cdot mL^{-1}$ GA_3 treatment, accession A had the shortest dormancy, only 32 d to sprout, but there were no significant differences among accessions B, C, and D, which required more than 41 d to sprouting (Table 1).

There were interactions between GA_3 and accession and between accession and tuber size for days required to sprouting. GA_3 significantly reduced time to sprouting, regardless of concentration, by an average of 10 d for accession B and 7 d for accession C, but not for accessions A and D. Under the conditions of this study, accession A did not require the application of exogenous GA_3 to accelerate or increase the emergence of sprouts. For accessions B and C, the 50 $\mu g \cdot mL^{-1}$ concentration of GA_3 was sufficient to increase sprouting by 33% and 41%, respectively. However, for accession D, none of the GA_3 concentrations was effective for shortening tuber dormancy. Acceleration of dormancy breaking with the use of GA_3 has been reported with doses lower than 50 $\mu g \cdot mL^{-1}$ for several potato varieties (Alexopoulos et al., 2008; Hartmann et al., 2011; Van Ittersum, 1992). A practical application of breaking dormancy of potato tubers was demonstrated by Mustefa et al. (2017), who indicated that applications of 10 and 20 $\mu g \cdot mL^{-1}$ of GA_3 on tubers of *Budu* significantly reduced the dormancy period from 102 d for non-treated seed to 83 and 70 d, respectively.

Larger tubers tended to require a shorter time to sprout than smaller tubers (Fig. 1F). In accession A, tuber dormancy was significantly shorter in medium and large tubers than in small tubers (Table 2). In accession B, tuber dormancy was significantly shorter in large than in small tubers. In accessions C and D, tuber size had no effect on tuber dormancy (Table 2).

The effect of tuber size on dormancy has also been observed for potato: smaller tubers required longer periods to produce sprouts and larger tubers exhibited shorter dormancy (Lommen, 1994; Nipa et al., 2013; Van Ittersum, 1992). However, as observed for accessions C and D, there is not always a significant and negative correlation between tuber size and the time required to remove dormancy. The absence of a relationship between time to sprouting and tuber size has been reported for some potato varieties (Van Ittersum, 1992).

Sprout number and length. The GA_3 concentration and soak time had no significant effects on sprout number. The sprout number was significantly affected by accession and tuber size only. Accession A had the highest number (±SD) of sprouts per tuber (1.9 ± 0.1); followed by accession B (1.6 ± 0.1), accession C (1.6 ± 0.1), and accession D (1.0 ± 0.0). The large tuber class produced the highest number of sprouts per tuber (1.8 ± 0.1), with no significant differences between medium (1.6 ± 0.1) and small (1.6 ± 0.1) tuber classes.

The GA_3 concentrations and accession had significant effects on the sprout length of *S. chacoense* (Table 2). The soak time of GA_3 showed no significant effect on sprout length. Without any GA_3 treatment, the average sprout length was 3.4 ± 0.3 mm. The GA_3 concentration of 50 $\mu g \cdot mL^{-1}$ resulted in the longest sprouts (5.9 ± 0.4 mm), which was significantly greater than the sprout length when treated with 100 $\mu g \cdot mL^{-1}$ (4.9 ± 0.4 mm) and 150 $\mu g \cdot mL^{-1}$ (5.3 ± 0.4 mm) of GA_3.

Across accessions, accession A produced the longest sprouts (6.9 ± 0.4 mm), followed by accession B (4.4 ± 0.3 mm), accession C (3.5 ± 0.3 mm), and accession D (2.0 ± 1.0 mm). There was a significant interaction between accession and tuber size on sprout length (Table 2). Accessions A and C showed no significant effect of tuber size class on the sprout length of *S. chacoense* (Table 2). In accession B, sprout length increased with increasing tuber size. The large tuber class was the only class to produce sprouts in accession D.

These results corroborated previous research that indicated that the application of exogenous GA_3 was used to break dormancy and elongate sprouts in several potato varieties (Galun, 2010; Sasani et al., 2009). It is noteworthy that previous research has shown that warm white fluorescents inhibit the elongation of potato sprouts even in the presence of exogenous GA_3 (Morris, 1967). Therefore, sprout lengths may have been negatively affected by the presence of artificial lights during the exposure time for the evaluations in this study.

Conclusions

The effectiveness of GA_3 treatments on dormancy breaking of *S. chacoense* was mostly dependent on the accession and tuber size. The accessions used in this study represented a small number of the total number of accessions of *S. chacoense* available in the USDA Potato Genebank, and they exhibited different levels of endodormancy and response to the dormancy breaking treatments. Accessions A and B exhibited weaker dormancy mechanisms overall, whereas accessions C and D exhibited moderate and strong dormancy mechanisms, respectively.

The GA_3 concentration of 50 $\mu g \cdot mL^{-1}$ successfully broke tuber dormancy in accessions B and C; however, for accessions A and D, there was no difference in time to sprouting due to GA_3 treatment. GA_3 concentrations more than 50 $\mu g \cdot mL^{-1}$ showed no increase in the sprouting rate or total percentage of tuber sprouted up to 46 DAT. Future studies should investigate GA_3 rates lower than 50 $\mu g \cdot mL^{-1}$. The soaking time showed no significant effect on breaking dormancy of *S. chacoense*, with 5 min of soaking time being sufficient for breaking dormancy. The tuber size significantly impacted the percentage of sprouting, time to sprouting, and number of sprouts on *S. chacoense*. For those accessions with strong dormancy, alternative methods to break dormancy may be required.

Literature cited

Alexopoulos, A.A., G. Aivalakis, K.A. Akoumianakis, and H.C. Passam. 2008. Effect of gibberellic acid on the duration of dormancy of potato tubers produced by plants derived from true potato seed. Postharvest Biol. Technol. 49:424–430.

Bamberg, J.B., C.A. Longtine, and E.B. Radcliffe. 1996. Fine screening *Solanum* (potato) germplasm accessions for resistance to colorado potato beetle. Amer. Potato J. 73:211–223.

Bamberg, J.B. 2010. Tuber dormancy lasting eight years in the wild potato *Solanum jamesii*. Amer. J. Potato Res. 87:226–228.

Bilski, J.J., D.C. Nelson, and R.L. Colon. 1988. Response of six wild potato species to chloride and sulfate salinity. Amer. Potato J. 65:605–612.

Bisognin, D.A., N.C. Manrique-Carpintero, and D.S. Douches. 2018. QTL analysis of tuber dormancy and sprouting in potato. Amer. J. Potato Res. 95:374–382.

Börnke, F., U. Sonnewald, and S. Biemelt. 2007. Potato, p. 297–315. In: E.C. Pua and M.R. Davey (eds.). Biotechnology in agriculture and forestry. Springer, Heidelberg, Germany.

Brian, P.W., H.G. Hemming, and M. Radley. 1955. A physiological comparison of gibberellic acid with some auxins. Physiol. Plant. 8:899–912.

Bryan, J.E. 1989. Breaking dormancy of potato tubers. CIP Res. Guide 16. Intl. Potato Ctr., Lima, Peru.

Brandt, T.L., G.E. Kleinkopf, N. Olsen, and S.L. Love. 2003. Storage management for Umatilla Russet potatoes. Univ. Idaho Agr. Commun. Bul. 839.

Brown, C.R. and P.E. Thomas. 1993. Resistance to potato leafroll virus derived from *Solanum chacoense*: Characterization and inheritance. Euphytica 74:51–57.

Capo, A., M. Cammareri, F. Delia-Rocca, A. Errico, A. Zoina, and C. Conicella. 2002. Evaluation for chipping and tuber soft rot (*Erwinia carotovora*) resistance in potato clones from unilateral sexual polyploidization (2x × 4x). Amer. J. Potato Res. 79:139–145.

Claassens, M.M. and D. Vreugdenhil. 2000. Is dormancy breaking of potato tubers the reverse of tuber initiation? Potato Res. 43:347–369.

Colon, L.T. and D.J. Budding. 1988. Resistance to late blight (*Phytophthora infestans*) in ten wild *Solanum* species. Euphytica 39:77–86.

Davidson, T.M.W. 1958. Dormancy in the potato tuber and the effects of storage conditions on initial sprouting and on subsequent sprout growth. Amer. Potato J. 35:451–465.

Errebhi, M., C.J. Rosen, F.I. Lauer, M.W. Martin, J.B. Bamberg, and D.E. Birong. 1998. Screening of exotic potato germplasm for nitrogen uptake and biomass production. Amer. J. Potato Res. 75:93–100.

Errebhi, M., C.J. Rosen, F.I. Lauer, M.W. Martin, and J.B. Bamberg. 1999. Evaluation of tuberbearing *Solanum* species for nitrogen use efficiency and biomass partitioning. Amer. J. Potato Res. 76:143–151.

Fernie, A.R. and L. Willmitzer. 2001. Molecular and biochemical triggers of potato tuber development. Plant Physiol. 127:1459–1465.

Galun, E. 2010. Patterning of flowers: Genes and phytohormones, p. 325–366.

In: E. Galun (ed.). Phytohormones and patterning: The role of hormones in plant architecture. World Scientific, Hackensack, NJ.

Gandarillas, H. and R.E. Nylund. 1949. Further studies on the influence of sprout inhibiting and sprout inducing treatments on the growth and yields of potatoes. Amer. Potato J. 26:7–16.

Hansen, J., Z. Ju, P.D. Petracek, P. Warrior, and D. Woolard. 2002. Promoting early establishment of potato crops by ethylene inhibitors. European Patent Office EP1534069B1. 23 Sept. 2019. <http://www.google.com/patents/EP1534069B1?cl=en>.

Hartmann, A., M. Senning, P. Hedden, U. Sonnewald, and S. Sonnewald. 2011. Reactivation of meristem activity and sprout growth in potato tubers requires both cytokinin and gibberellin. Plant Physiol. 155:776–796.

Hermundstad, S.A. and S.J. Peloquin. 1985. Germplasm enhancement with potato haploids. J. Hered. 76:463–467.

Jackson, S.D. 1999. Multiple signaling pathways control tuber induction in potato. Plant Physiol. 119:1–8.

Jansky, S. and A. Hamernik. 2014. Rapid cycling of potato tuber generations by overcoming dormancy. Amer. J. Potato Res. 92:148–152.

Kim, H.S., J.H. Jeon, K.H. Choi, Y.H. Joung, and H. Joung. 1999. Effects of rindite on breaking dormancy of potato tubers. Amer. J. Potato Res. 76:5–8.

Krijthe, N. 1962. Observations on the sprouting of seed potatoes. Eur. J. Potato Res. 5:316–333.

Leue, E.F. and S.J. Peloquin. 1980. Selection for 2n gametes and tuberization in *Solanum chacoense*. Amer. Potato J. 57: 189–195.

Lommen, W.J. 1994. Effect of weight of potato minitubers on sprout growth, emergence and plant characteristics at emergence. Potato Res. 37:315–322.

Lynch, D.R., L.M. Kawchuk, and J. Hachey. 1997. Identification of a gene conferring high levels of resistance to verticillium wilt in *Solanum chacoense*. Plant Dis. 81:1011–1014.

McDonald, J.G. and W.K. Coleman. 1988. A reevaluation of bromoethane in comparison to rindite for the post-harvest detection of potato virus Y in tubers by ELISA. Amer. Potato J. 65:547–550.

Morris, D.A. 1967. The influence of light, gibberellic acid and CCC on sprout growth and mobilization of tuber reserves in the potato (*Solanum tuberosum* L.). Planta 77:224–232.

Mustefa, G., W. Mohammed, N. Dechassa, and D. Gelmesa. 2017. Effects of different dormancy-breaking and storage methods on seed tuber sprouting and subsequent yield of two potato (*Solanum tuberosum* L.) varieties. Open Agr. 2:220–229.

Mweetwa, A.M., D. Hunter, R. Poe, K.C. Harich, I. Ginzberg, R.E. Veilleux, and J.G. Tokuhisa. 2011. Steroidal glycoalkaloids in *Solanum chacoense*. Phytochemistry 75:32–40.

Nipa, J.S., T.S. Roy, A.K. Amin, and M.R. Hasanuzzaman. 2013. Effect of lifting time and tuber size on ambient storage performance of potato derived from true potato seed. Intl. J. Sustainable Agr. 5:1–9.

Rappaport, L. 1956. Growth regulating metabolites. Calif. Agr. 10(12):7–11.

Rappaport, L., L.F. Lippert, and H. Timm. 1957. Sprouting, plant growth, and tuber production as affected by chemical treatment of white potato seed pieces. Amer. Potato J. 34:254–260.

Rappaport, L., H. Timm, and L.F. Lippert. 1958. Gibberellin on white potatoes. Calif. Agr. 12(2):4–6.

Sasani, R., H.R. Khazaee, and A. Nezami. 2009. Effect of gibberellin, benzyl adenine, zeatin and temperature on mini tuber dormancy breaking. J. Hort. Sci. 23:61–67.

Sato, M., K. Nishikawa, K. Komura, and K. Hosaka. 2006. Potato virus Y resistance gene, Ry_{chc}, mapped to the distal end of potato chromosome 9. Euphytica 149:367–372.

Shewry, P.R. 2003. Tuber storage proteins. Ann. Bot. 91:755–769.

Sinden, S.L., L.L. Sanford, and S.F. Osman. 1980. Glycoalkaloids and resistance to the colorado potato beetle in *Solanum chacoense* Bitt. Amer. Potato J. 57:331–343.

Sonnewald, U. 2001. Control of potato tuber sprouting. Trends Plant Sci. 6:333–335.

Suttle, J.C. 2011. Dormancy and sprouting, p. 288–308. In: D. Vreugdenhil, J. Bradshaw, C. Gebhardt, F. Govers, M. Taylor, D. MacKerron, and H. Ross (eds.). Potato physiology and biotechnology. Advances and perspectives. Elsevier, New York, NY.

Thompson, P., F. Haynes, and R. Moll. 1980. Estimation of genetic-variance components and heritability for tuber dormancy in diploid potatoes. Amer. Potato J. 57:39–46.

Uribe, P., S. Jansky, and D. Halterman. 2014. Two CAPS markers predict verticillium wilt resistance in wild *Solanum* species. Mol. Breed. 33:465–476.

Van Ittersum, M.K. 1992. Variation in the duration of tuber dormancy within a seed potato lot. European Potato J. 35:261–269.

Xu, X., A.A.M. Van Lammeren, E. Vermeer, and D. Vruegdenhil. 1998. The role of gibberellin, abscisic acid, and sucrose in the regulation of potato tuber formation in vitro. Plant Physiol. 117:575–584.

This paper was presented during the 2019 FSHS Annual Meeting and originally published in *HortTechnology* [HortTechnology 30(1):76–81. 2020. https://doi.org/10.21273/HORTTECH04448-19]. The paper is included in this Proceedings as a reprint (with permission).

Proc. Fla. State Hort. Soc. 132:172–176. 2019.

Postharvest Physiological Disorders in Mangoes in Florida

Jeffrey K. Brecht*

Horticultural Sciences Department, University of Florida/IFAS, PO Box 110690, Gainesville, FL 32611

Additional index words. chilling injury, heat injury, high CO_2 injury, internal breakdown, jelly seed, lenticel discoloration, low O_2 injury, *Mangifera indica* L., sapburn, soft nose, stem-end breakdown, stem-end cavity, spongy tissue, sunburn

Physiological disorders of mango (*Mangifera indica* L.) fruit may arise from either preharvest or postharvest conditions that induce abnormal physiological processes and may become apparent during postharvest handling. Induction of these disorders in fruit on the tree has been related to environmental conditions, orchard conditions, and nutritional programs, and fruit developmental stage at harvest (i.e., harvest of immature or overmature fruit). There may also be combinations of those factors involved as is the case for internal breakdown, which is thought to be induced by low calcium supply and/or oversupply of nitrogen to the fruit at an early stage of their development, but symptoms appear mainly in fruit allowed to initiate ripening on the tree. Besides internal breakdown, disorders of preharvest origin include sunburn and lenticel discoloration. Postharvest conditions leading to development of physiological disorders are usually environmental in nature, such as heat treatment, storage temperature, and storage atmosphere. Disorders of postharvest origin include sapburn, chilling injury, and heat injury (which may also result in lenticel discoloration), and MA/CA-associated injuries. The symptoms of mango postharvest physiological disorders are varied, but generally they involve tissue breakdown or necrosis of some sort. Avoidance of the factors that induce physiological disorders is the key to preventing their development.

Mango (*Mangifera indica* L.) originated and was first domesticated in India and southeast Asia (Mukherjee and Litz, 2009), and its history in Florida dates to the mid-1800s (Warschefsky and von Wettberg, 2019). It is one of the most economically important fruits cultivated across the tropics and subtropics and is renowned as the "king of fruits" (Purseglove, 1972). The mango is a climacteric fruit that exhibits increased respiration and ethylene production during ripening (Brecht and Yahia, 2009). When grown for local consumption, mangoes are typically allowed to ripen fully on the tree before harvest. However, mangoes grown for distant markets or for export may be harvested prior to the onset of ripening, when the fruit are physiologically mature, having reached full size and accumulated the maximum starch content. Both late- and early-harvested mangoes are subject to several physiological disorders (Brecht, 2018).

Physiological disorders result from abnormal physiological processes that become apparent during postharvest handling. Mango fruit physiological disorders may arise from either preharvest or postharvest conditions and involve tissue breakdown or necrosis of some sort. Physiological disorders in mango can be related to seasonal environmental conditions, orchard conditions, nutritional programs, and fruit development stage at harvest. Weather, soil type, water relations, fertilization practices (amount and timing), pruning, and fruit thinning can all affect development of physiological disorders (Galán Saúco, 2009; Shivashankar, 2014). Fruit maturity at harvest could also be considered as a factor leading to development of postharvest physiological disorders: immature mango fruit are predisposed to both chilling injury (Mohammed and Brecht, 2002) and heat injury such as from hot water insect quarantine treatment (Jacobi et al., 2001a), while mangos allowed to ripen on the tree prior to harvest are more likely to express internal breakdown symptoms (Raymond et al., 1998a). Harvested mangoes may be exposed to heat stress from high ambient temperatures plus sun exposure in the orchard and during transport to packing facilities, or heat may be applied to mangos for decay or insect control. Reducing fruit temperatures and maintaining them in refrigerated environments is the primary method used to preserve mango quality during storage and transport, but mangoes are chilling sensitive and are damaged by temperatures below their chilling threshold if the exposure time is sufficient (Mohammed and Brecht, 2002). Modified and controlled atmospheres with reduced oxygen and elevated carbon dioxide are used to supplement temperature management by further reducing the rates of metabolic reactions and slowing physiological processes, but can cause injuries if the tolerance limits of the mango fruit are exceeded. The maturity of mangos at harvest can differ greatly depending on the intended use, and both low and high maturity influence mango susceptibility to extreme temperatures and atmospheres (Brecht, 2018).

Internal Breakdown

Internal breakdown (IB) refers to several disorders involving mango fruit flesh breakdown that are known as soft nose or tip pulp, jelly seed, stem-end cavity or stem-end breakdown, and spongy tissue (Schaffer, 1994). Internal breakdown is largely confined to mango cultivars from India or those, like the cultivars developed in Florida, that have Indian pedigrees (Campbell, 1973; Schaffer, 1994). IB in Florida is generally a disorder of tree-ripe mangoes (Malo and Campbell, 1978). Ploetz (2003)

*Corresponding author. Email: jkbrecht@ufl.edu

reported that IB affects around 5 to 20% of mangoes in Florida, but the incidence can exceed 50% in fruit ripened on the tree.

Soft nose is a disorder in which the tissue near the distal end or "nose" of the fruit softens prematurely and becomes water-soaked as if overripe, eventually extending further into the tissue surrounding the seed. Fruit with soft nose can be identified by applying finger pressure because the nose of the fruit is easily depressed in the later stages of the disorder. Soft nose was first described in Florida by T.W. Young (Young, 1957). According to Raymond et al. (1998a), the distal tissues in affected fruit appear as a defined yellow area suggestive of incomplete ripening prior to developing the characteristic over-ripe appearance. Since calcium concentration in mango fruit follows a decreasing gradient from the proximal to distal regions, soft nose symptoms develop in the region of the fruit with the lowest calcium concentration (Burdon et al., 1991).

Jelly seed is the name for the disorder of mango fruit that results in the flesh adjacent to the stone becoming translucent with a jelly-like consistency. Development of the disordered tissue suggests premature ripening and overripening as the tissue exhibits an intense yellow color before turning brown as it softens and the cell wall completely disintegrates (Raymond et al., 1998a). Jelly seed is not easily identifiable unless the fruit is cut.

Stem-end cavity or *stem-end breakdown* begins as a patch of brown tissue between the stem attachment and the seed of the fruit followed by formation of a cavity and further discoloration to dark brown or even grayish (Mead and Winston, 1991). Eventually, tissue breakdown like that found in jelly seed extends into the tissue around the seed. Tissue collapse or easy depression of the fruit surface upon application of finger pressure can reveal the underlying cavitation.

Spongy tissue is a disorder typical of 'Alphonso' and a few other Indian varieties that occurs when the fruit are allowed to ripen on the tree (Krishnamurthy, 1981; Subramanayam et al., 1971). Since Florida mango varieties are of Indian lineage, it might be expected that spongy tissue would be a problem with Florida mangoes, but there are no reports of the disorder in Florida. However, Lakshminarayanan (1975) reported that the Florida mango varieties 'Haden', 'Irwin', and 'Kent' grown in Mexico developed spongy tissue when harvested tree-ripe. Symptoms of spongy tissue are slightly desiccated, yellowish-white, starchy regions in the mesocarp with spongy or corky texture and variable amounts of cavitation.

It has been generally accepted among mango researchers that the various types of IB are different manifestations of the same underlying problem. For example, Mead and Winston (1991) concluded from a review of the literature from Florida and the Canary Islands that soft nose, jelly seed, and stem-end cavity are different degrees of expression of the same disorder. However, when Raymond et al. (1998a) examined disorder symptom development during fruit development in 'Tommy Atkins', 'Van Dyke', and 'Irwin' mango fruit, they found both spatial and temporal differences in initiation and progression of jelly seed, soft nose, and stem-end cavity, suggesting that the disorders are different.

Most researchers have concluded that calcium deficiency or a high nitrogen:calcium ratio that reduces calcium availability in fruit by promoting vegetative growth is the basis for IB (see Schaffer, 1994). Low calcium in mango leaves and fruit has been found by some researchers to be predictive of fruit IB (see Raymond et al., 1998b). However, there have been many other studies examining the possible role of calcium in IB with contradictory results (see Raymond et al., 1998b). It is possible that there is a critical period early in fruit development during which calcium is most important for cell wall formation or alternatively when calcium is most likely to be deficient, for example, during rapid cell expansion. It has been reported that calcium redistribution occurs after tissue breakdown in mangoes with soft nose, resulting in accumulation of calcium in the symptomatic region (Burdon et al., 1991).

Lenticel Discoloration

Lenticel discoloration (LD) or lenticel spotting is a darkening of the tissue surrounding the lenticel openings in the mango peel. The discoloration is caused by accumulation of phenolic compounds (flavanones) in the cell walls of the mesophyll cells surrounding the lenticels and does not necessarily involve damage to the cell structure (DuPlooy et al., 2006; 2009). There have been no reports of research LD in Florida other than a report by McMillan et al. (1987) showing that heated fungicide treatments (53 °C for 3 min) induced the disorder in 'Tommy Atkins' and 'Keitt' mangoes. Lenticel spotting seems to be an almost ubiquitous response of mangoes to stress, with the discoloration tending to progress from a reddish appearing halo to brown then black as time progresses or in response to more severe stress (Pesis et al. 1997). Factors reported to be associated with LD including heat and cold stress, soil and fruit moisture status at harvest, high humidity, fruit wetting (water infiltration into lenticels), postharvest washing and brushing, and irradiation treatment (Cronje, 2009a,b; Rymbai et al., 2012).

Sunburn

Direct exposure of mango fruit to ultraviolet radiation from the sun can cause sunburn, which is a localized necrosis of the fruit surface. Sunburned fruit have areas that are bleached or yellowish. Sunburn can occur on the tree to fruit that are directly exposed to the sun. Pruning to avoid excessive sun exposure is helpful in avoiding sunburn. Mango fruit are sometimes sprayed with whitewash to reduce sunburn. Bagging fruit to protect against abrasions, insects, and diseases also protects the fruit against sunburn, but development of red blush is also inhibited by bagging. Keeping mango trees well-irrigated also tends to reduce sunburn, probably due to a certain amount of evaporative cooling of the fruit that may occur. Importantly, freshly harvested mangos must be kept shaded until they can be collected and during transport to the packinghouse in order to avoid sunburn. The temperature of the surface of fruit can reach 20 to 40 °C above the ambient air temperature within 1 to 2 hours if they are directly exposed to the sun.

Sapburn

Mangos have an extensive system of resin-containing ducts (lactifers) in both the fruit and stem, which contains an acidic sap with caustic terpenes, particularly terpinolene (Loveys et al., 1992). The sap can spurt several centimeters from the pedicel when mango fruit are harvested and, if the sap comes in contact with the fruit surface and enters the lenticel openings, chemical injury occurs and brown to black discoloration develops within a few hours to a day later (Brown et al., 1986; O'Hare, 1994). Although the damage is relatively superficial, the discoloration can render mangos unsalable. There is considerable cultivar variation in the severity of sapburn (O'Hare, 1994) that is due to differences in the sap composition and fruit features that influence injury development such as lenticel density and the amount of oxidative enzymes and their phenolic substrates that are present in the skin (Loveys et al., 1992; O'Hare, 1994; Saby John et al., 2002). The discoloration itself is due to cell damage by the terpenes that allows the enzymes to come in contact with their substrates.

Sapburn can be avoided by handling the fruit during removal from the tree so as to direct the spurting sap away from the fruit, followed by inverting the fruit, usually on a rack, to allow the remaining sap to finish dripping before further handling. It is also possible to reduce sap flow by picking fruit with a 5 cm or longer length of stem attached. The stem can then be trimmed several hours later with little or no sap flow. Another common approach is to quickly rinse the freshly harvested fruit with copious water to remove the latex; if neither waiting for sap flow to cease or rinsing with water is practical, the fruit may be dipped in solutions of detergent, hydrated lime (calcium hydroxide), or alum (potassium aluminum sulfate) to remove the latex (Brecht, 2018; Thompson, 2015). If the dipping is done within an hour or so of picking, little or no discoloration will develop.

Chilling Injury

Mango fruit that are exposed for sufficient time to low temperatures below a threshold temperature for unripe fruit of about 12.5 °C can develop chilling injury (CI) (Brecht and Yahia, 2009). Hatton et al. (1965) reported on the susceptibility of Florida mango varieties to low storage temperatures. Tolerance to CI increases as mango fruit develop and ripen and its severity increases as the duration of exposure increases (Bender et al., 2000a; Mohammed and Brecht, 2002). Since Florida-grown mangoes are rarely stored or shipped for extended periods, CI is not normally a problem for locally marketed fruit. However, CI is common in international marketing of the fruit because the chilling threshold temperature of 12.5 °C may not prevent the occurrence of ripening, decay, and senescence, causing shippers to send less mature mangoes at lower temperatures for longer shipping durations. Thus, imported mangoes in the domestic market may show symptoms of CI.

The symptoms of CI include, roughly in order of appearance or chilling severity, inhibition of aroma (Dea et al., 2010), lenticels turning red or brown then black (Pesis et al., 1997), poor flavor and color development, uneven ripening, surface pitting or pebbled appearance, and grayish, scald-like discoloration on the epidermis (Hatton et al., 1965; Medlicott et al., 1990; Pesis et al., 1997). Development of CI symptoms can be inhibited by the same low temperatures that induce the injury so that symptoms may often be observed only after the mangos have been removed from low temperature conditions and placed at warmer, usually ripening-inducing temperatures for some time. Inhibition of ripening is typical of CI in mango (Hatton et al., 1965; Medlicott et al., 1990), but the inhibition is not apparent until after the fruit have been transferred from the chilling temperature to a ripening conducive temperature. Mangos that have been chilled often show increased susceptibility to decay, both in storage and especially following transfer to higher temperature (Kane et al., 1982).

Susceptibility to CI varies among cultivars (Brecht and Yahia, 2009; Brecht et al., 2012), but when searching for information on the recommended storage and shipping temperature for different mango cultivars it is important to know the maturity stage at which the testing of chilling susceptibility was done and if the fruit were transferred from chilling to ripening temperature.

Besides avoiding exposure of mangos to chilling temperatures, the most practical way to avoid CI is to avoid harvesting and handling immature fruit, which are more susceptible to CI (Mohammed and Brecht, 2002). Mangoes harvested at more advanced stages of maturity or ripeness are less sensitive to CI (Bender et al., 2000a; Mohammed and Brecht, 2002). Anything that promotes ripening prior to chilling exposure reduces mango sensitivity to CI.

Heat Injury

Mangoes originated in an area of the world with relatively high ambient temperatures and thus are not particularly sensitive to high temperature. In many countries, harvested mangos are typically held and ripened under extremely high ambient temperature conditions, but with diurnal temperature fluctuations that may tend to mitigate negative high temperature effects (Kalra and Tandon, 1983). However, storage at a constant temperature of 27-30 °C can result in mottled skin and strong flavor, while ripening can be retarded above 30 °C (Brecht et al., 2013; Hatton et al., 1965; O'Hare, 1995).

Since mango fruit are quite tolerant of high temperatures, treatments for decay control and insect disinfestation treatments have been developed that utilize heated water or air (Brecht and Yahia, 2009). The most common insect quarantine treatment used for mangoes imported to the U.S. involves immersion of the fruit for from 65 to 110 minutes (depending on fruit size and shape) in 115°F (46.1 °C) water and was developed by USDA entomologists in Florida (Sharp, 1988). Hot water decay control treatments use higher temperatures and much shorter durations, typically 2 to 15 min at 50 to 55 °C. The first treatment (53 °C for 3 min) was first developed in Florida and Puerto Rico in order to control anthracnose rot which, once established in mango fruit, is extremely difficult to control with fungicides (Pennock and Maldonado, 1962; Smoot and Segall, 1963). Heat treatments are developed and chosen so as to kill the decay organism or insect but not to injure fruit. Thus, injury of mangos caused by heat treatment indicates misuse or mismanagement of the process; most commonly, this involves either operator error or treating immature mangos, which are less tolerant of heat than mature fruit (Jacobi et al., 2001a,b; Yahia and Campos, 2000). There are also indications that different mango cultivars vary either in their tolerance to heat treatment or in their propensity to exhibit injury symptoms (Jacobi et al., 2001b).

Injuries to mango fruit from forced hot air and vapor heat insect disinfestation treatments include peel scald and pitting, LD, and internal cavitation with white, starchy, unripe areas (Jacobi et al., 2001b). Injuries to mango fruit from hot water immersion treatments include LD, surface scald, surface pitting, discoloration of vascular strands, cavitation, starchy pockets in the otherwise ripe flesh, and flesh discoloration (Jacobi et al., 2001b). Cavitation typically occurs near the stem end (shoulders) of the fruit and is often followed by tissue collapse that makes the injury apparent visually from outside the fruit. Hot water treatment used for insect quarantine can increase LD, but the symptoms may differ from the LD seen in mango fruit with CI. Simao de Assis et al. (2009) reported that hot water treatment of Brazilian 'Tommy Atkins' fruit destined for the United States resulted in black LD compared with fruit destined for the Eurpoean Union (EU) that did not receive the HW treatment and exhibited principally red lenticel spot. It is possible that the red LD observed for fruit in the EU represented mild CI due to low shipping temperatures while the black LD in U.S.-bound fruit was caused by heat stress from the hot water treatment.

Modified and Controlled Atmosphere Injury

Modified and controlled atmospheres (MA and CA) involve reduced oxygen (O_2) and/or elevated carbon dioxide (CO_2) that may serve as a supplement to temperature management for extending shelf life of produce (Bishop, 1997), or as a method to disinfest stored crops of insects (Neven et al., 2009). The difference between MA and CA lies in their precision: CA involves active generation and feedback control of the atmosphere while MA is designed to roughly balance fruit respiration with restricted gas exchange of the storage space or packaging (modified atmosphere packaging: MAP) to reach a desired O_2 and CO_2 equilibrium. Optimum O_2 and CO_2 concentrations for mangoes depend on the cultivar, maturity or ripeness stage, storage period, and the purpose of using MA or CA (Yahia and Singh, 2009).

Optimum atmosphere composition for long term transport and storage of mature-green mangos is usually considered to be 3 to 5% O_2 plus 5 to 10% CO_2. Ripening mangoes can benefit from higher CO_2 levels (up to 15%) and lower storage or transport temperature, but O_2 cannot be lowered very much due to risk of initiation of fermentative metabolism in the fruit (Bender et al., 1994; Bender et al., 2000a,b). The ideal atmosphere for any particular purpose depends on several factors including fruit maturity or ripeness, cultivar, temperature, storage or shipping duration, and treatments given to the mangos prior to MA/CA application (such as heat treatment) (Yahia and Singh, 2009). Improper use of MA and CA can result in physiological disorders and quality deterioration.

Injuries to mango fruit from MA or CA can result from misapplication of the technology, but, for mangoes, O_2 and CO_2 injuries more commonly result from inadvertent exposure of mangos to reduced O_2 and/or elevated CO_2 such as keeping mangos in CA beyond the recommended exposure time or failing to open the fresh-air exchange on marine containers during lengthy international shipments. Mango fruit are quite prone to shifting from aerobic to anaerobic (fermentative) metabolism, which occurs even during normal ripening. Unripe mangos and those held at lower temperatures tend to be more tolerant of reduced O_2 but are less tolerant of elevated CO_2 than ripening fruit and those held at higher temperatures. There is also an exposure duration limit for holding mangos in any MA or CA—for proper MA and CA, storage is limited by ripening-related changes like fruit softening, but more extreme atmospheres can eventually damage the fruit, resulting in characteristic symptoms.

Initiation of fermentative metabolism resulting ethanolic odor and off-flavor is the first symptom of MA or CA injury of mango fruit. Using storage durations at 12 °C typical of marine shipments from South America to the United States, Bender et al. (1994) found that exposure of mature-green 'Kent' and 'Tommy Atkins' mangos to atmospheres with CO_2 concentrations higher than 25% at 12 °C for longer than 2 weeks result in elevated ethanol production and damage to skin color development. The injury to mango fruit caused by CO_2 appears to be more severe the lower the temperature (Bender et al., 1994; 1995). In complementary experiments, Bender et al. (2000b) found that holding 'Tommy Atkins' and 'Haden' mangoes in O_2 below 4% for 2 or more weeks at 12 °C caused no visible injury symptoms, but after ripening, a fermented off-flavor developed in the initially mature-green fruit stored in 2% O_2 and in ripening initiated fruit at 2 and 3% O_2. The extent of ripeness also determined the tolerance of mango to reduced O_2—'Haden' mangos at the onset of the climacteric peak produced 10 times more ethanol than preclimacteric fruit (Bender et al., 2000b).

More extreme MA and CA results in visible injury symptoms such as failure of fruit to recover ripening capacity, greyish flesh color from low O_2 injury, and peel discoloration from high CO_2 injury. The peel of mango fruit injured by high CO_2 takes on an abnormal, dull grayish green (Bender et al., 1994; 1995) or olive-khaki (O'Hare and Prasad 1993) appearance that appears to be brown discoloration of injured epidermal tissue overlaying normal green color from retarded ripening. Injurious CO_2 levels may also cause abnormal flesh softening (Reid and Serek, 1999; Thompson, 2010). The type of atmosphere injury most commonly seen in mango fruit is caused by failure to ventilate marine containers during long distance transport, resulting in symptoms that are apparently from a combination of low O_2 and high CO_2 damage.

Literature Cited

Bender, R.J., J.K. Brecht, E.A. Baldwin, and T.M.M. Malundo. 2000a. Aroma volatiles of mature-green and tree-ripe Tommy Atkins mangoes after controlled atmosphere vs. air storage. HortScience 35:684–686.

Bender, R.J., J.K. Brecht, and C.A. Campbell., 1994. Response of Kent and Tommy Atkins mangoes to reduced O_2 and elevated CO_2. Proc. Fla. State Hort. Soc. 107:274–277.

Bender, R.J., J.K. Brecht, and S.A. Sargent. 1995. Inhibition of ethylene production in mango fruit by elevated CO_2 and recovery during subsequent air storage. Proc. Fla. State Hort. Soc. 108:279–285.

Bender R.J., J.K. Brecht, S.A. Sargent, and D.J. Huber. 2000b. Mango tolerance to reduced oxygen levels in controlled atmosphere storage. J. Amer. Soc. Hort. Sci. 125:707–713.

Bishop, D. 1997. Controlled atmosphere storage, p. 53–92. In: C.V.J. Dellino (ed.), Cold and Chilled Storage Technology. Second edition. Chapman & Hall, London.

Brecht, J.K. 2018. Mango, p. 443–466. In: S. Pareek and S. Tonetto de Freitas (eds.). Postharvest Physiological Disorders in Fruits and Vegetables. CRC Press, Boca Raton, FL.

Brecht, J.K., M.C.N. Nunes, and F. Maul. 2012. Time-temperature combinations that induce chilling injury of mangos. Final Report to the National Mango Board, April 2012.

Brecht, J.K., S.A. Sargent, A.A. Kader, E.A. Mitcham, F. Maul, P.E. Brecht, and O. Menocal. 2013. Mango postharvest best management practices manual. Brecht, J. K. (ed.), HS1185. National Mango Board and UF/IFAS, Gainesville, FL. (revised).

Brecht, J.K. and E.M. Yahia. 2009. Postharvest physiology, p. 484–528. In: R.E. Litz (ed.). The Mango, Botany, Production and Uses, 2nd ed., CABI, Wallingford, U.K.

Brown, B.I., I.A. Wells, and C.F. Murray. 1986. Factors affecting the incidence and severity of mango sapburn and its control. ASEAN Food J. 2:127–132.

Burdon, J.N., K.G. Moore, and H. Wainright. 1991. Mineral distribution in mango fruit susceptible to the physiological disorder "soft nose". Scientia Horticulturae 48:329–336.

Campbell, C.W. 1973. The 'Tommy Atkins' mango. Proc. Fla. State Hort. Soc. 86:348–350.

Cronje, R.B. 2009a. Effect of harvesting practices and pre-packing storage on lenticel discolouration of mangoes. Acta Hortic. 820:653–664.

Cronje, R.B. 2009b. Effect of packhouse procedures on lenticel discolouration of mangoes. Acta Hortic. 820:673–680.

Dea, S., J.K, Brecht, M.C.N. Nunes, and E.A. Baldwin. 2010. Occurrence of chilling injury in fresh-cut 'Kent' mangoes. Postharvest Biol. Technol. 57:61–71.

DuPlooy, G.W., C.F. Van Der Merwe, and L. Korsten. 2006. Lenticel discolouration in mango (*Mangifera indica* L.) fruit—A cytological study of mesophyll cells from affected tissue. J. Hort. Sci. Biotechnol. 81:869–873.

DuPlooy, G.W., S. Combrinck, T. Regnier, and B.M. Botha. 2009. Linking lenticel discolouration of mango (*Mangifera indica* L.) fruit to reversed-phase HPLC profiles of phenolic compounds. J. Hort. Sci. Biotechnol. 84:421–426.

Galán Saúco, V. 2009. Physiological disorders, p. 303–316. In: R.E. Litz (ed.). The Mango, Botany, Production and Uses, 2nd ed., CABI, Wallingford, U.K.

Hatton, T.T. Jr., W.F. Reeder, and C.W. Campbell. 1965. Ripening and storage of Florida mangoes. Marketing Research Report 725, Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C.

Jacobi, K.K., E.A. MacRae, and S.E. Hetherington. 2001a. Effect of fruit maturity on the response of 'Kensington' mango fruit to heat treatment. Australian J. of Experimental Agric. 41:793–803.

Jacobi, K.K., E.A. MacRae, and S.E. Hetherington. 2001b. Postharvest heat disinfestation treatments of mango fruit. Scientia Horticulturae 89:171–193.

Jacobi, K.K. and L.S. Wong. 1992. Quality of 'Kensington' mango (*Mangifera indica* L.) following hot water and vapour-heat treatment. Postharvest Biol. Technol. 1:349–359

Kalra, S.K. and D.K. Tandon. 1983. Ripening-behaviour of 'Dashehari' mango in relation to harvest period. Scientia Horticulturae 19:263–269.

Kane, O., Boulet, M., and F. Castaigne. 1982. Effect of chilling injury on texture and fungal rot of mangoes (*Mangifera indica* L.). J. Food Sci. 47:992–995.

Krishnamurthy, S. 1981. Chemical studies on internal fruit breakdown in 'Alphonso' mango (*Mangifera indica* Linn.). J. Hort. Sci. 56:247-250.

Lakshminarayanan, S. 1975. Relation of time of harvest on respiration, chemical constituents and storage life of mangos. Proc. Fla. State Hort. Soc. 88:477–481.

Loveys, B.R., S.P. Robinson, J.J. Brophy, and E.K. Chacko. 1992. Mango sapburn: Components of fruit sap and their role in causing skin damage. Australian J. Plant Physiol. 19:449–457.

Malo, S.E. and C.W. Campbell. 1968. Studies of mango fruit breakdown in Florida. Proc. Trop. Reg. Amer. Soc. Hort. Sci. 22:1–5.

McMillan, R.T., Jr., D.H. Spalding, and W.F. Reeder. 1987. Effectiveness of various postharvest treatments for mango decay control. Proc. Fla. State Hort. Soc. 100:7–9.

Medlicott, A.P., S.B. Reynolds, and A.K. Thompson. 1986. Effects of temperature on the ripening of mango fruit (*Mangifera indica* L. var. Tommy Atkins). J. Sci. Food Agric. 37:469-474.

Mohammed, M. and J.K. Brecht. 2002. Reduction of chilling injury in 'Tommy Atkins' mangoes during ripening. Scientia Horticulturae 95:297–308.

Mukherjee, S.K. and R.E. Litz. 2009. Introduction: Botany and importance, p. 1–18. In: R.E. Litz (ed.). The Mango, Botany, Production and Uses, 2nd ed., CABI, Wallingford, U.K.

Neven, L.G., E.M. Yahia, and G.J. Hallman. 2009. Effects on insects, p. 233–266. In: E.M. Yahia (ed.). Modified and Controlled Atmospheres for the Storage, Transportation, and Packaging of Horticultural Commodities. CRC Press, Boca Raton, FL.

O'Hare, T.J. 1994. The susceptibility of Thai and Australian mango cultivars to sap injury and possible means of control. ACIAR Proceedings 58:21–24.

O'Hare, T.J. 1995. Effect of ripening temperature on quality and compositional changes of mango (*Mangifera indica* L.) cv. Kensington. Australian J. Experimental Agric. 35:259–263.

O'Hare, T.J. and A. Prasad. 1993. The effect of temperature and carbon dioxide on chilling symptoms in mango. Acta Hortic. 343:244–250.

Pennock, W. and G. Maldonado. 1962. Hot water treatment of mango fruits to reduce anthracnose decay. Univ. Puerto Rico Agric. J. 46:272–283.

Pesis, E., M. Faure, and R. Marinansky-Ben Arie. 1997. Induction of chilling tolerance in mango by temperature conditioning, heat, low O_2 and ethanol vapours. Acta Hortic. 455:629–634.

Ploetz, R.C. 2003. Diseases of mango. p. 327–363. In: R.C. Ploetz (ed.). Diseases of Tropical Fruit Crops. CABI, Wallingford, U.K.

Purseglove, J.W. 1972. Mangoes west of India. Acta Hortic. 24:107–174.

Raymond, L., B. Schaffer, J.K. Brecht, and J.H. Crane. 1998a. Internal breakdown in mango fruit: Symptomology and histology of jelly seed, soft nose and stem-end cavity. Postharvest Biol. Technol. 13:59–70.

Reid, M.S. and M. Serek. 1999. Guide to Food Transport. Controlled Atmosphere. Mercantila, Copenhagen.

Saby John, K., S.G. Bhat, and U.J.S. Prasada Rao. 2002. Involvement of peroxidase and polyphenol oxidase in mango sap injury. J. Food Biochem. 26:403–414.

Schaffer, B. 1994. Mango disorders caused by abiotic factors, p. 43–44. In: R.C. Ploetz, G.A. Zentmyer, W.T. Nishijima, K.G. Rohrbach, and H.D. Ohr (eds.). Compendium of Tropical Fruit Diseases, Part III. Mango. APS Press, St. Paul, MN.

Sharp, J. 1988. Status of hot water immersion quarantine treatment for Tephritidae immatures in mangos. Proc. Fla. State Hort. Soc. 101:195–197.

Shivashankar, S. 2014. Physiological disorders of mango fruit. Horticultural Reviews 42:313–348.

Simão de Assis, J., G. Self, and V.C. Caron. 2009. Effects of postharvest handling on lenticel spotting of 'Tommy Atkins' mangoes from northeast Brazil. Acta Hortic. 820:681-688.

Smoot, J.J. and R.H. Segall. 1963. Hot water as a postharvest control of mango anthracnose. Plant Disease Reporter. 47:739-742.

Subramanyam, H., S. Krishnamurthy, N.V. Subhadra, V.B. Dalal, G.S. Randhawa, and E.K. Chacko. 1971. Studies on internal breakdown, a physiological ripening disorder in 'Alphonso' mangoes (*Mangifera indica* L). Trop. Sci. 13:203–210.

Thompson, A.K. 2010. Effects and interactions of CA storage, p. 11–25. In: Controlled Atmosphere Storage of Fruits and Vegetables. CABI, Wallingford, U.K.

Thompson, A.K. 2015. Fruit and Vegetables: Harvesting, Handling and Storage, 3rd ed. Wiley, Chichester, West Sussex, U.K.

Warschefsky, E.J. and E.J.B. von Wettberg. 2019. Population genomic analysis of mango (*Mangifera indica*) suggests a complex history of domestication. New Phytologist 222:2023–2037.

Yahia, E.M. and P. Campos. 2000. The effect of hot water treatment used for insect control on the ripening and quality of mango fruit. Acta Hortic. 509:495–501.

Yahia, E.M. and S.P. Singh. 2009. Tropical fruits, p. 397–461. In: E.M. Yahia (ed.), Modified and Controlled Atmospheres for the Storage, Transportation, and Packaging of Horticultural Commodities. CRC Press, Boca Raton, FL.

Young, T.W. 1957. "Soft-nose," a physiological disorder in mango fruits. Proc. Fla. State Hort. Soc. 70:280–283.

Proc. Fla. State Hort. Soc. 132:177–181. 2019.

Accelerating Implementation of Huanglongbing-tolerant Hybrids As New Commercial Cultivars for Fresh and Processed Citrus

Elizabeth Baldwin, Ed Stover, Randy Driggers, Jinhe Bai, John Manthey, Xiuxiu Sun, Chris Ference, and Anne Plotto*

USDA–ARS, U.S. Horticultural Research Laboratory, 2001 South Rock Rd., Ft. Pierce, FL 34945

Additional index words. flavor, limonin, mandarin/tangerine, nomilin, orange, sugars, acids

Citrus greening or huanglongbing (HLB) has reduced citrus and especially orange yields, resulting in closure of packing houses and processing plants in Florida. To maintain the remaining packing and processing infrastructure, a National Institute of Food and Agriculture (NIFA), Citrus Disease Research and Extension (CDRE) grant was obtained to identify hybrids in breeding programs with HLB tolerance and commercial quality for either the fresh fruit or processed juice industry. The proposed research would also determine HLB resistance or tolerance DNA markers for marker assisted breeding or use in transgenic programs. This paper will discuss the first season performance of some of these hybrids for their intrinsic flavor quality and similarity to either orange or mandarin, since both orange and mandarin are present in the hybrids' genetic backgrounds. Both sensory and chemical evaluations for flavor were conducted. Several commercial cultivars were also harvested and evaluated for comparison. These hybrids were harvested once or multiple times over the season. Out of the 20 hybrids evaluated, 7 exhibited orange similarity, and most scored relatively high for orange flavor in at least one harvest. One of these hybrids has since been released as 'U.S. SunDragon'. Seven hybrids exhibited similarity to mandarin and/or mandarin flavor, with three that also had similarity to orange. Overall, the trial showed that there is much diversity in the breeding programs for flavor, sugars, acids, and bitter limonoids. There is also potential for both HLB tolerance and commercial quality. Hybrids with HLB tolerance and good flavor would be candidates for citrus juice blends.

Huanglongbing (HLB) or citrus greening disease has plagued Florida citrus industry since 2005, and citrus production acreage has dropped from 762,000 acres in 1999–2000 to 400,000 acres in 2017–18 (USDA NASS, 2019). In spite of this shrinkage, Florida remains the number two supplier of orange juice in the world (USDA FAS, 2020), with production that has stabilized at around 300,000 metric tons/ year (65 °Brix) since 2015 (this not counting the drop following hurricane Irma in 2017). However, this number is far below the most productive years, and several processing plants have closed due to the reduction in fruit volume in Florida resulting from HLB. A National Institute of Food and Agriculture (NIFA) grant was obtained to identify HLB-tolerant scion cultivars and or genetic material from the U.S. Department of Agriculture (USDA), University of California, Riverside (UCR) and University of Florida (UF) breeding programs that can be quickly mobilized for use by the U.S. citrus industry. The project is identifying HLB resistant/tolerant orange mandarin and grapefruit like hybrids with good flavor quality, identifying DNA markers associated with tolerance to HLB and evaluating fruit and juice quality of HLB tolerant scions. This material can then be used either as stand alone varieties, hybrid classification as "orange," "mandarin," or "grapefruit" and/or development of juice combinations to supplement the current processing stream. These results are continuously communicated to the industry to facilitate industry implementation of HLB tolerant scion cultivars.

This paper will present the first year flavor data for the HLB tolerant lines analyzed in the 2016–17 seasons for similarity to "orange" or "mandarin" and for overall flavor quality traits. We hope the research will result in a sustainable solution when used either alone or in combination with other disease mitigation methods. Hopefully, developing HLB resistant varieties with commercial quality will decrease production costs per acre, increase crop yields, enhance consumer satisfaction, and maintain production and processing infrastructure. In addition, analysis and comparison of sensory and chemical flavor analyses will improve understanding of citrus flavor.

Materials and Methods

Fruit from HLB tolerant trees from the USDA breeding program were harvested once or multiple times from November 2016 to February 2017 to determine optimal harvest dates, if not known, or at optimal harvest time if previously determined. Fruit were brought to the USDA laboratory, washed, juiced and frozen at –20 °C pending sensory and chemical analyses. For sensory analysis, juice was tested in comparison to orange and tangerine reference juices, using the polarized sensory positioning method (Teillet et al., 2010; Valentin et al., 2012). The reference juices were from fresh squeezed unpasteurized 'Valencia' oranges (Al's Family Farm, Fort Pierce, FL) and locally processed fresh

Financial support for this research was partially provided by USDA-NIFA SCRI Award #2018-70016-27453

*Corresponding author. Email: anne.plotto@usda.gov

tangerines “gourmet pasteurized” (Orchid Island Juice Co., Fort Pierce, FL). Six trained panelists, with three to 10 years of training and experience rating citrus juice products, rated the juice on a 0–15 scale for similarity to orange or tangerine references (0 = exactly the same flavor as reference, 15 = totally different from reference). Panelists then rated sweetness, sourness, bitterness, orange like flavor, mandarin like flavor and off flavor on a 0–15 scale, where lower and higher values indicate lower or higher intensity of perception for a flavor characteristic, respectively. Panelists were also asked to specify off flavor, when perceived, and were free to make any comment about the juice. Six to eight samples were tasted per day and within a day, samples were presented in a completely randomized order across panelists. Taste panels took place in isolated booths with red lighting to mask color differences among juices, and using Compusense© Cloud (West Guelph, ON, Canada) for data acquisition. Samples from the same juice were analyzed for pH and titratable acidity (TA) to a pH 8.1 endpoint using an autotitrator (Mettler Toledo DL50, Columbus, OH), soluble solids content (SSC) by refractomer (Atago PR 101, Tokyo, Japan), SSC/TA ratio, and for levels of the bitter compounds limonin and nomilin by chromatography as described in Raithore et al., (2020).

Sensory data were analyzed using SenpaQ v.5.01 (Qi Statistics, Berkshire, U.K.). Correlation tests and principal components analyses (PCA) were performed to compare instrument with sensory data using XLSTAT v. 2019 (Addinsoft, New York, NY).

Results and Discussion

An alternative to descriptive sensory analysis was tested in this project, where the samples were compared with either an orange or a tangerine juice reference (Valentin et al., 2012). Not knowing what the hybrids would taste like “a priori”, we hypothesized that this method would help in classifying the hybrids. A principal components analysis (PCA) showed that rating samples in comparison with a reference juice sample (“same as reference”), or simply rating the flavor by providing a conceptual reference standard gave similar results; indeed, correlations (Table 1) as well as the PCA graph (Fig. 1) showed that descriptors “same as orange” or “same as mandarin” were highly correlated with “orange like” and “mandarin like” flavors, respectively. However, in general, the similarity ratings were higher (up to 10.5 points on a 0–15 scale) than the absolute ratings (up to 6.3 points) (Table 2). Furthermore, more samples had high similarity to orange than mandarin and also had relatively high orange flavor ratings, whereas samples that had high similarity with mandarin juice did not all have high mandarin flavor ratings. The latter can be explained by the fact that the mandarin juice used as a reference was pasteurized, and pasteurization, even at low temperatures, can change the flavor profile of a juice (Perez Cacho and Rouseff, 2008).

Table 2 presents average ratings for sensory descriptors as well as instrumental data. Color coded sensory means indicate relatively high values: orange color for “same as orange” and “orange flavor”, peach color for “same as tangerine” and “tangerine flavor, yellow for high “sweet” ratings, and green, blue or grey for high “sour”, ”bitter” or “off flavor” ratings, respectively. Samples with the highest ratings for “same as orange” (≥ 7) were ‘Bower’ (Dec.), ‘Fortune’ (Jan. Feb.), FTP 6-49-96 (Nov. Dec., newly released ‘U.S. SunDragon’, which also had high “same as mandarin” rating for Nov.), FF 1-8-96 (Dec.), FF 1-10-61 (Nov. Dec.), FF 1-74-14 (Dec.), FF 1-75-113 (Nov.), FF 1-85-109 (Nov. Jan.) and FF 1-85-119 (Jan. Feb., which also had high rating for “same as mandarin” for Feb.). Of these, all but ‘Bower’ and FF 1-74-14 (Dec.) also had relatively high (≥ 5) “orange flavor” ratings in at least one harvest. This includes ‘Fortune’ (Jan.), FF 1-8-96 (Dec.), FF 1-10-61 (Nov. Dec.), FF 1-75-113 (Nov.), FF 1-85-109 (Jan.) and FF 1-85-119 (Jan. Feb.) (Table 2). For “same as mandarin”, ‘Nova’ (Dec.), FTP 6-49-96 ‘U.S. SunDragon’ (Nov.), FF 1-10-1 (Jan.), FF 1-78-62 (Dec.), FF 1-85-119 (Feb.), FF 1-8-70 (Feb.) and FF 5-51-2 (Dec.) had relatively high ratings (≥7), and among them, only ‘Nova’ (Dec.), FF 1-78-62 (Dec.) and FF 5-51-2 (Dec.) had relatively high ratings for “mandarin flavor” in addition to FF 1-8-96 (Dec.) (Table 2). The sweetest samples tended to also be high in mandarin, orange, or both flavors (Table 2). High and significant correlations between sweet and “same as orange” and with mandarin flavor were found (Table 1), as well as with the SSC/TA ratio. A SSC/TA ratio greater than 12.0 generally resulted in a high sweetness rating, but there were exceptions. Volatile compounds also contribute to the perception of sweetness, as demonstrated by Bartoshuk et al. (2017) in strawberries, or in orange juice (unpublished data).

Some samples had an exceedingly high level of sourness, greater than 9.5 on the 0 15 scale: FF 1-85-82 (Nov.), FF 1-8-70 (both harvests), FTP 6-45-137 (Jan), FTP 6-46-15 (both harvests), FTP 6-47-13 (both harvests), and FTP 6-47-9 (all harvests),

Table 1. Pearson correlation coefficients between sensory and instrumental measurements (n = 41).

Variables	Orange Flavor	Same as Mandarin	Mandarin Flavor	Sweet	Sour	Bitter	Off-flavor	SSC (°Brix)	TA (% Acid)	SSC/TA	Limonin	Nomilin
Same as orange	**0.797**[z]	0.360	0.432	**0.665**	**–0.628**	–0.500	**–0.623**	0.212	–0.371	0.258	–0.307	–0.352
Orange flavor		0.237	0.451	0.564	–0.424	–0.247	**–0.595**	0.401	–0.191	0.087	–0.170	–0.322
Same as mandarin			**0.680**	0.442	–0.495	–0.168	–0.387	0.323	–0.513	0.517	–0.007	0.010
Mandarin flavor				**0.701**	**–0.689**	–0.087	–0.445	0.304	**–0.600**	0.562	–0.089	–0.109
Sweet					**–0.894**	–0.222	–0.317	0.510	**–0.647**	**0.657**	–0.125	–0.242
Sour						0.318	0.357	–0.313	**0.782**	**–0.773**	0.233	0.283
Bitter							0.533	0.097	0.378	–0.286	**0.692**	0.401
Off–flavor								–0.018	0.346	–0.203	0.272	0.245
SSC (Brix)									–0.079	0.359	0.029	–0.077
TA (% Acid)										**3**	0.134	0.197
SSC/TA											–0.141	–0.194
Limonin												**0.696**

[z]Values in bold are different from 0 with a significance level α < 0.0001.

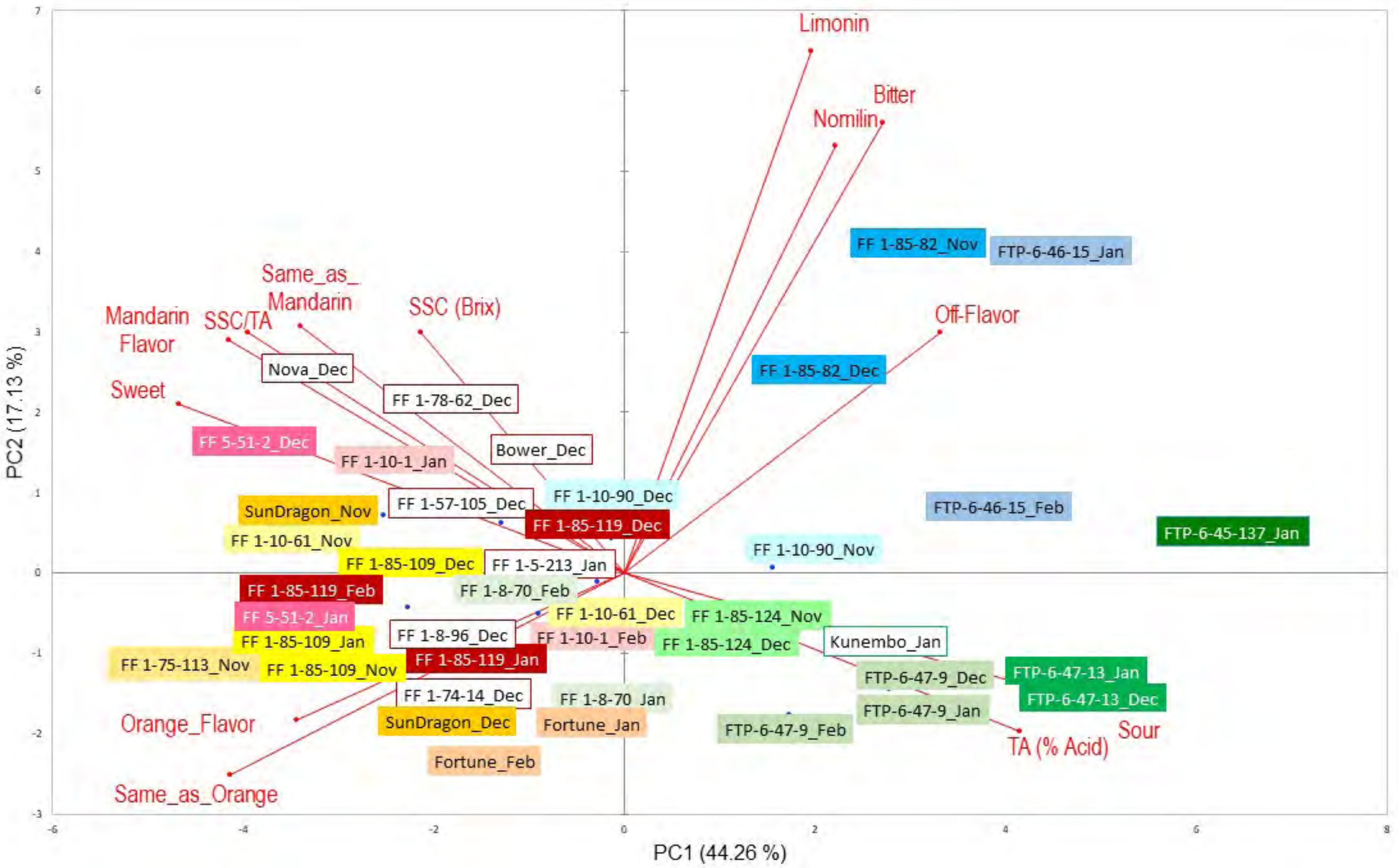

Fig. 1. Principal components analysis (PCA) of sensory and instrumental quality ratings of 24 citrus hybrids harvested multiple times.

associated with high TA levels (greater than 1.25% and up to 2.16%) and low SSC/TA ratio (less than 10.0) (Table 2). Some of these samples were additionally perceived as noticeably or highly bitter, depending on panelists, and had high limonin or limonin + nomilin levels: FF 1-10-90 (Nov.), FF 1-85-124 (Nov.), FF 1-85-82 (both harvests), FTP 6-45-137 (Jan.), FTP 6-46-15 (both harvests), and FF 6-47-13 (Jan.) (Table 2). Dea et al. (2013) found that in a complex matrix such as orange juice, limonin recognition threshold (i.e. concentration of limonin at which point it is perceived as bitter) was 4.7 mg·L^{1}. The current data indicate that most of the time, limonin greater than 3.43 mg·L^{1} induced perception of bitterness, but some samples with lower levels of limonin were also perceived as bitter (2.34 and 2.16 mg·L^{1} for FTP 6-45-137 (Jan.) and FTP 6–47–13 (Jan.), respectively). Interestingly, bitterness was perceived in FF 1–10–61 (Nov.) and FF 1–78–62 (Dec.) which had relatively high limonin levels (3.96 and 3.77 mg·L^{1}, respectively), but with high soluble solids content (15.1 and 13.7 °Brix, respectively) and high SSC/TA ratio (13.7 and 11.4, respectively), panelists gave those hybrids high sweetness ratings, as well as relatively high mandarin flavor (Table 2 and Fig. 1).

Excessive sourness and high bitterness also resulted in high off flavor ratings. Comments for FTP 6–45–137 (Jan.), which had sour and bitter ratings of 13.5 (the highest) and 4.8, respectively, were "so sour, it is difficult to perceive anything else". FTP 6–47–13 (Dec. and Jan.) was also found too sour but not bitter, with additional notes of vinegar, woody, "green" and sulfury (data not shown). Off flavor in FF 1-85-82 (Nov. and Dec.) was described as harsh, bitter, gasoline, soapy, woody, which might be reminiscent of off flavor that has been found in fruit from some Poncirus hybrids. Other samples in which panelists found poncirus like off flavor were FF 1-10-1 (also soapy, pumpkin like, musty), FF 6-46-15 (also bitter, harsh, metallic, soapy, sulfury), and FF 1-85-119 (also metallic, gasoline, sulfury, woody). Off flavor in 'Bower' was described as carrot/pumpkin, not unpleasant but not typical of orange juice, which is unsurprising for this largely mandarin cultivar. Finally, off flavor in 'Kunembo' was associated with high sourness, and it is possible it was harvested too early (Jan. 2017).

The PCA plot summarizes quality of these 24 samples harvested in 2016–17 (Fig. 1). The first two PCs accounted for 61.39% of the variability. All samples on the left side of the graph (negative PC1) had desirable attributes, sweet, mandarin like and orange flavor with high SSC and SSC/TA, while samples on the right side of the graph were highly sour with high TA (negative PC2) or highly bitter with high limonin and nomilin levels (positive PC2). This first year of observations allowed sorting out which hybrids produce fruit with negative attributes, in spite of having shown some HLB tolerance. FTP 6-45-137, FTP 6-46-15, FTP 6-47-13. and FF 1-85-82 were removed from the list of hybrids for future evaluation. FTP 6-47-9 was also a possible candidate for removal, but was given a second chance in 2018 2019 with later harvests (end of February and March) with the hope of decreasing sourness; however, sourness ratings were still quite high in 2018–19 (data not shown). FF 1-85-124 on the other hand, improved with a later harvest in 2019. Hybrids

Table 2. Sensory ratings (0–15 scale), soluble solids content (SSC), titratable acidity (TA), limonin (L) and nomilin (N) of 24 citrus hybrids harvested in the 2016–17 season.

Genotype	Harvest date	Same as Orange	Orange flavor	Same as mandarin	Mandarin Flavor	Sweet	Sour	Bitter	Off flavor	SSC (°Brix)	TA (% citric acid)	SSC/TA	Limonin (mg L−1)	Nomilin (mg L−1)
Bower	13 Dec.	8.0	3.5	7.0	3.2	8.3	4.7	2.3	6.2	13.4	1.02	13.2	3.37	2.40
Kunembo	12 Jan.	3.5	2.7	4.2	3.3	4.2	9.3	2.8	5.7	11.6	1.49	7.8	0.86	0.91
Nova	13 Dec.	6.5	4.2	8.2	6.0	9.2	3.0	2.7	3.5	12.6	0.71	17.7	2.80	2.40
Fortune	12 Jan.	7.7	5.0	5.5	3.3	5.8	9.2	0.7	1.8	13.6	1.63	8.4	1.53	1.05
	15 Feb.	9.7	4.8	6.7	3.5	6.0	7.2	0.7	0.7	12.7	1.55	8.1	0.45	0.76
FTP 6-49-96	17 Nov.	7.7	4.2	9.7	4.7	5.8	5.7	1.8	1.8	12.0	0.79	15.3	2.90	1.19
‘SunDragon’	22 Dec.	8.7	4.7	5.0	3.8	5.8	5.3	0.7	1.2	11.0	0.93	11.8	1.18	1.73
FF 1-5-213	25 Jan.	4.8	3.5	6.2	4.2	6.0	8.5	2.7	3.0	12.4	1.06	11.8	1.33	1.01
FF 1-8-96	22 Dec.	8.0	5.2	6.5	5.3	7.7	5.8	2.2	2.0	11.3	1.03	10.9	0.97	1.27
FF 1-10-1	25 Jan.	5.3	3.8	7.5	4.5	7.7	5.0	2.5	4.2	15.2	0.80	19.0	1.29	0.86
	15 Feb.	7.2	4.5	6.5	3.7	6.0	5.2	2.8	5.2	10.8	1.51	7.1	1.30	1.24
FF 1-10-61	17 Nov.	9.3	6.3	5.3	4.2	9.2	5.5	3.0	1.0	15.1	1.11	13.7	3.96	0.86
	22 Dec.	8.5	5.2	5.7	2.8	6.2	8.5	2.3	3.3	15.2	1.55	9.8	2.85	1.06
FF 1-10-90	17 Nov.	6.8	3.8	4.8	3.8	5.2	8.7	3.3	1.8	6.7	1.23	5.6	6.14	2.39
	22 Dec.	6.0	4.3	5.8	3.5	6.2	6.8	3.3	2.0	12.3	1.13	10.9	4.75	1.82
FF 1-57-105	22 Dec.	4.5	1.5	5.3	3.3	8.3	2.8	1.0	5.0	12.0	0.66	18.3	1.49	1.33
FF 1-74-14	22 Dec.	10.0	4.8	4.7	4.2	7.7	5.7	2.2	4.3	11.5	1.27	9.2	0.96	0.97
FF 1-75-113	17 Nov.	10.5	6.3	5.8	4.8	9.0	4.0	1.5	2.0	9.8	0.84	11.6		
FF 1-78-62	22 Dec.	6.5	3.8	8.3	5.8	8.0	4.3	4.5	1.8	13.7	1.21	11.4	3.77	1.39
FF 1-85-109	17 Nov.	7.8	4.8	6.5	3.8	6.3	5.7	1.0	2.7	12.7	1.04	12.2	1.08	1.53
	22 Dec.	6.2	4.7	7.2	4.8	6.7	6.5	1.0	3.5	13.8	1.38	10.1	1.41	1.24
	25 Jan.	7.8	5.7	5.3	4.3	7.7	5.0	1.7	2.0	14.8	1.17	12.7	0.94	0.76
FF 1-85-119	22 Dec.	6.2	3.2	6.8	4.0	6.7	7.3	2.0	4.0	12.5	1.28	9.8	1.23	2.87
	25 Jan.	8.0	5.5	6.2	3.2	6.3	7.0	0.7	3.2	12.4	1.15	10.8	2.58	2.43
	15 Feb.	9.8	5.8	8.3	4.7	7.7	7.0	1.3	2.5	14.1	1.34	10.5	1.75	1.31
FF 1-85-124	17 Nov.	5.5	3.8	6.7	4.0	3.8	9.0	3.2	2.7	10.4	1.25	8.4	0.84	1.44
	22 Dec.	5.0	3.5	6.0	3.5	5.0	9.0	2.2	2.7	11.6	1.25	9.3	0.55	1.39
FF 1-85-82	17 Nov.	3.0	2.5	6.2	2.5	4.0	10.5	6.5	8.2	12.3	1.13	10.9	8.23	3.45
	22 Dec.	4.0	3.3	5.8	4.0	6.0	7.8	5.0	6.8	14.2	1.44	9.9	5.18	1.85
FF 1-8-70	25 Jan.	7.2	4.8	6.2	3.7	5.0	9.7	1.2	2.0	11.0	1.36	8.1	0.98	1.23
	15 Feb.	4.0	3.3	7.7	4.2	4.5	10.0	0.7	2.2	13.2	0.91	14.5	1.48	1.09
FF 5-51-2	22 Dec.	7.2	2.5	8.5	5.5	7.0	3.3	1.2	3.2	11.8	0.52	22.5	2.03	1.76
	25 Jan.	6.8	2.8	6.3	3.8	7.0	3.0	0.5	2.2	11.6	0.50	23.3	0.48	0.71
FTP 6-45-137	12 Jan.	1.0	0.8	3.7	2.2	3.0	13.5	4.8	10.7	12.2	2.16	5.6	2.34	1.58
FTP 6-46-15	12 Jan.	3.0	2.2	6.3	3.7	4.0	11.7	4.3	4.2	13.0	1.82	7.1	7.86	6.76
	15 Feb.	2.8	3.3	5.3	3.0	3.3	10.8	5.0	3.2	13.2	2.09	6.3	3.43	2.80
FTP 6-47-13	13 Dec.	3.0	2.0	2.3	1.8	3.0	11.5	1.2	5.7	9.2	1.47	6.3	1.92	2.47
	12 Jan.	3.8	3.2	3.0	2.2	3.3	11.8	4.0	5.8	10.6	2.07	5.1	2.16	0.82
FTP 6-47-9	13 Dec.	4.0	1.8	6.3	3.0	2.8	12.2	1.3	2.8	8.4	1.48	5.7	2.31	1.66
	12 Jan.	3.8	2.2	5.3	1.7	3.5	11.2	1.3	3.8	10.6	1.62	6.5	2.42	1.25
	15 Feb.	7.2	2.2	6.7	2.2	3.8	9.5	1.3	3.2	11.2	1.76	6.3	0.80	1.65

Color legend

- Same as orange flavor (≥7.5)
- Orange flavor (≥5)
- Same as tangerine flavor (≥7.5)
- Tangerine flavor (≥5)
- High sweet (≥7)
- High sour (≥7)
- High bitter (≥3)
- High off flavor (≥3)

with positive or intermediate attributes will continue to be evaluated, both for tree performance and fruit quality. These include FF 5-51-2, FF 1-75-113, FF 1-85-109, FF 1-85-119, FF 1-5-213, FF 1-57-105, FF 1-78-62, FF 1-8-70, and FTP 6-49-96, which has since been released as 'U.S. SunDragon'.

In conclusion, there is great diversity among the breeding program hybrids, with some showing promise for commercialization along with HLB tolerance. 'U.S. SunDragon' has been released and shows much promise, but in addition, other hybrids from the breeding programs have potential. Optimal harvest dates need to be established for the new hybrids, as there is great variation over the harvest season and from season to season, necessitating multiple seasons of data. This is true not only for the flavor characteristics, but also for yield and HLB tolerance. The best performing hybrids and others have been sampled and analyzed in the 2017–18 and 2018–19 seasons, with plans to continue for the next few years.

Literature Cited

Bartoshuk, L., T.A. Colquhoun, D.G. Clark, M. Schwieterman, C.A. Sims, V. Whitaker, H.J. Klee, D. Tieman, and L. McIntyre. 2017. Composition and methods for modifying perception of sweet taste. Patent Application No. 2017 0119034 A1.

Dea, S., A. Plotto, J.A. Manthey, S. Raithore, M. Irey, and E. Baldwin. 2013. Interactions and thresholds of limonin and nomilin in bitterness perception in orange juice and other matrices. J. Sensory Studies 28:311–323. doi: 10.1111/joss.12046.

Perez Cacho, P.R. and R. Rouseff. 2008. Processing and storage effects on orange juice aroma: A review. J. Agric. Food Chem. 56:9785–9796. https://doi.org/10.1021/jf801244j

Raithore, S., J. Kiefl, J.A. Manthey, A. Plotto, J. Bai, W. Zhao, and E. Baldwin. 2020. Mitigation of off flavor in Huanglongbing affected orange juice using natural citrus non volatile compounds. J. Agric. Food Chem. In press. https://doi.org/10.1021/acs.jafc.9b07756.

Teillet, E., P. Schlich, C. Urbano, S. Cordelle, and E. Guichard. 2010. Sensory methodologies and the taste of water. Food Qual. Pref. 21:967 976. http://dx.doi.org/10.1016/j.foodqual.2010.04.012.

USDA FAS, 2020. Citrus: World Markets and Trade. Jan. 2020. <https://apps.fas.usda.gov/psdonline/circulars/citrus.pdf>

USDA NASS, 2019. Florida Citrus Statistics. May 2019. <https://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/Citrus_Statistics/2017 18/fcs1718.pdf>

Valentin, D., S. Chollet, M. Lelièvre, and H. Abdi. 2012. Quick and dirty but still pretty good: a review of new descriptive methods in food science. International Journal of Food Science & Technology 47:1563–1578. doi: 10.1111/j.1365 2621.2012.03022.x.

Proc. Fla. State Hort. Soc. 132:182–184. 2019.

Low Inlet Air Temperature Spray-drying for Improved Encapsulation of Carvacrol In a Pectin/Sodium Alginate Matrix

Xiuxiu Sun, Randall G. Cameron, and Jinhe Bai*

USDA-ARS, Horticultural Research Laboratory, 2001 S. Rock Rd., Ft. Pierce, FL, 34945

Additional index words. microencapsulation; carvacrol; spray drying; encapsulation efficiency

Even though the effectiveness of essential oils against a variety of microorganisms has been well documented in the literature, the application of essential oils for the preservation of food is restrictive due to their powerful or unpleasant aroma. Microencapsulation has been extensively utilized in the pharmaceutical and food industries to entrap essential oils related to flavoring. Spray-drying is one of the most commonly used microencapsulation techniques in the food industry. The inlet air temperature of spray-drying affects the physicochemical and functional properties of the microcapsules. In this research, four inlet air temperatures (100, 130, 160, and 190 °C) were applied for the encapsulation of carvacrol in a pectin/sodium alginate matrix. The lower temperatures (100 and 130 °C) resulted in microcapsules with better physicochemical, structural, and functional properties. Therefore, an inlet temperature between 100 and 130 °C is ideal for processing pectin/sodium alginate encapsulated carvacrol.

Microencapsulation is a process in which bioactive ingredients are surrounded by a coating to form micro scale capsules which possess numerous functional properties (Castro-Rosas et al., 2017). Microencapsulation by spray-drying is a common method where a homogenized mixture of encapsulating agent and encapsulated agent is converted into a powder with high efficiency and low cost (Assadpour and Jafari, 2019). The encapsulating agent surrounds the core material, with spraying and high heat utilized to quickly volatilize the solvent (Assadpour, et al., 2019; Shahidi and Han, 1993; Sun et al., 2019). The spray-drying conditions, particularly the inlet air temperature, are an important factor contributing to the efficiency of the encapsulation process (Correa-Filho et al., 2019; Paulo and Santos, 2017).

In this research, four inlet air temperatures 100, 130, 160, and 190 °C were used. The moisture content of the powder ranged from 4.1 to 6.3% for all inlet air temperatures (Table 1). The microcapsules spray dried at 100 °C showed significantly higher moisture content (6.3%) than other treatments (Table 1). The powder bulk density ranged from 0.28 to 0.35 $g \cdot cm^{-3}$ depending on inlet air temperature (Table 1). Increased inlet air temperature decreased bulk density (Table 1). The times of wettability at 100 °C and 130 °C are significantly lower than those at 160 °C and 190 °C inlet air temperatures (Table 1). The time of wettability (4.5–5 min) increased with increased inlet air temperature (Table 1), indicating that powder wettability decreased with increasing inlet air temperature. The hygroscopicity was between 0.06 and 0.11 $g \cdot g^{-1}$ powder solid depending on inlet air temperature (Table 1).

The retention efficiencies of pectin/sodium alginate encapsulated carvacrol were 88.69, 48.78, 19.21, and 10.05%, when the inlet air temperatures were 100, 130, 160, and 190 °C, respectively (Fig. 1). A higher inlet air temperature corresponded to a lower retention efficiency (Fig. 1). Increased drying temperatures caused increased carvacrol loss due to the high volatility of carvacrol. Therefore, the retention efficiency of carvacrol was reduced by increasing the inlet air temperature. The encapsulation efficiencies of carvacrol in a pectin/sodium alginate matrix were 45.73, 43.41, 67.73, and 82.79%, when the inlet air temperatures were 100, 130, 160, and 190 °C, respectively (Fig. 1). A higher inlet air temperature corresponded directly to a higher encapsulation efficiency. The encapsulation efficiency was significantly affected by inlet air temperature because of its effect on crust formation (Rosenberg et al., 1990).

With an inlet air temperature ≤ 130 °C the minimum inhibitory concentration (MIC) was ≤ 0.5 $mg \cdot mL^{-1}$ (Fig. 2). The results also show that the higher the inlet air temperature, the larger the MIC (Fig. 2). A lower MIC means stronger antimicrobial activity, which correlates to the higher retention efficiency of carvacrol at lower inlet air temperature. The microencapsulated carvacrol provided more than 90% inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) at an inlet air temperature of 100 °C (Fig. 2). The higher the inlet air temperature, the lower the DPPH as a result of lower retention of carvacrol on the particle.

At inlet air temperature of 100 and 130 °C, the particles exhibited a spherical and smooth surface with a contiguous integument (Fig. 3), which is necessary to reduce gas permeability and improve the protection and retention of core material (Correa-Filho, et al., 2019; Tonon et al., 2011). Conversely, the particles were shrunken or cracked when the temperature was higher than 130 °C (Fig. 3). At the highest inlet air temperatures, microcapsules normally had larger internal voids. Raising the inlet air temperature increased

Any mention of a trademark or proprietary product is for identification only and does not imply a guarantee or warranty of the product by the U.S. Department of Agriculture. The U.S. Department of Agriculture prohibits discrimination in all its programs and activities on the basis of race, color, national origin, gender, religion, age, disability, political beliefs, sexual orientation, and marital or family status.

*Corresponding author. Email: Jinhe.bai@ars.usda.gov

Table 1. The effect of inlet air temperature (100, 130, 160, and 190 °C) on the physical properties of carvacrol in pectin/sodium alginate matrix microcapsules. Different letters in the same column indicate significant differences by the Tukey's HSD test ($P < 0.05$).

Temperature (°C)	Bulk density ($g \cdot cm^{-3}$)	Moisture content (%)	Dissolution time (min)	Hygroscopicity ($g \cdot g^{-1}$)
100	0.35 ± 0.02 a	6.30 ± 0.20 a	4.50 ± 0.00 b	0.07 ± 0.01 b
130	0.33 ± 0.00 ab	4.80 ± 0.10 b	4.67 ± 0.06 b	0.09 ± 0.01 ab
160	0.31 ± 0.02 bc	4.33 ± 0.21 bc	4.91 ± 0.11 a	0.10 ± 0.01 a
190	0.28 ± 0.02 c	4.17 ± 0.18 c	5.01 ± 0.10 a	0.10 ± 0.01 a

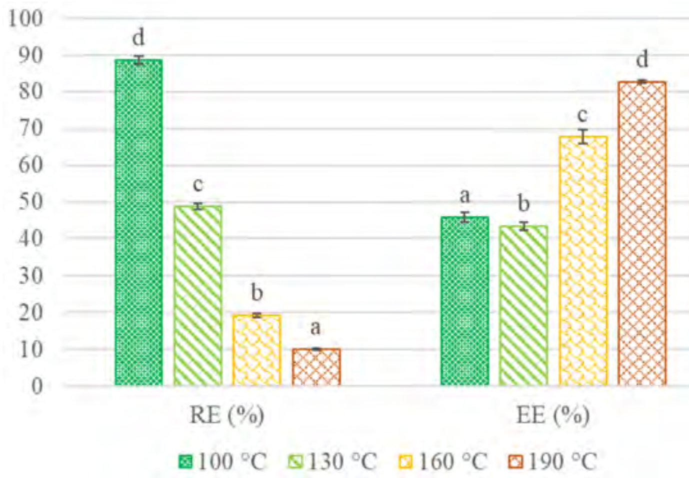

Fig. 1. Effect of inlet air temperature (100, 130, 160, and 190 °C) on retention efficiency and encapsulation efficiency of the carvacrol in pectin/sodium alginate matrix microcapsules. Bars with different letters indicate significant difference by the Tukey's HSD test ($P < 0.05$).

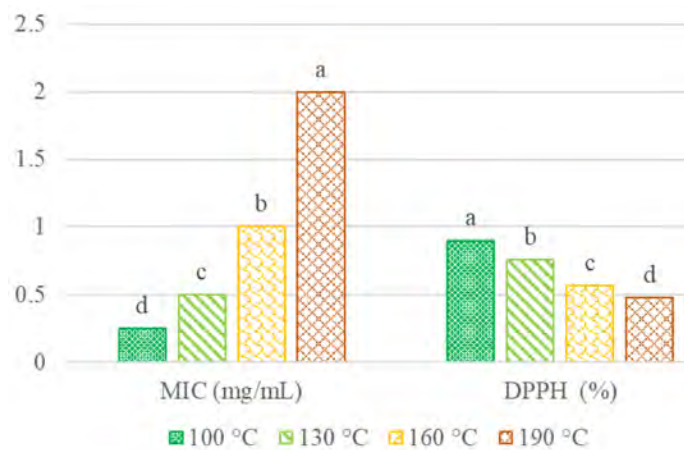

Fig. 2. Effect of inlet air temperature (100, 130, 160, and 190 °C) on antimicrobial and antioxidant properties of the carvacrol in pectin/sodium alginate matrix microcapsules. (MIC = minimum inhibitory concentration; DPPH = 2, 2-Diphenyl-1-picrylhydrazyl percentage inhibition). Bars with different letters indicate significant difference by the Tukey's HSD test ($P < 0.05$).

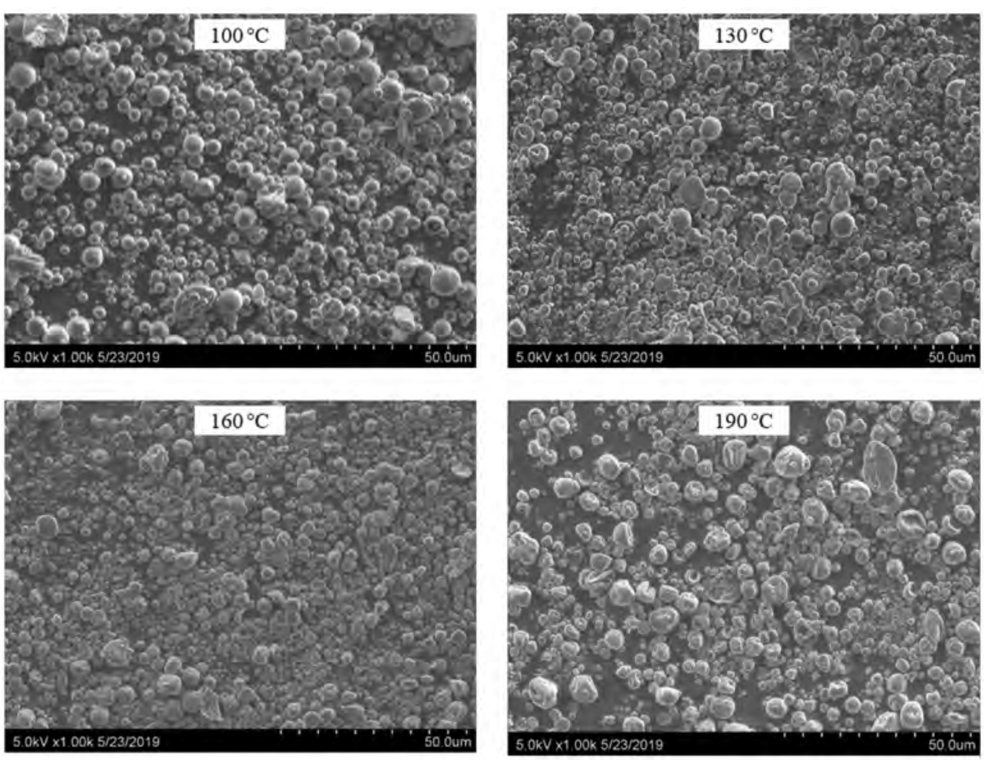

Fig. 3. Effect of inlet air temperature (100, 130, 160, and 190 °C) on microstructure of the carvacrol in pectin/sodium alginate matrix microcapsules.

both the average volume of the voids and the percentage of microcapsules which had voids (Chegini and Ghobadian, 2005). Microcapsules which formed voids early in the drying process occasionally burst and then contracted. Increasing the inlet air temperature frequently results in the quick formation of a dry surface layer on the droplet. The increase in average microcapsule surface area may also be a result of higher clumping at the upper inlet air temperatures in this study (Broadhead, et al., 1994).

This work suggests that the inlet air temperature significantly affects the physical, chemical, and functional properties of carvacrol encapsulated in a pectin/sodium alginate matrix. Increasing the inlet air temperature increased time-for-wettability, hygroscopicity, and encapsulation efficiency, but reduced moisture content, bulk density, and retention efficiency. The powder dried at lower temperatures (100 and 130 °C) showed higher antimicrobial and antioxidant properties with lower MIC and higher DPPH-PI. The SEM revealed that the microcapsules at 100 and 130 °C were regular round spheres with fairly smooth surfaces. Therefore, the lower inlet air temperatures between 100 °C and 130 °C were appropriate for the processing of carvacrol encapsulated in a pectin/sodium alginate matrix.

Literature Cited

Assadpour, E., and S.M. Jafari. 2019. Advances in spray-drying encapsulation of food bioactive ingredients: From microcapsules to nanocapsules. Annu. Rev. Food Sci. Technol. 10:103–131.

Broadhead, J., S.K. Rouan, I. Hau, and C.T. Rhodes. 1994. The effect of process and formulation variables on the properties of spray-dried beta-galactosidase. J. Pharm. Pharmacol. 46(6):458–467.

Castro-Rosas, J., C.R. Ferreira-Grosso, C.A. Gomez-Aldapa, E. Rangel-Vargas, M.L. Rodriguez-Marin, F.A. Guzman-Ortiz, and R.N. Falfan-Cortes. 2017. Recent advances in microencapsulation of natural sources of antimicrobial compounds used in food—A review. Food Res. Int. 102:575–587.

Chegini, G.R., and B. Ghobadian. 2005. Effect of spray-drying conditions on physical properties of orange juice powder. Drying Techno., 23:657–668.

Correa-Filho, L. C., M. M. Lourenco, M. Moldao-Martins, and V.D. Alves. 2019. Microencapsulation of beta-carotene by spray drying: Effect of wall material concentration and drying inlet temperature. Intl. J. Food Sci. 2019:8914852.

Paulo, F., and L. Santos. 2017. Design of experiments for microencapsulation applications: A review. Mater Sci. Eng. C Mater Biol. Appl. 77:1327–1340.

Rosenberg, M., J. Kopelman, and Y. Talmon. 1990. Factors affecting retention in spray-drying microencapsulation of volatile materials. J. Agric. Food Chem. 38:1288–1294.

Shahidi, F., and X. Q. Han. 1993. Encapsulation of food ingredients. Crit. Rev. Food Sci. Nutr. 33(6):501–547.

Sun, X., R.G. Cameron, and J. Bai. 2019. Microencapsulation and antimicrobial activity of carvacrol in a pectin-alginate matrix. Food Hydrocolloids. 92:69–73.

Tonon, R.V., C.R.F. Grosso, and M.D. Hubinger. 2011. Influence of emulsion composition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying. Food Res. Int. 44:282–289.

Proc. Fla. State Hort. Soc. 132:185–188. 2019.

Optimizing Essential Oil Applications to Prevent Postharvest Decay In Strawberries

Anna Marín[1], Xiuxiu Sun[1], Marcela Miranda[1], Chris Ference[1], Elizabeth Baldwin[1], Jinhe Bai[1], Mark A. Ritenour[2], Jiuxu Zhang[2], and Anne Plotto[1]*

[1]*USDA–ARS, U.S. Horticultural Research Laboratory, 2001 South Rock Rd., Ft. Pierce, FL 34945*

[2]*Department of Horticultural Sciences, University of Florida, Indian River Research and Education Center, 2199 S. Rock Rd., Ft. Pierce, FL 34945*

Additional index words. active packaging, alginate, fungal decay, pectin, spray drying, thymol, whey protein isolate

Strawberry is a fragile commodity highly susceptible to postharvest decay. With antifungal activity, thymol is a potential candidate for postharvest decay prevention. A coating consisting of whey protein isolate (WPI) (1% w/v) with 0.05, 0.1 and 0.2% thymol, was sprayed on strawberries cv. Radiance using an airbrush. Fruit were air dried and repacked in 1-lb commercial clamshells. Another treatment consisted of sachets (pouches) containing 0.2, 0.4, and 0.8 g of spray-dried pectin with 11% thymol, attached to the lids of 1-lb clamshells. Fruit were stored at 7 °C for 9 d, then moved to 21 °C for 3 d. Taste panels were performed after 1, 5 and 8 d and decay incidence/severity recorded. None of the treatments reduced decay in comparison with the control (untreated) when fruit were stored at 7 °C. However, after 3 d stored at 21 °C, fungal decay was reduced by the 0.8 g sachet treatment. Off-flavor due to thymol was perceived in all WPI-treated fruit the day after coating application, while none of the fruit with sachet treatments had off-flavor. By day 8, strawberries packed with sachets in clamshells at all concentrations of thymol had better appearance than those treated with WPI coatings and control. Essential oils microencapsulated in a polysaccharide and packed in a sachet in a clamshell is a promising method to apply antimicrobials to fragile fruit such as strawberries.

Strawberry is one of the most consumed fruit in the United States, as reflected by the continuous increase in consumption, which amounted to about 8.34 pounds per capita in 2017 (USDA, 2018). Strawberries are considered an attractive commodity because of their high nutritional value and appealing sensory attributes (Shahbazi, 2018). However, they are highly perishable and susceptible to fungal spoilage, whose control relies mainly on the use of synthetic pesticides in the field. *Botrytis cinerea* and *Rhizopus* sp. are the most common fungi causing postharvest losses of strawberries (Badawy et al., 2017; Petrasch et al., 2019).

Consumers increasing demand for less synthetic pesticide use on farms, together with the emergence of pesticide-resistant fungal strains and stricter regulatory policies, have led to intensive search on more eco-friendly alternative methods based on the use of natural antimicrobials. In this context, essential oils (EOs) and their constituents have gained remarkable popularity owing to their widely demonstrated effectiveness in suppressing fruit fungal pathogens (Sivakumar and Bautista-Baños, 2014). EOs and their constituents to prevent postharvest decay of fruit may be applied by means of different strategies such as exposure to vapors or fumigation, incorporation into edible coatings (ECs) and release from active packaging. Examples of the application of these approaches on strawberries can be found in Aguilar-González et al. (2015), Badawy et al. (2017) and Campos-Requena et al. (2017). Thymol, the predominant monoterpenoid of thyme EO, is one of the most studied natural antifungal substances owing to its demonstrated effectiveness against *B. cinerea*, among other fungal pathogens (Sivakumar and Baustista-Baños, 2014). One of the disadvantages of EOs and their constituents is their high volatility, which can result in a rapid decrease in concentration in the packaging and as a consequence, a decrease in their effectiveness. Furthermore, some EOs present a strong smell that can have a deleterious effect on fruit flavor, especially if applied at high concentrations, which is usually necessary for antifungal activity. For this reason, when using these antifungal EOs, it is necessary to determine the most appropriate application method as well as the concentration that allows for an effective fungal control without modifying the sensory profile of the fruit.

In the present study, the control of strawberry fungal decay was evaluated by the use of thymol. Two strategies were tested: (a) incorporation into an EC based on whey protein isolate (WPI) and applied by spraying onto the fruit; and (b) encapsulation in pectin-alginate microcapsules incorporated into sachets or pouches attached to the packaging containing the fruit. A recommendation was provided as to which method of application combined with thymol concentrations is the best compromise for strawberry fungal decay control.

This research was supported in part by a USDA/FDACS Florida Specialty Crop Block Grant #024845.

Mention of a trademark or proprietary product is for identification only and does not imply a guarantee or warranty of the product by the U.S. Department of Agriculture. The U.S. Department of Agriculture prohibits discrimination in all its programs and activities on the basis of race, color, national origin, gender, religion, age, disability, political beliefs, sexual orientation, and marital or family status.

*Corresponding author. Email: anne.plotto@usda.gov

Material and Methods

Materials. Strawberries (*Fragaria* × *ananassa*, cultivar 'Radiance') were harvested on 27 Nov. 2018 in Dover, FL, brought to the USDA Horticulture Research Laboratory (Fort Pierce, FL) and stored overnight at 5 °C until treatment application. Whey protein isolate (WPI) was obtained from The Isopure Company LLC (Downers Groove, IL). Citrus pectin (≥ 74%), sodium alginate, thymol and Tween 80 were purchased from Sigma Aldrich (St. Louis, MO).

Preparation of WPI coating solutions. WPI coating solutions were prepared at 1 % (w/v) by dissolving the protein in sterile deionized water and stirring for 90 min at 30 °C. Upon cooling, thymol was incorporated into WPI solutions at different concentrations [0.05, 0.10, and 0.20 % (w/v)]. Because of its low water solubility, thymol was first mixed with Tween 80 (ratio of thymol to Tween 80 1:1), then the mixture was added to the coating solutions, which were homogenized at 12,000 rpm for 4 min (PRO Scientific, Oxford, CT, USA).

Preparation of pectin–alginate microcapsules by spray-drying. solution of 3% (w/v) pectin-alginate was prepared by mixing both polymers in distilled water using ratio of pectin to sodium alginate 1:1 (w/w), and kept overnight at room temperature (20 °C) by gently shaking to achieve full hydration of the biopolymer molecules. One gram of thymol was mixed with 5 g of Tween® 80, and the mixture was slowly introduced by dripping into 100 mL of the biopolymer blend solution. Emulsification was done with a Bio-Den Series Pro200 homogenizer (PRO Scientific, Oxford, CT,) at 12,000 rpm for 4 min. The emulsion was spray-dried using a model B-290 Mini Spray Dryer (BUCHI Corporation, New Castle, DE) with a two-fluid nozzle consisting of a 1.5 mm diameter nozzle cap with a 0.7 mm diameter nozzle tip hole. The operating parameters were set according to previous research (Sun et al., 2019).

Application of thymol based treatments. Strawberries were selected as uniform in size and maturity (neither very ripe nor green) and with no signs of mechanical damage or decay. Strawberries were placed on stainless steel racks, well separated from each other, in a room at 7 °C, and sprayed for 3 seconds with the coating solutions using a Paasche VL-100D airbrush system (Paasche Airbrush Company, Kenosha, WI, USA). After drying for 1 h, they were placed in 1-lb modified humidity clamshells with 0.44% opening ratio (Packaging Plus, Yakima, WA). Such opening ratio, 0.44% versus 2.83% for commercial clamshells, usually reduces fruit water loss (Bai et al., 2019), but in this experiment, it was intended to maintain the volatiles without completely closing the container to avoid an anaerobic environment. For the treatments consisting of thymol encapsulated in pectin-alginate microcapsules, 0.2, 0.4, or 0.8 g of powder with 11% of thymol were placed in Miracloth (Millipore, Billerica, MA) pouches or "sachets" (SAC) which were attached to the clamshells lids. Clamshells were stored at 7 °C for 9 d and then moved to 21 °C. Assessments were performed after 1, 5, 8, and 10 d. Each clamshell contained 20 strawberries and there were 7 treatments, including control samples with no treatment.

Sensory evaluation. After 1, 5, and 8 d of storage, strawberries were washed, cut into quarters, and placed on plates identified with 3-digit codes. Fifteen to thirty panelists participated in the sensory assessments. Panelists were asked to rate samples for strawberry flavor and off flavor intensities using a 0–10 scale, with the words "low", "medium", and "high" at anchoring the scale. Overall liking was also asked using the 1–9 hedonic scale (Meilgaard et al., 2007).

Decay incidence and severity. Incidence was visually evaluated by counting the number of fruits showing symptoms of fungal decay. Severity was estimated as the percentage of strawberry surface affected by mold using a 1–4 scale were 1 = 0 to 25%, 2 = 26 to 50%, 3 = 51 to 75% and 4 = >75%.

Microbial counts. Microbial assessment was evaluated following our previous method (Sun, et al., 2014) at the end of the storage at 7 °C and after 3 days at 21 °C. Briefly, 10 fruit samples from each replicate were agitated for 1 h in 99 mL of 0.01 M sterile phosphate buffer (pH 7.2) on an orbital shaker, and plate count agar (PCA), potato dextrose agar (PDA), and nutrient agar (NA) were used for enumerating total bacteria, yeast, and mold.

Statistical analysis. Decay incidence data were analyzed by a one-way analysis of variance (ANOVA) with XLSTAT v. 2018.5 (Addinsoft, New York, NY) and a least significant difference (LSD) test was used to identify significant differences between samples ($P < 0.05$). Severity of decay non-parametric data were analyzed by Kruskal-Wallis due to the ordinal level of the variable and independent samples using R Core Team v. 3.6.2-2019 (Vienna, Austria) and package "agricolae" (Mendiburu, 2020). Significance was defined at $P < 0.05$. Sensory data were analyzed using SenPAQ v. 5.01 (Qi Statistics, Reading, UK). A one-way ANOVA was performed for each sensory attribute within each storage date using a mixed model where "panelists" are random and the main effect is tested against the interaction (Panelist × Sample). Fisher's LSD multiple comparison test ($P < 0.05$) was used for means separation.

Results and Discussion

Sensory evaluation. Due to the strong aroma of thymol, its application within a spray dried matrix consisting on pectin and alginate (Sun et al., 2019) that enables a slower release of the compound was proposed and compared to its direct application on the fruit in a coating matrix. Sensory evaluation results are shown in Fig. 1. Because thymol flavor was so strong, samples coated with the formulation containing 0.20% of thymol were not tasted on day 1 and 5. On day 1, strawberries packed with 0.40 g and 0.80 g of pectin alginate powder containing encapsulated thymol in sachets (0.40-SAC and 0.80-SAC, respectively) had the highest strawberry flavor, followed by 0.20 g (0.20-SAC). No differences between fruit packed with 0.20 g of encapsulated thymol, coated with WPI-0.05 and control were detected by panelists for strawberry flavor. Likewise, little off flavor was perceived in control, encapsulated thymol in sachets (all concentrations) and WPI-0.05. However, samples coated with 0.10% of thymol had the lowest strawberry flavor and highest off flavor ($P < 0.001$). Off-flavor had an average rating of 5.3 (on a 0–10 intensity scale) in the fruit coated with 0.10% thymol, and some panelists were able to identify the presence of thymol by describing an herbal taste in theses samples. On day 5, control samples had the highest strawberry flavor scores, compared to strawberries packed with sachets containing thymol, while no differences were perceived among coated samples and between coated samples and control. After 8 d, non-treated strawberries were again found to have high strawberry flavor, as well as strawberries packed with 0.40 g encapsulated thymol and those coated with 0.05 and 0.10 % thymol. As storage progressed, the high off flavor perceived in

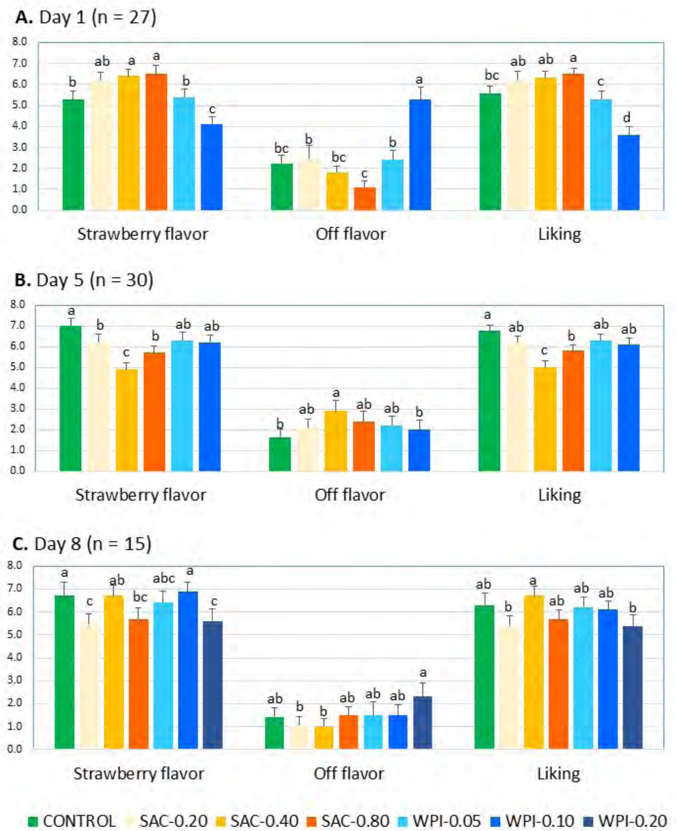

Fig. 1. Sensory evaluation [intensity scale from 0 (none) to 10 (high)] of strawberries placed in clamshells with sachets (SAC) containing 0.2, 0.4, or 0.8 g of encapsulated thymol (11%) in pectin alginate, or coated with whey protein isolate (WPI) containing thymol at 0.05, 0.10, or 0.20%, and tasted after 1, 5, and 8 d in storage at 5 °C. Bars with a different letter are significantly different using a least significant difference (LSD) test ($P < 0.05$). Strawberries coated with WPI containing 0.20% thymol were not tasted at day 1 and day 5 because of strong thymol flavor.

WPI-0.10 coated fruit decreased and no statistically significant differences were perceived by panelists between WPI-0.10 and any of the samples. Overall, lower ratings for off flavor were given after 8 days, indicating the loss of thymol over time. However, fruit coated with 0.20% thymol still had some residual off flavor compared to other treatments (2.3 for WPI-0.20 versus <1.5 for the other treatments including control). Degree of liking also was consistent with the two other sensory attributes. At day 1, coated strawberries were given the lowest liking ratings (5.3 for WPI-0.05 and 3.6 for WPI-0.10), and no significant differences were perceived in the rest of the samples, whose liking ratings ranged between 5.6 and 6.5. After 5 and 8 d, no statistical significant differences were found among samples, and only SAC-0.40 was given a significantly lower rating at day 5, in agreement with its lower strawberry flavor and higher off flavor. Application of thymol incorporated into ECs caused an initial negative impact on strawberries sensory profile because of its direct contact with the fruit. However, when encapsulated in a powder placed inside clamshells, a slower release might have taken place and the loss in sensory quality was minimized. In line with our results, Campos-Requena et al. (2017) incorporated thymol into a nanocomposite film for active packaging strawberries and did not observe alteration of their organoleptic characteristics. Sangsuwan et al. (2016), who obtained chitosan beads loaded with several EOs, reported similar results with lavender EOs, while red thyme oil failed the panelists acceptance. Most of the published studies using thymol incorporated into ECs on strawberries do not include sensory assessment, but other work using other EOs constituents can be found: working with alginate and pectin coatings enriched with citral and eugenol, Guerreiro et al. (2015) reported that panelists acceptance was clearly influenced by the concentration of active compound; and Perdones et al. (2012) described a decrease in judges preference for strawberries coated with chitosan and lemon EO. These results demonstrate that not only the type of EO and/or its constituents but also the application method play an important role in their sensory compatibility with fruit.

Decay control. In in vitro preliminary studies, thymol showed effective activities against *Botrytis cinerea* and *Rhizopus stolonifer* which are the major postharvest fungal pathogens on Florida strawberries (data not shown). In vivo fungal decay of strawberries treated with thymol, expressed as incidence and severity of infection (% of strawberry surface affected by fungal decay), is summarized in **Fig. 2**. Since after 9 d in cold storage no signs of fungal decay development were observed in any of the samples, including control, clamshells were moved to 21 °C. After three days at 21 °C, all samples presented high levels of fungal growth and decay, which was mainly identified as *B. cinerea* and *Pestalotia longidetula*. Incidence values ranged from 90 to 100 % and only SAC-0.80 had less incidence (87.9%, $P < 0.05$)

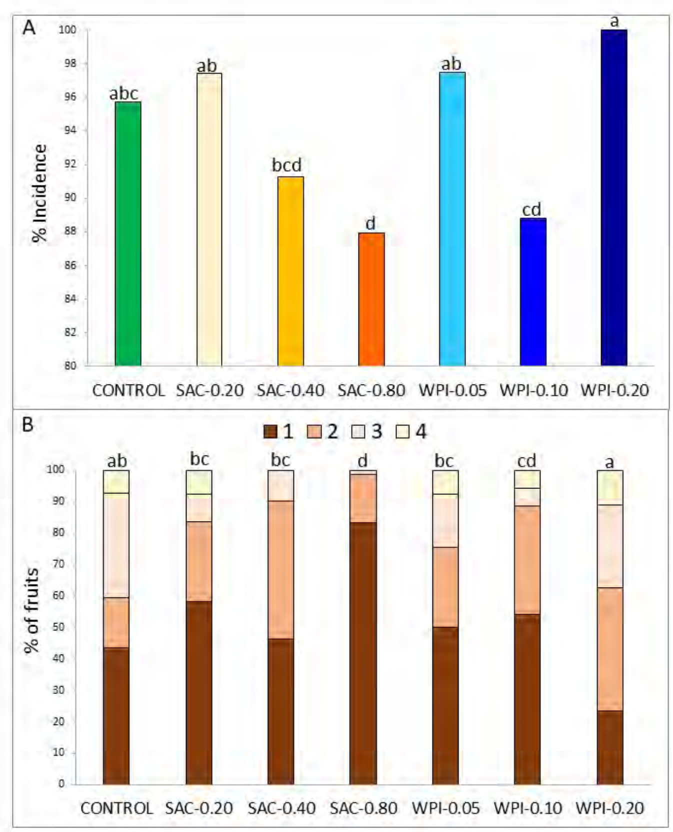

Fig. 2. Disease incidence (**A**) and severity (**B**) on strawberries placed in clamshells with sachets (SAC) containing 0.2, 0.4, or 0.8 g of encapsulated pectin alginate with 11% thymol, or coated with whey protein isolate (WPI) containing thymol at 0.05, 0.10, or 0.20%, after 9 d of storage at 7 °C followed with 3 d at 21 °C. Severity scale (% fruit surface with mold): 1 = 0 to 25%, 2 = 26 to 50%, 3 = 51 to 75%, 4 = > 75%.

than the untreated control (95.7%) (Fig. 2A). When strawberries were exposed to encapsulated thymol (SAC), a clear dose effect of active compound was observed, with lower decay incidence at higher concentration. However, the concentration effect when thymol was applied into ECs was inconsistent. The highest concentration (WPI-0.20) gave rise to 100% incidence that was not significantly different from that of WPI-0.05 (97.5%) and the control. In addition, these samples presented high levels of infection severity and the highest percentage of fruits in severity group 4 (more than 75% of surface affected) (Fig. 2B). The highest thymol concentrations in coatings possibly were phytotoxic to the fruit surface and hence favored fungal infection. In fact, we observed that the strawberry calices treated with coatings had darker green color, also indicating a possible phytotoxic effect of thymol. Phytotoxicity on fruit surface may lead to damage of the cell wall barrier and cell membrane leakage, creating a port of entry for fungal hyphae and making strawberries more sensitive to pathogen growth. Samples treated with coatings with intermediate thymol concentration (WPI-0.10) showed lower severity of infection, with most of the fruit in severity level 1. Among samples stored with encapsulated thymol, SAC-0.80 had 83% of fruit with the lowest severity, mostly at level 1, and no fruit at severity level 4 (Fig. 2B). Low severity levels (1, 2, and 3) were also observed with SAC-0.40 and SAC-0.20 but overall were not significantly different from control, while control samples exhibited greater levels of incidence and had 33% of fruit with severity level 3. Hence, SAC-0.80 encapsulated thymol significantly reduced fungal decay incidence at 21 °C, followed by WPI-0.10. Microbial counts on PDA, PCA or NA media were inconsistent and lower than 1200 $mL^{-1}\cdot g^{-1}$ cfu. Treatments with 0.8 g microencapsulated thymol powder (SAC-0.80) as well as WPI-0.05 and WPI-0.2 had lower overall microbial count than control (data not shown).

In conclusion, thymol encapsulated in a spray dried pectin-alginate matrix can be a feasible antimicrobial to be used in a controlled-release active food packaging system for fresh strawberries. In addition to low impacts on fruit flavor, this system shows several advantages compared to the application of thymol into ECs, such as less fruit handling and reduced risks of cross contamination. The slow diffusion of volatiles in the packaging avoids phytotoxicity to the fruit and enables a prolonged antifungal effect over time.

Literature Cited

Aguilar-González, A.E., E. Palou, and A. López-Malo. 2015. Antifungal activity of essential oils of clove (*Syzygium aromaticum*) and/or mustard (*Brassica nigra*) in vapor phase against gray mold (*Botrytis cinerea*) in strawberries. Innov. Food Sci. Emerg. Technol. 32:181–185.

Badawy, M.E.I., E.I. Rabea, M.A.M. El-Nouby, R.I.A. Ismail, and N.E.M. Taktak. 2017. Strawberry shelf life, composition, and enzymes activity in response to edible chitosan coatings. Int. J. Fruit Sci. 17(2):117–136.

Bai, J., E. Baldwin, E. Tsantili, A. Plotto, X. Sun, L. Wang, M. Kafkaletou, Z. Wang, J. Narciso, W. Zhao, S. Xu, C. Seavert, and W. Yang. 2019. Modified humidity clamshells to reduce moisture loss and extend storage life of small fruits. Food Packaging and Shelf Life 22:100376.

Campos-Requena, V.H., B.L. Rivas, M.A. Pérez, C.R. Figueroa, N.E. Figueroa, and E.A. Sanfuentes. 2017. Thermoplastic starch/clay nanocomposites loaded with essential oil constituents as packaging for strawberries—In vivo antimicrobial synergy over *Botrytis cinerea*. Postharvest Biol. Technol. 129:29–36.

Guerreiro, A.C., C.M.L. Gago, M.L. Faleiro, M.G.C. Miguel, and M.D.C. Antunes. 2015. The use of polysaccharide-based edible coatings enriched with essential oils to improve shelf-life of strawberries. Postharvest Biol. Technol. 110:51–60.

Meilgaard, M.C., G.V. Civille, and B.T. Carr. 2007. Sensory evaluation techniques. 3rd ed. CRC Press, Boca Raton, FL.

de Mendiburu, F. 2020. Agricolae: Statistical Procedures for Agricultural Research. R package version 1.3-2. <https://CRAN.R-project.org/package=agricolae>

Perdones, A., L. Sánchez-González, A. Chiralt, and M. Vargas. 2012. Effect of chitosan—Lemon essential oil coatings on storage-keeping quality of strawberry. Postharvest Biol. Technol. 70:32–41.

Petrasch, S., S.J. Knapp, J.A.L. Van Kan, and B. Blanco-Ulate. 2019. Grey mould of strawberry, a devastating disease caused by the ubiquitous necrotrophic fungal pathogen *Botrytis cinerea*. Mol. Plant Pathol. 2019:1–16.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical.Computing, Vienna, Austria. <https://www.R-project.org/>

Sangsuwan, J., T. Pongsapakworawat, and P. Bangmo. 2016. Effect of chitosan beads incorporated with lavender or red thyme essential oils in inhibiting *Botrytis cinerea* and their application in strawberry packaging system. LWT Food Sci. Technol. 74:14–20.

Shahbazi, Y. 2018. Application of carboxymethyl cellulose and chitosan coatings containing *Mentha spicata* essential oil in fresh strawberries. Int. J. Biol. Macromol. 112:264–272.

Sivakumar, D., and S. Bautista-Baños. 2014. A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. Crop Prot. 64:27–37.

Sun, X., J. Bai, C. Ference, Z. Wang, Y. Zhang, J. Narciso, and K. Zhou. 2014. Antimicrobial activity of controlled-release chlorine dioxide gas on fresh blueberries. J. Food Prot. 77:1127–1132.

Sun, X., R.G. Cameron, and J. Bai. 2019. Microencapsulation and antimicrobial activity of carvacrol in a pectin-alginate matrix. Food Hydrocoll. 92:69–73.

U.S. Department of Agriculture, Economic Research Service. 2018. Fruit and Tree Nuts Outlook. Table A-1D. 13 May 2019. <https://www.ers.usda.gov/data-products/fruit-and-tree-nut-data/fruit-and-tree-nut-yearbook-tables/#Berries>

Proc. Fla. State Hort. Soc. 132:189. 2019.

—Scientific Note—

Filling in the Gaps Toward Produce Safety Rule Implementation

Travis Chapin*[1], Matt Krug[2], Renee Goodrich[3], Keith Schneider[3], and Michelle Danyluk[1,3]

[1]*Citrus Research and Education Center, University of Florida/IFAS, 700 Experiment Station Rd., Winter Haven, FL 33850*

[2]*Southwest Florida Research and Education Center, University of Florida/IFAS, 2685 State Rd. 29 North, Immokalee FL 34142*

[3]*Food Science and Human Nutrition Department, University of Florida/IFAS, P.O. Box 110370, Gainesville, FL 32611*

Additional index words. food safety, FSMA, Produce Safety Rule

The Food Safety Modernization Act (FSMA) was signed into law in 2011 and granted the US Food and Drug Administration (FDA) authority to create seven new, foundational food safety regulations, including the Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption, commonly referred to as the Produce Safety Rule (PSR). Following public comment periods after the initial Proposed Produce Safety Rule (ca. 2013) and the Supplemental Proposed Rule (ca. 2014), the Final PSR was published in the Federal Register in 2015 and covers activities during growing, harvesting, and packing fruits and vegetables intended to be consumed raw. The PSR requirements are devoted to preventing microbial contamination and are specifically related to worker training, health, and hygiene; biological soil amendments of animal origin; wildlife and domesticated animals; agricultural water quality; sanitation; and recordkeeping. The PSR will be implemented on tens of thousands of diverse farms across the US and around the world. Based on data provided in the 2012 USDA National Agricultural Statistics Service Agricultural Census, the National Association of State Departments of Agriculture estimates there are 8467 farms in Florida that grow the types of fruits and vegetables covered by the PSR (unpublished). Due to several potential regulatory exemptions and exclusions, the total number of farm operations in Florida covered under the PSR is estimated at 3,92. The volume and diversity of farms that will need to implement PSR requirements necessitates that the PSR provide significant flexibility in how farmers may approach minimizing food safety risks in their operations, but individual farms have questions and concerns about how the new requirements can be achieved in various types of unique operations.

To address these challenges in Florida, the University of Florida/IFAS (UF/IFAS) Extension Produce Food Safety Team has been building capacity to provide outreach and technical assistance. The team offers a number of resources to assist Florida producers implement the PSR. One of the key requirements of the Produce Safety Rule is for at least one responsible person from every farm covered by the rule to attend a one-day food safety training recognized as adequate by FDA. The UF/IFAS, Florida Fruit & Vegetable Association, and Florida Department of Agriculture & Consumer Services (FDACS) have partnered with the Produce Safety Alliance (PSA) to offer a standardized food safety training that fulfills one of the PSR training requirements. The current team consists of 38 UF/IFAS Extension Agents and Specialists across the state who have met the requirements to be trainers or lead trainers of the PSA Grower Training curriculum. A total of 18 PSA trainings were conducted across Florida for 331 participants in 2019. The majority of these participants are from Florida and represented farms, packinghouses, and others directly involved in agriculture and food production. All of these participants received a Certificate from the Association of Food and Drug Officials for receiving the standardized food safety training recognized as adequate by the FDA that is required under the PSR. This helps primary producers in Florida achieve regulatory compliance with the mandated food safety training requirement, along with improving knowledge of on-farm food safety principles and practices. On post-workshop evaluations, 66% of participants (186/280) indicated that this was their first produce safety training. Eighty participants indicated that the buyers of their products have specifically requested compliance with the PSR. Ninety-five percent of respondents (289/303) self-reported that the level of PSR information provided in the training was sufficient to guide their efforts towards implementation of produce food safety requirements.

In order to offer an opportunity for more personalized technical assistance to farmers following their PSA training, UF/IFAS and FDACS have also partnered to offer on-site, voluntary visits called "On Farm Readiness Reviews" (OFRRs) to help interested farmers assess their readiness for PSR regulatory inspections. Combined, the outreach provided through PSA training and OFRRs provide the initial foundation for strengthening the food safety culture across the Florida fruit and vegetable production industry. These efforts help ensure that the nearly $432 billion of citrus and other fruits, nuts, vegetables, and melons grown and shipped from Florida each season will continue to meet escalating market-driven and regulatory food safety standards and contribute to a safe supply of fresh fruits and vegetables in the market.

*Corresponding author. Email: tkchapin@ufl.edu

Proc. Fla. State Hort. Soc. 132:190–191. 2019.

—Scientific Note—

Establishing a Scientific Basis for Buffer Zones Following Animal Intrusion

Matthew Krug*[1], Eugene McAvoy[2], Travis Chapin[3], Lorrie Freidrich[3], Min Li[4], Arie Havelaar[4,5], and Michelle Danyluk[3,6]

[1]*University of Florida/IFAS Southwest Florida Research and Education Center, 2685 SR 29 N, Immokalee, FL 34142*

[2]*University of Florida /IFAS Hendry County Extension, 1085 Pratt Blvd, LaBelle, FL 33935*

[3]*University of Florida /IFAS Citrus Research and Education Center, 700 Experiment Station Rd. Lake Alfred, FL 33850*

[4]*University of Florida /IFAS Emerging Pathogens Institute and Department of Animal Sciences, 2055 Mowry Rd., Gainesville, FL 32610*

[5]*University of Florida /IFAS Institute for Sustainable Food Systems, Gainesville, FL*

[6]*University of Florida /IFAS Department of Food Science and Human Nutrition*

Additional index words. cross-contamination, *Escherichia coli*, salmonella

Wild animals can carry human pathogens in their feces and can spread that contamination by depositing feces as they move through a field. The Food Safety Modernization Act (FSMA)'s Produce Safety Rule (PSR) (USFDA, 2015) highlights the need to reduce risks associated with wild animal intrusion into fresh produce fields and specifies a requirement to not harvest produce with fecal material present. While the requirements of the PSR seem straight forward, growers must also consider if there was the potential for spread of the fecal material to a wider area and determine if a no-harvest buffer zone around the contamination is necessary or sufficient to reduce the risk. The PSR does not require no-harvest buffer zones be established, however buffer zones between 0–25 ft are highlighted as an effective means of minimizing the cross-contamination risk from fecal contamination in a produce field. Requirements in the Florida Tomato Good Agricultural Practices (FDACS, 2008) simply state "Wild animal presence cannot be excluded but shall be minimized to the degree possible by methods identified by wildlife experts." There is no mention of establishing a buffer zone for tomatoes. Other industry metrics (LGMA, 2011; PrimusGFS, 2015) specifically require a five-foot buffer zone around the point of the fecal contamination. When these metrics are applied to tomatoes, they may include not only the tomato plant where the feces have been deposited, but also adjacent plants. No scientific basis is given for determining the size of an appropriate buffer zone and currently no published literature exists to aid Florida tomato growers to make this determination to ensure food safety. The objective of this study was to determine microbial dispersal due to wild animal fecal deposits on or near tomato plants in commercial tomato fields.

*Corresponding author. Email: mkrug@ufl.edu

Over the 2018–19 Florida tomato season, 40 animal intrusion events were investigated in commercial tomato fields. Samples collected from bird dropping events (n = 38) included fruit or leaves with visible droppings; fruit from the contaminated plant sampled < 30 cm below, < 30 cm above, > 30 cm below, or > 30 cm above the point of visible contamination; and fruit from adjacent plants. The remaining events (n = 2) were found in the row (> 30 cm from tomatoes) and assumed to be mammalian; the fecal samples were collected along with fruit from adjacent plants. All samples were evaluated for aerobic plate count (APC), coliforms, generic *Escherichia coli E. coli*, and salmonella. Control fruit were collected from a plant ~25 ft away that had no visible contamination.

Salmonella was not isolated from any sample collected in the study (n = 341). Populations of APC (4.75–6.66 log CFU/g) and coliforms (3.40–4.84 log MPN/g) were not significantly different between sample groups and the controls ($P > 0.05$). Populations of generic *E. coli* were below the limit of detection (< 1 MPN/sample) in 92.3% of samples where there was no visible contamination present (n = 274). There was no evidence in the two observed events that contamination from mammalian droppings in rows was transported to tomatoes on adjacent plants in the field.

The low frequency of generic *E. coli* and *Salmonella* indicate that these microbial populations are not dispersed in high numbers by bird droppings in the fields evaluated. These data suggest that a 5-ft buffer zone for fecal contamination in Florida tomato fields may be more stringent than necessary. The requirements of FSMA's Produce Safety Rule, not harvesting visibly contaminated fruit, should adequately minimize risk from potential dispersal of pathogens while at the same time minimizing potential economic impacts resulting from excessive no-harvest buffer zones around an instance of animal intrusion and droppings.

Literature Cited

California Leafy Green Products Handler Marketing Agreement (LGMA). 2011. Commodity specific food safety guidelines for the production and harvest of lettuce and leafy greens. <http://www.lgma.ca.gov/wp-content/uploads/2014/09/California-LGMA-metrics-08-26-13-Final.pdf>.

Florida Department of Agriculture and Consumer Services (FADCS). 2008. Tomato best practices manual (TGAPs). <http://www.freshfromflorida.com/fs/TomatoBestPractices.pdf>.

PrimusGFS. 2015. PrimusGFS audit GAP (Module 2) guidelines V2.1-2, Edition v1.0. <http://www.primusgfs.com/PDFs/PrimusGFSGuidelinesv2.1-2_GAPModule2.pdf>

United States Food and Drug Administration. 2015. Title 21 of the Code of Federal Regulations, Parts 11, 16, and 112 -Standards for the growing, harvesting, packing, and holding of produce for human consumption; final rule. <https://www.gpo.gov/fdsys/pkg/FR-2015-11-27/pdf/2015-28159.pdf>

Proc. Fla. State Hort. Soc. 132:192. 2019.

—Scientific Note—

The Use and Stability of Monk Fruit Plant-derived Sweetener In a Prototype Orange Juice Beverage

Zhou Zou and Renée Goodrich-Schneider*

University of Florida/IFAS, Food Science & Human Nutrition Department, PO Box 110370, Gainesville, FL, 32611

Additional Index words. monk fruit; orange juice beverage; plant-based sweetener

Monk fruit (*Siraitia grosvenorii*) is a cucurbitaceous edible herb widely planted in China, which produces high-potency sweeteners increasingly popular in the food industry as additives in low-calorie drinks or foods. Mogroside V is a cucurbitane triterpenoid saponin and the major bioactive constituent of monk fruit, which is approximately 400-times sweeter than sucrose. Use of a high-potency sweetener combined with orange juice, water, and peel oil can potentially produce an orange juice-containing beverage with a different nutritional profile (fewer kilocalories and fewer carbohydrates) than 100% orange juice, while still providing awareness of Florida citrus. This study aims to evaluate the stability of mogroside V after thermal processing and storage and to demonstrate the applicability of a monk fruit extract (as a commercial source of mogroside V) in orange juice beverages.

The stability of pure mogroside V in citric acidified model systems (pH 3.5 and 5.0) was evaluated chemically after two heat treatment regimens and during subsequent shelf-life storage over 90 days. Processing methods and storage conditions were chosen to encompass the typical shelf life of orange juice products. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to monitor the chemical degradation of these pure mogroside V compounds. In this study, prototype orange juice beverages were developed using not-from-concentrate, single-strength orange juice and commercial monk fruit extract. Pasteurized samples and commercial products were compared by sensory evaluation. The result demonstrated that mogroside V was generally stable under the conditions tested and when evaluated with respect of product pH, two thermal processing times/temperatures, and storage time. Sensory testing with commercial products and a prototype formula indicated the prototype, monk fruit-sweetened product was acceptable to customers in terms of overall liking, sweetness, and orange flavor ratings.

The thermal stability of monk fruit extract under typical juice beverage processing and storage conditions will be validated in other juice systems, including juices of interest to Florida agriculture including peach, strawberry, and other citrus juices such as tangerine.

*Corresponding author. Email: goodrich@ufl.edu

Proc. Fla. State Hort. Soc. 132:193. 2019.

—Scientific Note—

Control of Strawberry Postharvest Decay Caused by *Botrytis cinerea* and *Rhizopus stolonifer* Using Essential Oils (Carvacrol and Thymol)

Jingjing Kou[1], Jiuxu Zhang[1], Tian Zhong[2], Xiuxiu Sun[3], Jeffrey K. Brecht[4], Steven A. Sargent[4], Anne Plotto[3], Jinhe Bai[3], and Mark A. Ritenour*[1]

[1]*University of Florida/IFAS Indian River Research and Education Center, 2199 Rock Rd., Ft Pierce, FL 34945*

[2]*School of Pharmacy and Food Science, Zhuhai College of Jilin University, China, 510940*

[3]*USDA–ARS, U.S. Horticultural Research Laboratory, 2001 South Rock Rd., Fort Pierce, FL 34945*

[4]*University of Florida/IFAS Horticultural Sciences Department, P.O. Box 110690, Gainesville, FL 32611-0690*

Additional index words. *Fragaria* ×*ananassa*, disease, vapor phase, fruit quality

Strawberry (*Fragaria* ×*ananassa* Duch.) is one of the most commonly consumed and appreciated fruits worldwide. Florida is the major winter domestic supplier of fresh-market strawberries. During shipping and storage, strawberries are highly perishable and susceptible to fungal pathogen attack. *Botrytis cinerea* and *Rhizopus stolonifer* are the two major storage fungal pathogens causing strawberry decay and fruit loss. To develop a new safe, effective and biodegradable method to control postharvest decay on strawberries, essential oils (EOs) have been extensively studied in recent years. In the current study, the in vitro antifungal activities of thymol and carvacrol were determined on potato dextrose agar (PDA), and in vivo decay control was evaluated on fresh strawberry fruit.

For the in vitro study, thymol and carvacrol were evaluated for their contact (incorporated into PDA) and volatile phase (applied to filter paper) antifungal capacities against *B. cinerea* (gray mold) and *R. stolonifer* (soft rot or leak) isolated from diseased strawberries. Application as a volatile was more effective than contact against fungal mycelial growth. Thymol vapor at concentrations of 10 $mg\cdot L^{-1}$ and 20 $mg\cdot L^{-1}$, respectively, completely killed *B. cinerea* and *R. stolonifer* in vitro. The concentration of carvacrol vapor required to completely kill both fungal pathogens was 50 $mg\cdot L^{-1}$. Thymol or carvacrol incorporated into PDA were fungicidal at 100 $mg\cdot L^{-1}$ for *B. cinerea* and 200 $mg\cdot L^{-1}$ for *R. stolonifer*.

For the in vivo study, strawberries inoculated with *B. cinerea* or *R. stolonifer* were exposed to carvacrol or thymol vapors (100, 300, and 500 $mg\cdot L^{-1}$) at room temperature (21 to 22 °C) for 24 or 48 h. Carvacrol vapors at 300 $mg\cdot L^{-1}$ for 24 h significantly reduced the incidence and severity of *B. cinerea* in inoculated strawberries by 43% and 61%, respectively, and reduced the incidence and severity of *R. stolonifer* by 58% and 69%, respectively. Thymol vapors at 300 $mg\cdot L^{-1}$ for 24 h significantly reduced the incidence and severity of *B. cinerea* by 46% and 68%, respectively, and incidence and severity of *R. stolonifer* by 47% and 65%, respectively. In addition, both EOs at 300 $mg\cdot L^{-1}$ for 48 h did not significantly affect the fruit quality characteristics firmness, total soluble solids, titratable acidity, and weight loss, but did increase calyx browning.

This research confirms the antifungal effects of carvacrol and thymol both in vitro and in vivo on fresh strawberries. Overall results indicate that carvacrol and thymol suppressed fungal pathogen infection, and *B. cinerea* and *R. stolonifer* development on strawberries. The potential practical application of essential oils might be a new commercial strategy for the control of postharvest fungal decays of strawberry resulting in prolonged shelf life.

*Corresponding author. Email: ritenour@ufl.edu

Proc. Fla. State Hort. Soc. 132:194. 2019.

—Scientific Note—

The Effect of Chlorine Dioxide Gas on Postharvest Preservation of Citrus Fruit During Ethylene Degreening

Zhike Zhang[1], Cuifen Hu[2], Wilmer Chu[2], Tian Zhong[3], Jiuxu Zhang[2], Jingjing Kou[1], and Mark A. Ritenour[2]*

[1]*College of Horticulture, South China Agricultural University, Guangzhou, PR. China, 510642*

[2]*University of Florida/IFAS Indian River Research and Education Center, 2199 S. Rock Rd., Fort Pierce, FL 34945*

[3]*School of Pharmacy and Food Science, Zhuhai College of Jilin University, China, 510940*

[4]*College of Horticulture, Agricultural University of Hebei, PR. China*

Additional index words. decay, ClO_2, 'Early-pride' and 'Tango' mandarin, 'Ray Ruby' grapefruit, 'Valencia' orange, fruit quality

Chlorine dioxide (ClO_2) is a strong oxidant often used as a broad-spectrum disinfectant in a variety of applications. This study evaluated the potential for gaseous ClO_2 application during ethylene degreening to control postharvest decay of four varieties of *Citrus*. Separate experiments were used to test fruit from 'Early-pride' and 'Tango' mandarin, 'Ray Ruby' grapefruit, or 'Valencia' oranges. The fruit did not receive any fungicide treatments after harvest. For each experiment, fruit of one variety were randomly placed within modified 44.6-L containers and exposed to one of four conditions with three replicates per condition. Chlorine dioxide gas was generated from ClO_2 slow- or fast-release product (ICA TriNova, Newnan, GA), where each product came as two dried media (A and B) – ClO_2 gas was released when equal amounts of A and B were mixed and reacted with each other. Chlorine dioxide treatments are expressed as grams of slow- or fast-release product per kilogram of fruit. The media was placed within a sachet and mixed at the beginning of each experiment and the sachets were affixed to the underside of each lid. No additional product was added afterward. The chambers were sealed, but two 40 mm ports were installed, one each on opposite side walls of the container. A 40 mm fan was affixed to one port and a hose was affixed over that with the end routed outside the room to slowly draw atmosphere out of the chamber and out of the room so a ClO_2 treatment in one container did not affect other treatments. The ports and fan allowed for a small negative pressure within each chamber to continually draw room air into the chambers. In these experiments, most chambers were placed within continuous degreening conditions containing 5 ppm ethylene, while control chambers were held in similar conditions (temperature and relative humidity) but without ethylene. An additional fan affixed on a middle side wall of each container facilitated air circulation within each chamber.

Fruit of 'Early Pride" placed within degreening conditions at 30 °C with 95% RH but also exposed to 0.5 g/kg of slow-release or 1.25 g/kg of fast-release ClO_2 product each developed significantly less decay than fruit exposed to degreening conditions alone. The specific type of decay was not identified but diplodia stem-end rot (*Lasiodiplodia theobromae*) was most commonly observed. While trends were apparent after 4 d when decay incidence was less than 3%, significant reductions in decay became apparent for the slow-release product after 6 d, and after 10 d for the fast-release product. Between 10 and 14 d (end of observations), there were no significant differences between the non-degreened control (no ethylene) and the slow- or fast-release products, which all had significantly less decay than fruit exposed to degreening conditions without ClO_2. Depending on the variety and time of year, citrus fruit are normally exposed to no more than 1–4 d degreening, but the excessive durations were used in the current experiments to promote greater decay to better show significant differences. Exposing citrus fruit to ethylene during degreening is known to induce decay and it appears the ClO_2 treatments compensated for the ethylene exposure. While there was a clear and significant delay in color development in fruit that were not exposed to ethylene, the ClO_2 treatments were not significantly different from the ethylene-treated control. The only potentially negative effect of the ClO_2 treatments was a significant increase in 'Early Pride' fruit weight loss compared to both controls; the reason for this is unclear and this was usually not observed with the other varieties. Overall, tests with the other varieties also showed both ClO_2 products generally significantly reduced fruit decay compared to the control, but there were no significant differences with 'Valencia', likely because decay pressure was so low that the fruit generally desiccated before decaying.

*Corresponding author. Email: ritenour@ufl.edu

Proc. Fla. State Hort. Soc. 132:195. 2019.

—Scientific Note—

A Slow-release Chlorine Dioxide Gas Treatment Can Reduce Postharvest Decay of Fresh Strawberries

Jiuxu Zhang[1], Jingjing Kou[1], Tian Zhong[2], Jeffrey K. Brecht[3], Steven A. Sargent[3], Anne Plotto[4], Jinhe Bai[4], Xiuxiu Sun[4], and Mark A. Ritenour*[1]

[1]*University of Florida/IFAS Indian River Research and Education Center, 2199 S. Rock Rd., Ft. Pierce, FL 34945*

[2]*School of Pharmacy and Food Science, Zhuhai College of Jilin University, China, 510940*

[3]*University of Florida/IFAS Horticultural Sciences Department, P.O. Box 110690, Gainesville, FL 32611-0690*

[4]*USDA–ARS, U.S. Horticultural Research Laboratory, 2001 South Rock Rd., Fort Pierce, FL 34945*

Additional index words. *Fragaria ×ananassa*, ClO_2, disease, fruit quality, fruit shelf life

Strawberries are one of the most important specialty crops both in Florida and in the United States. Postharvest fungal decay is one of the major factors affecting fruit shelf life and losses of fresh strawberries. To develop safe, effective and applicable technologies for postharvest protection of strawberry fruit from decay, a chlorine dioxide (ClO_2)-based product was evaluated in a simulated clamshell packing and storage system. Strawberry fruit in clamshells were exposed to 0.5 g of a ClO_2 slow-release product (ICA TriNova, Newnan, GA) per clamshell. The product comes in two dried media (A and B) and the ClO_2 gas is released when A and B are mixed and react with each other. The product was contained within a sachet attached to the inside lid of each clamshell. Four clamshells were used for each treatment and each clamshell contained 15 fruit. The control consisted of clamshells without ClO_2. Fruit were stored at 5 °C for up to 10 d, then transferred to 21 to 22 °C for up to 6 d depending on the tests. Fruit decay incidence and severity, receptacle and calyx deterioration, and quality properties including weight loss, fruit color and firmness, total soluble solids (TSS), and titratable acidity (TA) were evaluated during the tests.

Over three separate experiments, ClO_2 at 0.5 g product/clamshell reduced overall fruit decay incidence by 25% to 46%, and decay severity by 30% to 52% after 5 °C and then 21 to 22 °C storage. Increasing the ClO_2 application rate from 0.5 to 1 g or 2 g per clamshell did not improve decay control compared to that of 0.5 g product/clamshell. The ClO_2 treatment of 0.5 g product/clamshell did not affect strawberry weight loss, fruit or calyx color, fruit firmness, TSS or TA over time. While the ClO_2 product at 1 g or 2 g per clamshell did not cause phytotoxic fruit receptacle injury, calyx browning was sometimes observed.

The results of these studies suggest that the ClO_2 slow-release product may have good potential to be developed for applications in commercial strawberry packing and delivery conditions to reduce postharvest strawberry fruit decay and prolong fruit shelf life.

*Corresponding author. Email: ritenour@ufl.edu

Proc. Fla. State Hort. Soc. 132:196–200. 2019.

Variation among Strawberry Cultivars in Bruising Susceptibility Related to Wound Ethylene Production and Sensitivity

Lan-Yen Chang and Jeffrey K. Brecht
Horticultural Sciences Department, University of Florida/IFAS, Gainesville, FL 32611

Additional index words. calyx yellowing, decay, *Fragaria* ×*ananassa*, mechanical injury, respiration, water soaking

***Abstract*. Bruising of strawberry (*Fragaria ×ananassa* Duch.) fruit is a common mechanical injury that reduces product value. Wound-induced ethylene may accelerate deterioration or decay, affecting strawberry quality and shelf life. However, bruising susceptibility varies among strawberry cultivars. In this study, cultivars Monterey, Sweet Sensation, Radiance, and two proprietary cultivars (Cultivar A and Cultivar B) from a private breeding program were investigated to evaluate their bruising susceptibility and wound response. Bruising consisted of dropping a 28-g steel ball from 27 cm onto individual fruit; unbruised fruit were the primary control, while fruit exposed to 1 $\mu L{\cdot}L^{-1}$ ethylene were used as a check for ethylene response. All fruit were stored at 20 °C, 90% relative humidity (RH), with respiration and ethylene production measured at 2-hour intervals for 24 hours. Appearance observations were recorded daily until decay onset. Peak respiration rates of 30–40 mL $CO_2{\cdot}kg^{-1}{\cdot}h^{-1}$ mostly occurred within 4 hours (‘Cultivar B’) to 6 hours (‘Cultivar A’ and ‘Sweet Sensation’) after bruising, except ‘Monterey’, which peaked at 60 mL $CO_2{\cdot}kg^{-1}{\cdot}h^{-1}$ in 2 hours, and ‘Radiance’, which reached 70 mL $CO_2{\cdot}kg^{-1}{\cdot}h^{-1}$ in 6 hours. Maximum ethylene production rates after bruising were 0.05 to 0.06 $\mu L{\cdot}kg^{-1}{\cdot}h^{-1}$ for ‘Monterey’, ‘Cultivar A’, and ‘Cultivar B’, 0.10 $\mu L{\cdot}kg^{-1}{\cdot}h^{-1}$ for ‘Sweet Sensation’, and 0.20 to 0.37 $\mu L{\cdot}kg^{-1}{\cdot}h^{-1}$ for ‘Radiance’. ‘Cultivar B’, with the lowest ethylene production, exhibited the lowest overall bruising severity, whereas ‘Radiance’, with the highest ethylene production, exhibited the most severe bruising-induced water soaking, and the other cultivars were intermediate, although ‘Monterey’ bruises were more discolored than those of the other cultivars. ‘Monterey’, ‘Radiance’, and ‘Sweet Sensation’ showed more yellowing and browning of the calyx in response to both bruising and ethylene exposure than ‘Cultivar A’ and ‘Cultivar B’. Except for ‘Cultivar B’, bruising and ethylene exposure increased decay severity.**

Strawberry (*Fragaria ×ananassa* Duch.), the third most consumed fresh fruit in the U.S. market in 2017 (USDA, 2019), is attractive and highly valued, but it is delicate. Large cells with thin cell walls result in the fragile structure of strawberry fruit (Szczesniak and Smith, 1969), which become more susceptible to mechanical injury as the ripening process proceeds. Strawberries are harvested when mostly or fully ripe according to the commercial standard for U.S. No. 1 grade, which states that strawberry fruit must be at least 3/4 red (USDA, 2006). Bruising occurs mainly during harvesting, packing, and transportation for horticultural crops (Prussia and Shewfelt, 1993). When plant tissues are wounded, the physical and metabolic reactions change in the damaged tissues and trigger ethylene production, resulting in major postharvest losses, decay, and accelerated senescence, thus affecting strawberry quality and shelf life (Ferreira et al., 2009; Wills and Kim, 1995). The severity of wounding in strawberry depends on various factors, including the type of force applied on the fruit, cooling method, and rate of lowering pulp temperatures, along with the storage temperature (Ferreira et al., 2009). Internal fruit properties, such as texture, fruit maturity, water content, firmness, size, and shape (Hung and Prussia, 1989; Van Linden et al., 2006), and the nature of cultivars (Jiménez-Jiménez et al., 2013; Kunze et al., 1975) also contribute to the bruising response. Bruising severity and the tissues being affected can be determined by dissecting the fruit and measuring the bruise diameter, depth, and volume, which have strong positive correlations with impact energy (Schoorl and Holt, 1980).

Mechanical injury, such as cutting, abrasion, or bruising, has been shown to cause climacteric fruits such as banana and tomato to produce an increasing amount of ethylene (Moretti et al., 1998; Palmer and McGlasson, 1969). However, strawberry fruit is often regarded as a nonclimacteric and ethylene-insensitive crop. In previous research, some researchers have suggested that ethylene could not only stimulate the respiration rate of nonclimacteric fruit, but also accelerate fruit color development, softening, and ion leakage, with a deleterious logarithmic linear response to 10 to <0.005 $\mu L{\cdot}L^{-1}$ ethylene in terms of shelf life (Wills and Kim, 1995; Wills and Wong, 1996; Wills et al., 1999). In contrast, other researchers have presented evidence showing little or no effect of ethylene on strawberries (El-Kazzaz et al., 1983; Tian et al., 2000). The role of ethylene in nonclimacteric fruit wounding response is uncertain, and the differential response toward ethylene in previous studies was reported to be affected by maturity, storage time, and cultivar characteristics (El-Kazzaz et al., 1983; Picón et al., 1993; Tian et al., 2000).

In tests to determine the potential benefits of removing ethylene from the strawberry postharvest environment that were conducted before the present research (Brecht et al., 2016), we observed significant cultivar variation in response to ethylene scrubbing in terms of bruising severity and calyx yellowing and browning. We hypothesized that those differences in response to ethylene scrubbing might be related to differences in wound ethylene production or ethylene sensitivity among the cultivars that had been tested. Therefore, in this study, several major commercial strawberry cultivars, including Monterey, Sweet Sensation (Florida 127), Florida Radiance, and two proprietary cultivars (Cultivar A and Cultivar B) from a private breeding program, were investigated to determine their bruising susceptibility and wound response in terms of timing and the rate of wound ethylene production to inform further improvements in postharvest procedures.

Materials and Methods

Plant material. Strawberry fruit of the University of California licensed cultivar Monterey (received 24 Oct. 2017), two cultivars licensed by the University of Florida, namely Sweet Sensation (Florida 127) (Sweet Sensation) (received 11 Dec. 2017) and Florida Radiance (Radiance) (received 21 Mar. 2018), and two proprietary cultivars, Cultivar A (4 Oct. 2017) and Cultivar B (11 Dec. 2017), were received from distribution centers of retailers at the beginning of the harvest season for each variety and immediately transported to the University of Florida Postharvest Horticulture Laboratory in Gainesville by air-conditioned van at ≈18 °C within 2 h. Five separate experiments were conducted. Additional experiments conducted with ‘Radiance’ (once) and ‘Sweet Sensation’ (twice) showed similar results and are not presented here. On arrival at the laboratory, fruit were stored at

Received for publication 15 Nov. 2019. Accepted for publication 3 Jan. 2020.
Published online 25 February 2020.
We thank It’s Fresh Ltd. for their support by providing research funding for this work.
J.K.B. is the corresponding author. E-mail: jkbrecht@ufl.edu.

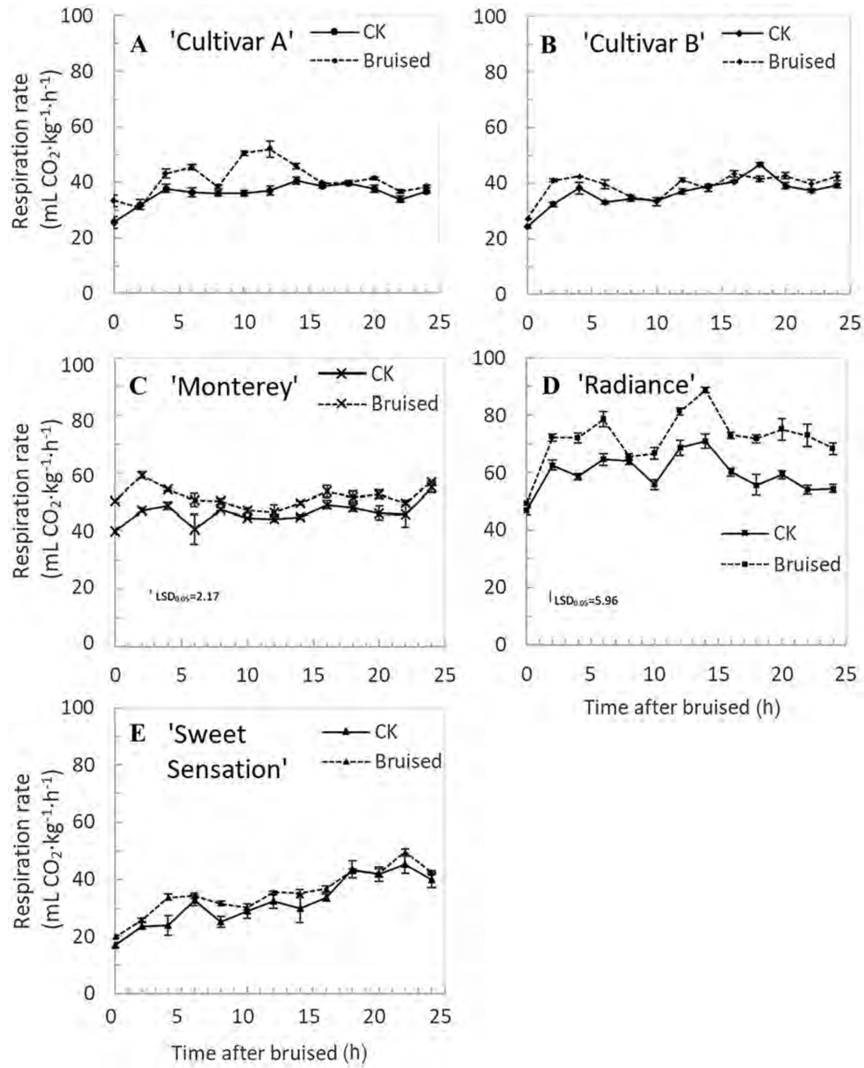

Fig. 1. Time course within 24 h for the respiration rate of (**A**) 'Cultivar A', (**B**) 'Cultivar B', (**C**) 'Monterey', (**D**) 'Radiance', and (**E**) 'Sweet Sensation' fruit with control (CK, solid line) and bruised (Bruised, dash line) fruit during storage at 20 °C and 95% relative humidity. Each data point represents the mean of three observations. Vertical bars represent SE.

1 °C overnight to reduce the disturbance of the physiological response during transportation. Fruit were rewarmed to ambient temperature (24 °C) the following day and fully red fruit, selected for uniform color, size, and freedom from defects, were grouped into three replicate groups of similar weight per cultivar (900 g for 'Cultivar A', 800 g for 'Monterey', and 650 to 670 g for 'Sweet Sensation', 'Radiance', and 'Cultivar B').

Treatments. To examine whether the wound response in strawberry fruit involves ethylene, bruised fruit (BR), fruit treated with 1 $\mu L \cdot L^{-1}$ ethylene (ETH), and untreated fruit as control (CK) were used in the experiments. Bruising treatment followed the procedure of Ferreira et al. (2008) with modifications to deliver the same impact energy to the fruit. A 28-g steel ball was released from a height of 26.67 cm within a plastic tube directed to the proper impact point on individual fruit at 24 °C on the fullest part of the side of the fruit; the applied impact energy was ≈0.74 J. Then, the BR fruit and the unbruised treatments (CK and ETH) were collected in rigid plastic clamshell containers as experimental units and stored at 20 °C. For ethylene application, three experimental units were placed in a 20-L closed plastic bucket at 20 °C and ventilated with air with ≈95% to 100% RH containing 1 $\mu L \cdot L^{-1}$ ethylene for 24 h. The other three unbruised units were regarded as CK. The CK and BR treatments were placed in separate ventilated chambers at 20 °C with humidity ≈95% to 100% RH. The ETH treatment was transferred to identical ventilated chambers after the 24-h ethylene application for further observation.

Assessment of respiration and ethylene production. Containers of BR and CK fruit were sealed for 1 h at each 2-h interval during the first 24 h of each experiment. Ethylene and carbon dioxide concentrations were determined by injecting a 3-mL sample of headspace into a Varian CP-3800 gas chromatograph (Varian Inc., Walnut Creek, CA) equipped with a pulsed discharge helium ionization detector (PDHID) and a thermal conductivity detector (TCD). Using an automated sample-loop and valve system, a 1-mL portion of the injected sample for ethylene determination passed through Hayesep Q Ultimetal (1 m × 3.18 mm) [particle size, 149–177 µm (80/100 mesh)] and Hayesep Q Ultimetal (1 m × 3.18 mm) [particle size, 149–177 µm (80/100 mesh)] columns (Varian) coupled in series to the PDHID. Another portion (1 mL) of the injected sample for CO_2 determination passed through Hayesep Q Ultimetal (1 m × 3.18 mm) [particle size, 149–177 µm (80/100 mesh)] and Molsieve 13 (1.5 m × 3.18 mm) [particle size, 149–177 µm (80/100 mesh)] columns (Varian) coupled in series to the TCD. The carrier gas (helium) flow rate was 0.25 $mL \cdot s^{-1}$. The injector and oven were operated at 220 °C and 50 °C, respectively. The PDHID was operated at 120 °C and the TCD was operated at 130 °C.

Assessment of wound response. At 24 h after treatment, the bruise diameters of each fruit were measured from edge to edge on two perpendicular axes with a caliper and averaged. Defects of BR, CK, and ETH strawberry fruit, including the bruised area on BR fruit, water soaking or darker color of bruised areas, calyx degreening, and decay were photographed and evaluated for each fruit at 24 h after treatment and daily until the onset of decay using the incidence rates of the defects. Evaluations of bruising symptoms for CK and ETH treatments were performed for incidentally bruised areas that were not evident during initial sorting because those fruit had not been intentionally bruised like the BR fruit.

Statistical analysis. Carbon dioxide and ethylene production rate data over time were analyzed by a one-way repeated measure analysis of variance (ANOVA) of each cultivar, and bruise diameters among cultivars were analyzed by one-way ANOVA with the JMP Pro 14.1.0 (SAS Institute, 2019) software and Microsoft Excel (version 1910; Microsoft Office 365; Microsoft, Redmond, WA). Fisher's least significant differences ($P \leq 0.05$) were determined to compare differences between treatment means following a significant ANOVA effect. Mean data are presented (±SEM).

Results and Discussion

Respiration and ethylene production during the first 24 h after treatment among different cultivars. Bruising treatment stimulated respiration of all cultivars. Respiration rates for each cultivar differed (Fig. 1), with 'Radiance' having the highest rate, followed by 'Monterey', 'Cultivar A', and 'Cultivar B'; 'Sweet Sensation' had the lowest rate. Peak respiration rates of 34.14 and 45.38 mL $CO_2 \cdot kg^{-1} \cdot h^{-1}$ occurred at 6 h after bruising for 'Sweet Sensation' and 'Cultivar A', respectively, and 42.47 mL $CO_2 \cdot kg^{-1} \cdot h^{-1}$ occurred at 4 h after bruising for 'Cultivar B'. Peak respiration rates after bruising for 'Monterey'

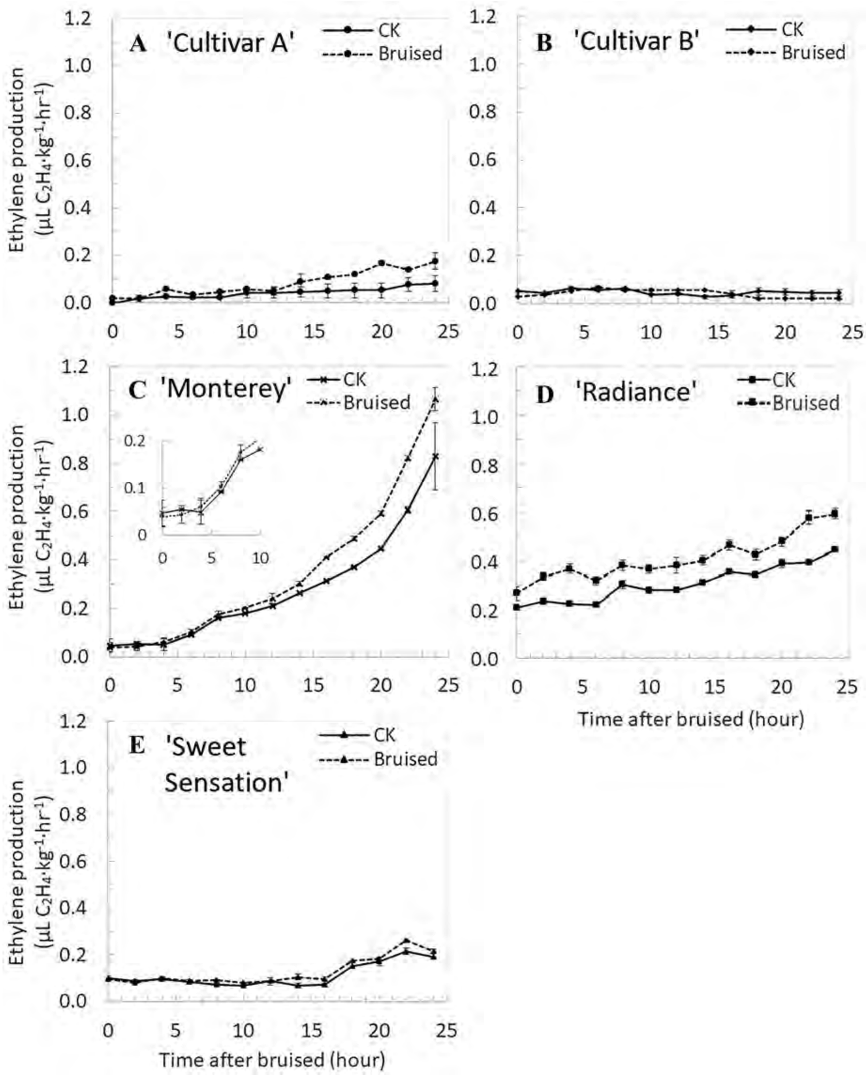

Fig. 2. Time course within 24 h for the ethylene production rate of (**A**) 'Cultivar A', (**B**) 'Cultivar B', (**C**) 'Monterey', (**D**) 'Radiance', and (**E**) 'Sweet Sensation' fruit with control (CK, solid line) and bruised (Bruised, dash line) fruit during storage at 20 °C and 95% relative humidity. Each data point represents the mean of three observations. Vertical bars represent SE.

Table 1. Bruise diameters of 'Cultivar A', 'Cultivar B', 'Monterey', 'Radiance', and 'Sweet Sensation' stored at 20 °C and 95% relative humidity measured 24 h after bruising.

Cultivar	Bruise diam (mm)
Cultivar A	12.34 ± 0.55 a[z]
Cultivar B	8.98 ± 0.22 c
Monterey	10.55 ± 0.39 b
Radiance	9.10 ± 0.20 c
Sweet Sensation	9.03 ± 0.26 c
$LSD_{0.05}$	1.34

[z]Means followed by the same letter in the column are not significantly different according to the least significant difference (LSD) test ($P > 0.05$). n = 15 fruits.

(59.58 mL $CO_2 \cdot kg^{-1} \cdot h^{-1}$ in 2 h) and 'Radiance' (78.63 mL $CO_2 \cdot kg^{-1} \cdot h^{-1}$ in 6 h) were higher than those for the other cultivars. Respiration rates of 'Radiance' and 'Monterey' were also significantly higher than those for the other cultivars over the entire 24 h after treatment.

Ethylene production was slightly higher in BR fruit in most cultivars except for Cultivar B, which maintained relatively low ethylene production in both BR and CK (Fig. 2). Among the cultivars, ethylene production was highest for 'Radiance', followed by 'Sweet Sensation' and 'Cultivar A', 'Cultivar B', and 'Monterey'. Maximum ethylene production after bruising occurred at ≈4 h, up to 0.060 $\mu L \cdot kg^{-1} \cdot h^{-1}$ in 'Monterey', 0.056 $\mu L \cdot kg^{-1} \cdot h^{-1}$ in 'Cultivar A', 0.066 $\mu L \cdot kg^{-1} \cdot h^{-1}$ in 'Cultivar B', 0.10 $\mu L \cdot kg^{-1} \cdot h^{-1}$ in 'Sweet Sensation', and 0.37 $\mu L \cdot kg^{-1} \cdot h^{-1}$ in 'Radiance'. There were different numbers of fruit of each cultivar in each replicate due to variations in fruit size, which meant there were different numbers of applied bruises per replicate. Therefore, we also calculated the respiration and ethylene production rates on a per fruit basis, but this did not reveal any differences in the relative rates for the bruised vs. unbruised treatments or between the cultivars (data not shown).

The ethylene production after 12 h for 'Cultivar A', 'Monterey', 'Sweet Sensation', and 'Radiance' increased up to 2- to 10-fold, presumably due to the development of decay caused by gray mold (*Botrytis cinerea*). Infection of *B. cinerea* has been reported to accelerate the ethylene production by the affected host plant tissues and by the fungus itself, with ethylene promoting decay of nearby tissues or fruit (Chague et al., 2002; Qadir et al., 1997).

Tian et al. (2000) indicated that enhanced respiratory rate in response to exogenous ethylene exposure depended on the ethylene concentration and the treatment duration, with fruit treated with 1 $\mu L \cdot L^{-1}$ ethylene for 2 d observed to produce more CO_2. Even though ethylene production rates for 'Radiance' and 'Monterey' in the present work were comparatively higher than those for the other cultivars, it did not appear to affect the respiration rate. However, ethylene and CO_2 production rates of 'Sweet Sensation' increased synchronously after 16 h (Figs. 1C and 2C), which might have been due to greater sensitivity to ethylene in that cultivar.

Comparison of appearances among different treatments within each cultivar. Bruise diameters measured at 24 h after bruising were significantly larger for 'Cultivar A' and 'Monterey' compared with 'Cultivar B', 'Radiance', and 'Sweet Sensation', which were not significantly different from each other (Table 1).

Defects among cultivars and treatments were categorized into four major symptoms, including decay, calyx yellowing or browning, fruit with water soaking, and fruit with darker damaged areas (Table 2). Decay was mostly represented by Botrytis fruit rot (gray mold), which manifested the symptoms of light brown spots or gray fuzzy coating on the fruit surface. Decay developed more rapidly in 'Cultivar A', 'Monterey', and 'Radiance' than in 'Cultivar B' and 'Sweet Sensation'. Fruit of 'Cultivar B' had the least amount of fruit infected by the fungus, whereas 'Monterey' and 'Radiance' were more susceptible.

The growth of *B. cinerea* was reported to be accelerated by 20 $\mu L \cdot L^{-1}$ ethylene (El-Kazzaz et al., 1983). 'Radiance' produced the most ethylene among the cultivars, and the BR fruit maintained ethylene production at higher rates than the CK fruit. After the initial wound ethylene production following bruising, an additional increase in ethylene production started after 12 h, which was associated with accelerated fungal growth. Initial ethylene production of 'Monterey' was relatively low, at ≈0.03 $\mu L \cdot kg^{-1} \cdot h^{-1}$, but the production increased starting from the sixth hour, with BK fruit producing more ethylene than CK fruit, which may have been related to enhanced growth of the fungus. However, neither bruising treatment nor ethylene exposure consistently promoted decay in the strawberry cultivars tested (Table 2). 'Cultivar B', which did not exhibit increased ethylene production in response to bruising, also did not exhibit increased decay in either the BR or the ETH treatments, whereas 'Sweet Sensation' showed increased decay

Table 2. Severity ratings of strawberry defects after bruising or ethylene treatment evaluated at the onset of decay.

Cultivar	Days before decay onset	Major defects[z]			
		Decay	Calyx yellowing/browning	Fruit water soaking	Fruit with darker damage area
Cultivar A					
Control	2	1	2	2	1
Bruised (0.74 J)		3	3	3	2
Ethylene (1 ppm)		3	3	2	2
Cultivar B					
Control	3	2	2	1	1
Bruised (0.74 J)		2	2	2	1
Ethylene (1 ppm)		2	3	2	1
Monterey					
Control	2	3	2	1	2
Bruised (0.74 J)		4	4	2	3
Ethylene (1 ppm)		4	4	2	3
Radiance					
Control	2	3	2	2	2
Bruised (0.74 J)		4	4	4	1
Ethylene (1 ppm)		4	4	2	1
Sweet Sensation					
Control	3	2	4	0	1
Bruised (0.74 J)		4	5	1	1
Ethylene (1 ppm)		2	5	0	2
Least significant difference at $P = 0.05$		0.71	0.64	1.29	0.50
Cultivar		****	****	****	****
Treatment		****	****	*	**
Cultivar × treatment		**	NS	NS	****

[z]The proportion of total fruit presenting symptoms: 1: 0% to 20%; 2: 21% to 40%; 3: 41% to 60%; 4: 61% to 80%; 5: 81% to 100%. n = 3.
NS, *, **, ***, ****Nonsignificant or significant at $P = 0.05$, 0.01, 0.001, or 0.0001, respectively, according to the F-test.

in response to bruising, but not to ethylene exposure. These results indicate that for some strawberry cultivars, the amount of ethylene required to accelerate the growth of *B. cinerea* might be lower than the 20 $\mu L \cdot L^{-1}$ ethylene reported previously by El-Kazzaz et al. (1983), but ethylene may have a small or no role in the development of Botrytis rot in other cultivars. However, the reduced levels of both wound ethylene production and decay observed in 'Cultivar B' suggest that the selection of strawberry germplasm with reduced wound ethylene production may represent an avenue for developing strawberry cultivars with reduced postharvest decay.

'Monterey' fruit had the most yellowing and browning of the calyx in the BR and ETH treatments, followed by 'Radiance' and 'Sweet Sensation' (Table 2). Cultivar A and Cultivar B exhibited less calyx yellowing and browning than the other cultivars. The occurrence of calyx yellowing or browning probably resulted from the breakdown of chlorophyll in response to ethylene, which was also observed by Bower et al. (2003), followed by leaf senescence.

The bruised area was evaluated in terms of two different defects: water soaking, in which damaged tissues were moist and translucent, and dry damaged area, in which there was dry and shrunken tissue. 'Radiance', followed by 'Cultivar A', had relatively more bruised fruit that developed water soaking, whereas 'Monterey' had more fruit with darker damaged areas than other cultivars.

Major defects observed in each cultivar in response to bruising were different. Decay, calyx yellowing/browning, and fruit with darker damaged areas were the major symptoms observed in 'Monterey'. For 'Radiance', decay, calyx yellowing/browning, and fruit with water soaking were the major symptoms, while 'Cultivar A' had more fruit with water soaking. Among all tested cultivars, Cultivar B exhibited the lowest bruising severity, whereas 'Monterey', 'Radiance', and 'Cultivar A' were more susceptible to bruising but responded with different symptoms. Saltveit (1997) described the first physical effects of wounding, which include removal of the protective epidermal layer, deposition of surface moisture (from the contents of broken cells), and exposure of the inner tissues to contaminants, including decay organisms. Water evaporation from the wound surface occurs later and the plant starts to respond physiologically to the wound. Surface water is first deposited and then lost as activation of healing proceeds. In this trial, most bruised fruit of 'Cultivar A' and 'Radiance' exhibited surface water in the damaged area where decay usually started. Other cultivars had more fruit with darker damaged areas where decay randomly spread. The healing processes activated after wounding among these cultivars were different and were presumably affected by the earlier wound response.

It would seem plausible that relative differences in wound ethylene production among strawberry cultivars may be, at least partly, a result of differences in the amount of damaged tissue resulting from the application of the same force. However, our results indicated that the cultivars with larger bruises (i.e., 'Cultivar A' and 'Monterey') had lower rates of wound ethylene production, suggesting that there are inherent genetic differences among these cultivars regarding wound ethylene production capacity.

In conclusion, bruising susceptibility varied among strawberry cultivars in terms of their responses, namely, respiration rate, ethylene production, and the phenotypic wound defects after being bruised. 'Monterey', 'Radiance', and 'Cultivar A' were more susceptible to bruising than 'Sweet Sensation' and 'Cultivar B', and they showed more ethylene-enhanced symptoms, including darker color or more severe water soaking at the injured area, or yellowing/browning of the calyx compared with unbruised CK fruit. 'Cultivar B', with the lowest ethylene production, also exhibited the lowest bruising severity, calyx yellowing/browning, and decay, whereas 'Radiance', with the highest ethylene production, exhibited the most severe bruise water soaking and among the most severe calyx yellowing/browning and decay.

Literature Cited

Bower, J.H., W.V. Biasi, and E.J. Mitcham. 2003. Effect of ethylene and 1-MCP on the quality and storage life of strawberries. Postharvest Biol. Technol. 28:417–423.

Brecht, J.K., S.A. Sargent, D.J. Huber, and R. Suthar. 2016. Efficacy of It's Fresh! palladium ethylene scrubber in reducing ethylene and extending strawberry quality. ISHS VIII Intl. Strawberry Symp., Quebec City, Quebec, Canada, 16 Aug. (abstr.).

Chague, V., Y. Elad, R. Barakat, P. Tudzynski, and A. Sharon. 2002. Ethylene biosynthesis in *Botrytis cinerea*. FEMS Microbiol. Ecol. 40:143–149.

El-Kazzaz, M.K., N.F. Sommer, and R.J. Fortlage. 1983. Effect of different atmospheres on postharvest decay and quality of fresh strawberries. Phytopathology 73:282–285.

Ferreira, M.D., S.A. Sargent, J.K. Brecht, and C.K. Chandler. 2008. Strawberry fruit resistance to simulated handling. Sci. Agr. (Piracicaba, Braz.) 65:490–495.

Ferreira, M.D., S.A. Sargent, J.K. Brecht, and C.K. Chandler. 2009. Strawberry bruising sensitivity depends on the type of force applied, cooling method, and pulp temperature. HortScience 44:1953–1956.

Hung, Y.C. and S.E. Prussia. 1989. Effect of maturity and storage time on the bruise susceptibility of peaches (cv. 'Red Globe'). Trans. ASAE 32:1377–1382.

Jiménez-Jiménez, F., S. Castro-García, and J.A. Gil-Ribes. 2013. Table olive cultivar susceptibility to impact bruising. Postharvest Biol. Technol. 86:100–106.

Kunze, D.R., W.H. Aldred, and E.D. Reeder. 1975. Bruising characteristics of peaches related to mechanical harvesting. Trans. ASAE 18:939–945.

Moretti, C.L., S.A. Sargent, D.J. Huber, A.G. Calbo, and R. Puschmann. 1998. Chemical composition and physical properties of pericarp, locule and placental tissues of tomatoes with internal bruising. J. Amer. Soc. Hort. Sci. 123:656–660.

Palmer, J.K. and W.B. McGlasson. 1969. Respiration and ripening of banana fruit slices. Austral. J. Biol. Sci. 22:87–99.

Picón, A., J.M. Martínez-Jávega, J. Cuquerella, M.A. Del Río, and P. Navarro. 1993. Effects of precooling, packaging film, modified atmosphere and ethylene absorber on the quality of refrigerated Chandler and Douglas strawberries. Food Chem. 48:189–193.

Prussia, S.E. and R.L. Shewfelt. 1993. Systems approach to postharvest handling, p. 44–71. In: R.L. Shewfelt and S.E. Prussia (eds.). Postharvest handling: A systems approach. Academic Press, San Diego, CA.

Qadir, A., E.W. Hewett, and P.G. Long. 1997. Ethylene production by *Botrytis cinerea*. Postharvest Biol. Technol. 11:85–91.

Saltveit, M.E. 1997. Physical and physiological changes in minimally processed fruits and vegetables, p. 205–220. In: F.A. Tomaìs-Barberaìn and R.J. Robbins (eds.). Phytochemistry of fruit and vegetables. Oxford University Press, Oxford, UK.

Schoorl, D. and J.E. Holt. 1980. Bruise resistance measurements in apples. J. Texture Stud. 11:389–394.

Szczesniak, A.S. and B.J. Smith. 1969. Observation on strawberry texture: A three-pronged approach. J. Texture Stud. 1:65–89.

Tian, M.S., S. Prakash, H.J. Elgar, H. Young, D.M. Burmeister, and G.S. Ross. 2000. Responses of strawberry fruit to 1-methylcyclopropene (1-MCP) and ethylene. Plant Growth Regulat. 32:83–90.

U.S. Department of Agriculture (USDA). 2006. United States Standards for grades of strawberries. 10 Nov. 2019. <https://www.ams.usda.gov/sites/default/files/media/Strawberry_Standard%5B1%5D.pdf>.

U.S. Department of Agriculture (USDA). 2019. Food availability (Per capita) data system: Fruit (fresh). 10 Nov. 2019. <https://www.ers.usda.gov/data-products/food-availability-per-capita-data-system/>.

Van Linden, V., N. Scheerlinck, M. Desmet, and J. De Baerdemaeker. 2006. Factors that affect tomato bruise development as a result of mechanical impact. Postharvest Biol. Technol. 42:260–270.

Wills, R.B.H. and G.H. Kim. 1995. Effect of ethylene on postharvest life of strawberries. Postharvest Biol. Technol. 6:249–255.

Wills, R.B.H. and T. Wong. 1996. Effect of low ethylene levels on the storage life of the Asian leafy vegetables Bak Choi (*Brassica chinensis*), Choi Sum (*Brassica parachinensis*) and Gai Lan (*Brassica alboglabra*). ASEAN Food J. 11:145–147.

Wills, R.B.H., V.V.V. Ku, D. Shohet, and G.H. Kim. 1999. Importance of low ethylene levels to delay senescence of non-climacteric fruit and vegetables. Austral. J. Exp. Agr. 39:221–224.

This paper was presented during the 2019 FSHS Annual Meeting and originally published in HortScience [HortScience 55(4):444–448. 2020. https://doi.org/10.21273/HORTSCI14733-19]. The paper is included in this Proceedings as a reprint (with permission).

Proc. Fla. State Hort. Soc. 132:201–202. 2019.

Bringing Extension Downtown

Kate Rotindo* and Edward A. Skvarch

University of Florida/IFAS, St. Lucie County Extension,
8400 Picos Rd., Suite 101, Fort Pierce, FL 34945

Additional index words. relationships, self-watering garden container, urban agriculture

In 2017 and 2018, the University of Florida/IFAS St. Lucie County Extension attempted to address the need to develop relationships with new, potential Extension advocates within the community. The revitalization of downtown business districts combined with the emergence of interest in locally produced products has yielded an increase in patrons to downtown businesses. These patrons are looking to be informed about local issues, goods, and services. University of Florida Extension faculty in St Lucie County created the "Sip and Sow" program to develop relationships with new potential Extension advocates within this downtown market. The program was designed to teach participants the benefits of container gardening through a hands-on demonstration project where participants were shown how to build a self-watering container garden out of a 5-gallon bucket. A relationship was developed with a local downtown micro-brewery to host the events, and the Extension agents held demonstration programs on multiple occasions. The program has reached 67 community members and agents plan on expanding this program in the future.

Increases in the interest of modern health mindfulness and urban food systems have spurred a growing interest in community and backyard gardening (Hou, 2014). Conversations and surveys were conducted by the local Extension faculty to examine interest in small-scale community and backyard gardening. The survey results have identified an expressed need, from community members, to learn methods that instruct them to grow their own produce. Participants were eager to have local backyard gardening resources available to them.

The practice of urban agriculture is spreading, and it has been embraced by government and civil society alike as a source of food, for its role in ecosystem services, and jobs, particularly in times of economic uncertainty (McClintock, 2010). As urban areas expand and encroach on production agricultural land, urban agriculture and community gardening will increase to supplement these important benefits as well as provide increased food security, and locally produced foodstuffs. Also, in urban areas, local businesses often seek out community involvement to show they are active participants in local communities and networking with Extension to provide educational programs helps to achieve this goal.

Materials and Methods

In 2017, the St. Lucie County Extension Urban Horticulture agent contacted Sailfish Brewing Company, a local brewery in downtown Fort Pierce, FL, to discuss bringing a container gardening program to be called "Sip and Sow" to their venue, specifically on the large outdoor front porch area that faces the middle of the main street in downtown. The Urban Horticulture agent and Commercial Horticulture agent brought all supplies needed to teach participants to build a 5-gallon, "bucket-style" self-watering garden container. The agents also supplied printed instructions, and vegetable or herb seedlings for participants to take home.

To increase participation of typical working families, the start time of the program was set for the evening, between 6:00 and 7:00 pm. Sailfish Brewing Company and St. Lucie County Extension agents used the social media platforms Facebook and Instagram to advertise and promote the program event dates and registration. Agents handled all pre-registration for event through Eventbrite, an online platform for organizers to register and charge fees for events. "On-site" registration was also accepted. All participants paid a $5 fee for the program.

Results and Discussion

To date, 67 community members have attended the "Sip and Sow" program (Fig. 1), with several of them reporting that they have built and used multiple self-watering bucket containers for

Fig. 1. University of Florida/IFAS St. Lucie County Urban Horticulture agent Kate Rotindo and Commercial Horticulture agent Edward Skvarch demonstrate container garden bucket at Sip and Sow program. Photo credit: K. Rotindo.

Acknowledgements. Many thanks to Sailfish Brewing Company and HopCat (Port St. Lucie) for being gracious hosts for the "Sip and Sow" program.

*Corresponding author. Email: krotindo@ufl.edu

vegetable gardening. New relationships were formed with community members who were previously unaware of the Extension Service, the programs provided, or available resources. The Extension agents plan to expand the program to other venues and incorporate other growing systems to teach participants. In future "Sip and Sow" programs, agents anticipate collecting more follow-up data to observe knowledge gained and reported behavioral changes.

Literature Cited

Hou, J. 2014. Making and supporting community gardens as informal urban landscapes, p. 79–96. In: Mukhija, V. and A. Loukaitou-Sideris (eds.). The Informal American City: Beyond Taco Trucks and Day Labor. MIT Press, Cambridge, MA.

McClintock, N. 2010. Why farm the city? Theorizing urban agriculture through a lens of metabolic rift. Cambridge J. Regions, Economy and Soc. 3(2):191–207. https://doi.org/10.1093/cjres/rsq005

Proc. Fla. State Hort. Soc. 132:203–204. 2019.

Rain Impacts Container Plant Irrigation

Thomas H. Yeager*

Department of Environmental Horticulture, University of Florida/IFAS, P.O. Box 110670, Gainesville, FL 32611

Additional index words. *Ilex cornuta* 'Burfordii Nana', overhead sprinkler irrigation

Producers of container nursery crops in Florida and other areas with large amounts of rain should consider strategies and management practices to utilize rain effectively. In this study, approximately 1.0 inch of irrigation was saved in 106 days when the CIRRIG irrigation control system used effective rain to compensate for plant water loss.

Water use by the nursery industry continues to be scrutinized and the scrutiny will likely increase in the future. Many nurseries are located near population centers that are experiencing rapid expansion. Hence, the competition for water resources has increased. Best management practices indicate that irrigation application amounts should be adjusted to match plant demand. The application should also include compensation for effective rain.

In Florida, the average rainfall is approximately 53 inches per year and nurseries in southwest locations of the state are often permitted for approximately 70 inches of irrigation per year. Thus, there is substantial rain to sustain and grow crops, but the distribution of rain is often ineffective or the amount of rain only partially effective for container plants that are usually irrigated daily. In addition, rain often occurs in the afternoons or evenings during summer in Florida and if automatic rain measuring devices are not integrated with irrigation control, the irrigation system will deliver an inappropriate amount of irrigation. An irrigation control system was developed [Container IRRIGation (CIRRIG)] that accounts for effective rain and outputs daily an irrigation operation duration (Million and Yeager, 2015a) considering plant need based on evapotranspiration and rain. The objective was to evaluate irrigation data from a CIRRIG zone and a producer-controlled irrigation zone to determine the impact of rain on irrigation applications.

Materials and Methods

Irrigation application amounts for two sprinkler irrigation zones with similar plants were compared at Hibernia Nursery in Webster, Florida. Hibernia Nursery staff controlled one irrigation zone and determined how much water to apply daily using a container substrate moisture rating protocol. Twice each week substrate was removed from random containers and substrate moisture rated, then irrigation operation times were determined and time clock manually adjusted and set to activate in early mornings. The other zone was controlled automatically with CIRRIG. CIRRIG used on-site hourly weather and leaching fraction results as inputs (Million and Yeager, 2019). On-site weather including rain was monitored with an Advantage Pro2 weather station (Davis Instruments, Hayward, CA). Leaching fraction tests were conducted by Hibernia Nursery staff (Million and Yeager, 2015b)

CIRRIG output of daily irrigation operation duration was transferred during early morning to a solenoid valve that controlled sprinkler irrigation. Irrigation applied to each of the approximately 0.56 acre zones was metered (McCrometer, Hemet, California). Meter readings were recorded manually approximately each week and readings converted to inches based on previous tests where irrigation was captured in pails distributed in each zone. Application amounts (inches/day) were calculated as the inches applied between meter readings divided by the number of days between readings.

The study was conducted for 106 days (Mar.–June 2016) with *Ilex cornuta* Lindl. & Paxton 'Burfordii Nana' grown in a pine bark (60%) and compost (40%) substrate amended with dolomitic limestone, controlled-release fertilizer, and micronutrients in spaced #3 containers. Plant height and two perpendicular widths for 20 plants in each zone were measured every 3 weeks. Initial plant heights plus average initial widths were 28 inches.

Results and Discussion

Plant growth data indicated that plants exhibited similar growth for CIRRIG and Hibernia zones (Table 1). Plant heights plus

Table 1. *Ilex cornuta* 'Burfordii Nana' size for plants irrigated with a CIRRIG irrigation control system or manually by Hibernia Nursery staff during Mar.–June 2016.

	Height + width, inches	
Day of experiment	CIRRIG	Hibernia
0	28	28
21	28	28
41	28	28
65	35	35
85	38	38
106	38	38

The author gratefully acknowledges the financial support of Southwest Florida Water Management District (project B404) and the assistance of Hibernia Nursery staff. Companies and products are mentioned for informational purposes only and do not constitute an endorsement.

*Corresponding author. Email: yeagert@ufl.edu

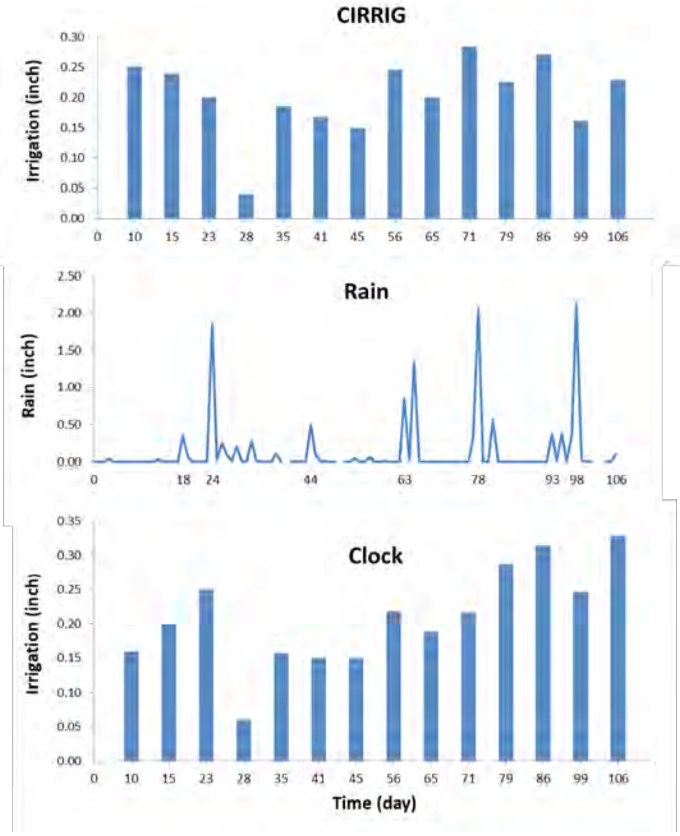

Fig. 1. Irrigation application amounts that resulted from a CIRRIG irrigation control system (top) and a manually operated nursery time clock (bottom) control systems are presented along with rain (center) that occurred during106 days (Mar.–June 2016) of *Ilex cornuta* 'Burfordii Nana' production at a central Florida nursery.

average widths for twenty plants from each zone ranged from 28 inches on day 0 to 38 inches on day 106.

Irrigation application amounts and rain amounts are presented in Fig. 1. Rain events larger than 1.5 inches occurred on days 24, 78, and 98. Another smaller, yet substantial event of 1.35 inches occurred on day 65. Other smaller events occurred periodically with events exceeding 0.2 inch occurring on days 18, 26, 29, 32, 44, 63, 77, 81, 93, 95, and 97. Thus, rain was distributed throughout the 106 days of production.

A large reduction in irrigation applied by CIRRIG and nursery staff occurred for days 24–28. This followed 1.85 inches and 0.26 inch on days 24 and 26, respectively. On day 44, there was a 0.5 inch rain event and concomitant decrease in irrigation for CIRRIG during days 42–45. However, it is interesting to note that nursery staff adjusting the clock did not reduce application amount and the amount applied (0.15 inch for days 42–45) was the same for both methods of controlling irrigation. On days 63 and 65, 0.85, and 1.35 inches of rain, respectively, occurred and both irrigation control methods exhibited a reduction in irrigation for this period (days 57–65) compared with the preceding period (days 46–56). On day 77, 0.3 inch of rain occurred and on day 78, 2.06 inches of rain occurred. However, with the nursery staff adjusting the clock more water was applied than with CIRRIG for days 72–79. On days 87–99, both methods of adjustment exhibited reductions compared to days 80–86. This reduction followed 3.25 inches of rain during days 93–98.

These data indicate the nursery was monitoring rain and usually reduced irrigation amount to compensate for rain. However, CIRRIG automatically determined effective rain and resulted in 0.9 inch less irrigation applied in 106 days. Assuming irrigation was applied for 320 days per year, this could equate to 2.7 inches of savings or additional water available. When a nursery is permitted for 70 inches per year, 2.7 inches is 3.8% of the permitted amount or approximately 13 days of water based on average application amounts of 0.21 inch/day for CIRRIG during the 106 days. That water could be very important, particularly when 70 inches annually might be inadequate for some crops.

Literature Cited

Million, J.B. and T.H.Yeager. 2015a. CIRRIG: Weather-based irrigation management program for container nurseries. HortTechnology 25(4):528–535.

Million, J. and T. Yeager. 2015b. Monitoring leaching fraction for irrigation scheduling in container nurseries. Extension fact sheet ENH 1268, University of Florida, IFAS, Gainesville, FL.

Million, J.B and T.H. Yeager. 2019. Testing an automated irrigation system based on leaching fraction testing and weather in a container nursery. HortTechnology 29(2):114–121.

Ornamental, Garden & Landscape Section

Proc. Fla. State Hort. Soc. 132:205–209. 2019.

Relationships between Leaf Chlorophyll Content, Fertilizer Application Rates and Visual Grades of Greenhouse-grown *Homalomena* 'Emerald Gem'

Kenneth J. Sweeney[1], Morgan Jones[2], Adam Cruz[2], Carolyn Cureton[2], Malcolm M. Manners[3], and John L. Griffis, Jr.*[4]

[1]*Biology Department, Florida Gulf Coast University, 10501 FGCU Blvd. S., Fort Myers, FL 33965*

[2]*Biology Department, Florida Southern College, 111 Lake Hollingsworth Dr., Lakeland, FL 33801*

[3]*Horticultural Science Department, Florida Southern College, 111 Lake Hollingsworth Drive, Lakeland, FL 33801*

[4]*Marine and Ecological Sciences Department, Florida Gulf Coast University, 10501 FGCU Blvd. S., Fort Myers, FL 33965*

Additional index words. daily light integral, DLI, foliage plants

***Homalomena* cultivars are relatively new plants in the foliage industry. Because there are no specific published growing recommendations for *H.* 'Emerald Gem', we set out to develop recommendations for successfully producing this plant in a greenhouse. Tissue cultured plantlets were potted up, placed in a greenhouse and grown under two light levels (30% and 60% shade) using three fertilizer rates. Over six months, we measured light intensity, temperature, chlorophyll content in leaves, and plant growth parameters (height, diameter, and number of shoots). We then calculated the daily light integral (DLI) for each light level. Plant quality was evaluated using a potential-purchaser survey. Plants produced under the higher light level at all fertilizer rates were larger than those produced under the lower light level. Leaf chlorophyll content and consumer purchase ratings were similar for all fertilizer rates within each light level. Based on the parameters measured during this trial, we can recommend production of *H.* 'Emerald Gem' at the higher light level and at the lower fertilizer rate.**

There are many reasons why researchers seek to evaluate the concentration of chlorophyll in leaves. Chlorophyll levels are related to overall visible plant stress, as well as abiotic factors such as light and water needs (Pinkard and Mohammed, 2006). The ability to quantify chlorophyll content allows researchers to gather valuable information about how plants are interacting with their environment (Coste et al., 2010). Additionally, thylakoid membrane nitrogen is proportional to the chlorophyll content to a first approximation (Evans, 1989). Nitrogen levels within the plant can be inferred by assessing chlorophyll content within leaves. Chlorophyll content within leaves is measured either by destructive or non-destructive methods. Destructive measurements involve collecting leaf samples, extracting chlorophyll with a solvent, and using spectrophotometric analysis (Wellburn, 1994). The non-destructive method involves using a meter to read absorbance, transmittance, or reflectance of a leaf without damaging it (Coste et al., 2010).

This study aimed to identify which light conditions and fertilizer treatments were most effective for commercial production of 'Emerald Gem' in a greenhouse setting. Prior to this study, light and fertilizer recommendations had not been established for *H.* 'Emerald Gem'. Florida produces over half of the country's indoor foliage plants (Chen et al., 2005), and 'Emerald Gem' has become somewhat popular among those. Since the foliage market is such a large part of Florida's horticultural economy, proper growth conditions should be established for the benefit of local foliage growers. This would allow growers to modify their current production practices in order to increase efficiency, lower production costs and minimize environmental pollution from fertilizer runoff.

Materials and Methods

Experimental design. Seventy-two tissue-cultured *Homalomena* 'Emerald Gem' plugs (Oglesby Plants International, Altha, FL) were potted up into 15.24 cm diameter standard pots on 21 Sept. 2018. A soilless mix of peat and perlite (Sunshine Mix #8, Sun Gro Horticulture, Agawam, MA) with additional nutrients (1/4 tsp/pot Soluble Trace Elements Mix, Everris 99900 Peters Professional S.T.E.M. Water Soluble Professional Fertilizer, Geldermalsen, The Netherlands) was distributed into 84 pots. Varying levels of 19–6–12 Osmocote fertilizer (Scotts Miracle-Gro Co., Marysville, OH) was added to the pots (Conover and Poole, 1990). The "Low"

*Corresponding author. Email: jgriffis@fgcu.edu

fertilizer rate (2.4 g³/pot/3 mo) was incorporated into the soilless mix in 28 pots. The same was done for the "Medium" rate (4.0 g³/pot/3 mo) and the "High" rate (5.7 g³/pot/3 mo). Twenty-four plugs were added to each group of 28 pots and the additional four pots were retained as controls with no plants. Each group of 24 was split in half and placed under inside-the-greenhouse structures covered with either 30% woven shade cloth or 60% woven shade cloth (Envirotech Greenhouse Solutions, Richmond, CA). The 12 replicates of each treatment grown under each light regime were randomly grouped into three rows of four plants in a split-plot design (Chang, 2008). This was repeated for all fertilizer levels, for a total of nine rows of plants growing under each light regime. A visual example of the setup within the 30% woven shade cloth treatment is shown in Fig. 1. The plants were measured bi-weekly and their height from the base of the plant to the tip of the tallest leaf was recorded in centimeters. In Dec. 2018, fertilizer was added to all pots at the same rates as initially applied, since the Osmocote formulation was rated for only 3–4 months. In Jan. 2019, *Scirtothrips dorsalis* Hood (chili thrips) were found on the plants, and 1/4 tsp imidacloprid solution/gallon of water was prepared and each plant was drenched with 100 mL of this solution.

Non-destructive chlorophyll measurements. Additionally, the newest fully expanded leaf on each plant was selected and marked. Five non-destructive chlorophyll measurements using a SPAD-502 Plus (Konica Minolta, Tokyo, Japan) were taken from between visible leaf veins, and the five measurements were averaged. All readings were taken within the same marked area on the chosen leaf to insure consistency in the measurements. Monthly, for a period of six months, the newest fully expanded leaf was again selected on each plant and non-destructive chlorophyll measurements were taken.

Fig. 1. Homalomena 'Emerald Gem' grown under 30% woven shade cloth treatment. Note HOBO positioning in the center of the growing area.

Table 1. Average SPAD readings and average DMSO extracted total chlorophyll from leaves of *Homalomena* 'Emerald Gem'.

Plant Treatments	Average SPAD	TotChl (µg/g leaf tissue)
Group 1	63.35277	21207.58
Group 2	63.60944	20610.52
Group 3	62.51111	21223.84
Group 4	65.60278	23669.44
Group 5	64.44167	22595.84
Group 6	64.46943	22941.94

Destructive chlorophyll measurements and dimethyl sulfoxide extraction of chlorophyll. On a bimonthly basis, so as not to damage the plants by removing too many leaves, the newest fully expanded leaf used for SPAD measurements was removed from the plant. A 9-mm cork borer was used to collect 100 mg of fresh leaf tissue disks from each leaf. Leaf tissue disks were collected within the same leaf area that the SPAD meter readings were obtained (~4 cm² area). Leaf tissue disks were placed into individual glass screw-cap test tubes that were pre-filled with 7 mL of dimethyl sulfoxide (DMSO) and placed in racks in a bag to exclude light. Samples were transported from the Florida Southern College greenhouses to Florida Gulf Coast University and allowed to extract at room temperature overnight. The following day, samples were diluted with an additional 3 mL DMSO, to be able to achieve accurate spectrophotometric readings without violating Beer's Law (Tait and Hik, 2003). Six Genesys 20VIS spectrophotometers (Thermo Fisher Scientific, Waltham, MA) were set up in the laboratory. Three of the spectrophotometers were set to read absorbance of chlorophyll *a* (665 nm), and three were set to read absorbance of chlorophyll *b* (649 nm). Absorbance was converted to µgChla/mL, µgChlb/mL using standard equations (Wellburn, 1994). A calibration curve was used to convert µgTotChl/mL to µgTotChl/cm² (Table 1).

Light and temperature measurement. HOBO Pendant® Temperature/Light 8K Data Loggers (Onset Computer Corp., Bourne, MA) were used to obtain light and temperature data every 30 min for the duration of the study. One HOBO was placed under the 30% shade structure on top of a standard pot, another one was placed under the 60% shade structure on top of a standard pot, and a third HOBO was placed on a greenhouse bench uncovered. Light levels were obtained in foot-candles per half hour. This data was converted to daily light integral (DLI) (Torres and Lopez, 2010) and averaged both monthly and with all six months of the experimental period combined. Similarly, temperature data were measured and averaged both monthly and with all six months of temperature data combined (unpublished data).

Visual grading. Thirty four undergraduate students majoring in biology at Florida Southern College were asked to participate in a visual grading survey approved by the Florida Southern College Institutional Review Board. The entire 72 plants were lined up on a table in numerical order, and students were asked to rank each of the plants on a scale of 1 to 4 where a score of 4 indicated that the student would gladly purchase this plant and a score of 1indicated that the student would never purchase this plant. (Scores of 2 and 3 were intermediate for the purchase decision. Students were not given instructions concerning plant height or number of shoots, so as not to bias purchase decisions for those particular visual characteristics.) The survey instrument also asked the students to indicate their gender. On the same day, the total number of shoots per plant was recorded. Statistical analysis was performed using Minitab and Microsoft Excel to determine if any of the plant treatment groups was significantly more appealing to potential student purchasers.

Results and Discussion

The measured SPAD readings were closely related to the lab-extracted chlorophyll determinations. The number of shoots per plant as well as the irradiance level under which the plant was grown had a significant impact on the overall marketability of each plant. The plants grown under 30% shade had more shoots per plant generally than the plants grown under 60% shade. Plants grown under 30% shade received higher visual grades and were more likely to be purchased by the student evaluators. The

Table 2. Regression analysis of treatments used in growing *Homalomena* 'Emerald Gem' using Minitab.

Predictor	Response	*P*-value	R^2
Fertilizer	Shoot #	0.00	4.57%
Shoot #	Visual grade	0.00	63.81%
Height	Visual grade	0.02	13.08%
Shade cloth	Visual grade	0.00	64.57%
Fertilizer	Visual grade	0.059	5.01%
SPAD reading	Visual grade	0.00	19.18%
SPAD reading	Total Chl	0.025	75.47%

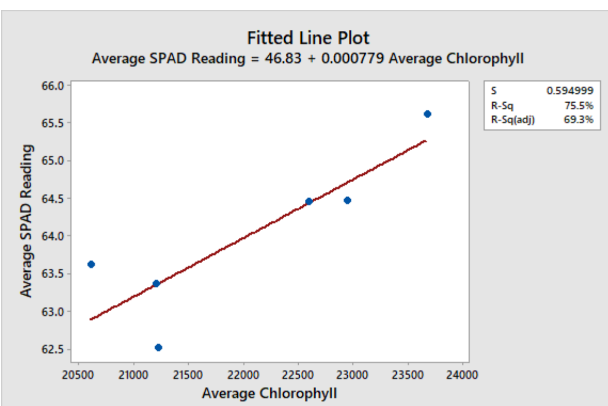

Fig.2. Regression analysis of SPAD-502 readings vs. total average chlorophyll extracted from leaves of Homalomena 'Emerald Gem'.

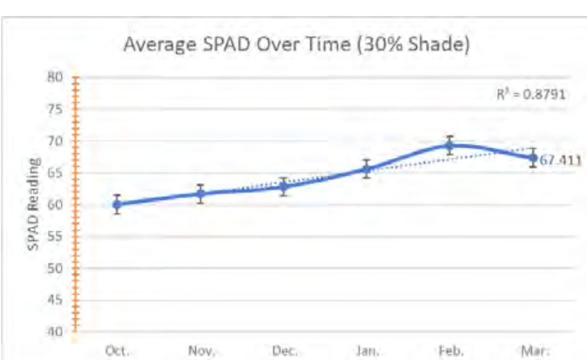

Fig. 3. Average SPAD readings for leaves of Homalomena 'Emerald Gem' grown in 30% shade over time for all fertilizer levels.

Table 3. Shade cloth levels vs. daily light integral (DLI) level (September–December) used in growing *Homalomena* 'Emerald Gem'. HOBO meters were obscured by plant growth as the study progressed.

Shade cloth	Average DLI (mol/day)
No shade	8.608
30%	4.588
60%	2.805

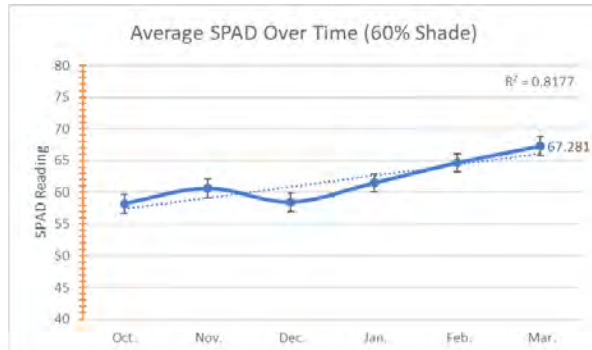

Fig. 4. Average SPAD readings for leaves of Homalomena 'Emerald Gem' grown in 60% shade over time for all fertilizer levels.

linear regression model was also used to establish a relationship between the fertilizer treatment groups and the visual grades that the plants received (Table 2). This analysis indicated that the various fertilizer rates applied to produce the crop did not have a significant effect on the overall attractiveness of the plants. The lowest of the three fertilizer rates evaluated yielded plants that were as attractive to potential buyers as plants produced with "Medium" or "High" fertilizer rates.

As the plants grew more shoots and were ultimately denser under the 30% shade cloth treatment, obscuring of the HOBO light sensor occurred. This was not noticed immediately, and therefore, not rectified. Thus, the light data from the last two months of the study were not included in determining the optimal DLI for growth of *H*. 'Emerald Gem'. The obscuring of light is reflected in Fig. 5; the plants grew over the HOBO meter at the point where the 30% shade treatment measurements begins to decrease (January–February).

A linear regression model established a relationship between the visual grades and the number of shoots on the plants (Table 2). The number of shoots was significantly related to the visual grades of the plants. The plants with more shoots appeared larger due to foliage density, and thus scored higher with potential buyers (Fig. 6). A regression analysis was performed, and it was determined that the production light levels significantly affected the number of shoots per plant (Table 2). Surprisingly, the overall heights of the plants did not significantly affect visual grading. A two-sample t-test was used to test for differences between average male and average female visual grades. While it appeared that males tended to grade slightly higher, there was no statistically significant difference between the two groups (data not shown).

There was no significant difference between the chlorophyll levels of the six treatment groups (Table 1). SPAD readings were also not significantly different from the beginning to the end of the trial (Fig. 3, Fig. 4). It can be discerned from this, that the three fertilizer rates used for this trial did not affect the

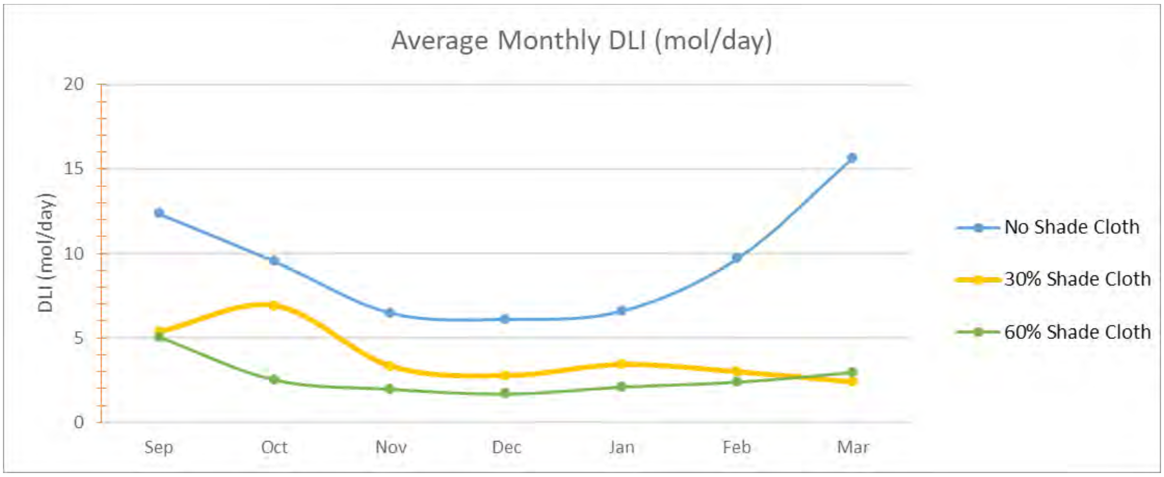

Fig. 5. Average daily light integral (DLI) Homalomena 'Emerald Gem' across all months. Note the decrease in DLI of 30% shade cloth treatment is due to obscuring of the HOBO meter from plant growth.

Fig. 6. Plant 18 from the 60% shade group (left) and Plant 56 from the 30% shade group (right).

chlorophyll levels in the leaves of the plants differently. Growers do not need to apply any more fertilizer than this study's lowest treatment rate, since higher fertilizer rates did not increase either the overall attractiveness and marketability of the plant or the amount of chlorophyll present in the leaves.

Conclusions

The SPAD-502 and the lab-extracted chlorophyll readings were well correlated. This means that growers do not need to perform a time-consuming DMSO extraction to reliably assess chlorophyll content within leaves of *H*. 'Emerald Gem'. Additionally, the three tested fertilizer rates applied to the crop did not have a significant effect on the overall attractiveness and marketability of the plants.

Subsequent studies will need to be done to assess if even lower fertilizer rates are sufficient for commercial production of *H*. 'Emerald Gem'. The light level under which the plants were produced did correlate with the overall appearance of the plants, as well as the visual grades of the plants. The plants produced under the higher light regime were superior to those grown under the lower light regime, all other treatments being equal. It can be recommended that *H*. 'Emerald Gem' be grown under the higher of the light levels evaluated in this study (Table 3). The DLI measured under the 30% shade (4.588 mol/day) is consistent with the minimum "good quality" to "high quality" (DLI 4-6, respectively) light conditions for growth of *Spathiphyllum* (Torres and Lopez, 2010), which is also in the family Araceae. To summarize, this study recommends producing *Homalomena* 'Emerald Gem' in a standard peat/perlite soilless potting mix supplemented with both S.T.E.M. at 1/4 tsp/pot /pot and 19–6–12 Osmocote fertilizer at a rate of 2.4 g^3/pot/3 mo and grown under a daily light integral of at least 4.588 mol/day.

Literature Cited

Chen, J., D.B. McConnell, R.J. Henny, and D.J. Norman. 2005. The foliage plant industry. Hort. Rev. 31:47–112.

Cheng, C. 2008. Complete factorial experiments in split-plots and strip-plots. 10 Sept. 2018. <https://www.stat.berkeley.edu/~cheng/Lecture13.pdf>

Conover, C.A. and R.T. Poole. 1990. Light and fertilizer recommendations for production of acclimatized potted foliage plants. University of Florida, IFAS, Central Florida Research and Education Center–Apopka, CFREC-A: Research Report RH-90-1. 4 Apr. 2018. <https://mrec.ifas.ufl.edu/foliage/resrpts/rh_90_1.htm>

Coste, S., C. Baraloto, C. Leroy, É. Marcon, A. Renaud, A.D. Richardson, and B. Hérault. 2010. Assessing foliar chlorophyll contents with the SPAD-502 chlorophyll meter: A calibration test with thirteen tree species of tropical rainforest in French Guiana. Annals of Forest Science 67(6):607–607. https://doi.org/10.1051/forest/2010020

Evans, J.R. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78(1):9–19. https://doi.org/doi:10.1007/bf00377192

Pinkard, E.A. and C.L. Mohammed. 2006. Photosynthesis of *Eucalyptus globulus* with mycosphaerella leaf disease. New Phytologist 170(1):119-127. https://doi.org/10.1111/j.1469-8137.2006.01645.x

Tait, M.A. and D.S. Hik. 2003. Is dimethylsulfoxide a reliable solvent for extracting chlorophyll under field conditions? Photosynthesis Research 78:87–91. https://doi.org/10.1023/A:1026045624155

Torres, A.P. and R.G. Lopez. 2010. Measuring daily light integral in a greenhouse. Purdue Agr. Extension Publication HO-238-W. Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, Ind.

Wellburn, A.R. 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144(3):307–313. https://doi.org/10.1016/s0176-1617(11)81192-2

Proc. Fla. State Hort. Soc. 132:210–212. 2019.

Development of a Model Mutagenesis System for Snapdragon

Zhaoyuan Lian*[1,2], Heqiang Huo[1,2], Sandra Wilson[2], and Jianjun Chen[1,2]

[1]*Department of Environmental Horticulture, University of Florida/IFAS, P.O. Box 110670, Gainesville, FL 32611*

[2]*Mid-Florida Research and Education Center, University of Florida/IFAS, 2725 S. Binion Rd., Apopka, FL 3270*

Additional index words *Antirrhinum majus*, Tam3 transposon

Snapdragon (*Antirrhinum majus* L.), a popular bedding and fresh cut ornamental plant, has been widely used as a model plant species due to its substantial genetic diversity and well-established transposon mutagenesis system. In this paper, a novel and highly efficient method for snapdragon (*Antirrhinum majus*) regeneration and transformation is introduced.

Snapdragon (*Antirrhinum majus*) has long been a very popular perennial in the United States due to its unique flower shape with a range of striking colors (Huo and Chen, 2018). Based on their height, snapdragons are typically classified into three categories: dwarf (6–15 in.), medium (1–2 ft.), and tall (6–15 ft.) (Fig. 1). These different heights allow for a range of uses. The dwarf variety has a dense, bushy growth pattern, producing numerous flower spikes. They grow on average 6–15 in. tall and are ideal plants for use as low borders or in containers. Mid-sized varieties grow 1–2 ft tall and are typically used in borders (either alone or with other bedding plants) and sometimes as cut flowers. Tall varieties range anywhere from 2–3 ft in height (Gilman et al. 2018). The magnificent flowers with a wide range of petal colors atop the long green spikes make the tall variety a desirable cut flower for container, bouquets, or gardens. In 2015, fresh-cut snapdragon sales increased 51.7% from 2010 and reached $12.93 million, making it a top 10 fresh cut flower in the United States, USDA, 2015).

With all of their many aesthetic attributes and versatility, snapdragons are also an important model system for genetics and molecular studies of various plant processes. For example, snapdragon pigmentation mutants produced by transposon (a type of mobile DNA) mutagenesis have provided researchers a good way to study anthocyanin biosynthesis and subsequently aid plant breeders in developing new varieties with novel flower colors (Jackson et al., 1992). Furthermore, snapdragon has a mechanism by which transposable mutations can be regulated into active and inactive states through temperature control (Hashida et al., 2006). Advantages of this elegant transposon mutagenesis system and how it relates to plant breeding are described below.

The snapdragon JI2 mutagenesis system. Transposons are mobile DNA elements that can move and insert into new loci within a genome. Plant genomes contain a number of active transposons that have the potential for gene disruption, chromosome breakage, and genome rearrangement. Several active transposons have been identified in snapdragon, including Tam3, which is of particular interest. Tam3 is a well-known transposon in snapdragon, whose transposition is strictly regulated by temperatures. Activated only when plants are grown at low temperatures (≤ 15 °C) for more than 60 days, Tam3 is strongly inhibited by high temperatures (≥ 25 °C) (Hashida et al., 2006). When activated at low temperature, Tam3 will move and insert into a new position in the snapdragon genome randomly, which can produce a variety of plant mutants. The unique snapdragon stock JI2 (*Pallida recurrens*) containing a highly active Tam3, causes flower color to change from red in the low temperature (≤ 15 °C) and white in the high temperature (≥ 25 °C) (Fig. 2). This serves as visible marker for monitoring the Tam3 activity.

Advantages of the snapdragon JI2 mutagenesis system

Convenience in operating. For breeding with traditional mutagenesis, chemical mutagens like ethyl methane sulfonate (EMS), methyl methane sulfonate (MMS), diethylsulfate (DES) or physical mutagens like UV rays, X-rays, γ-rays are used to generate mutations. It is noteworthy that these chemicals and radiation instruments are hazardous, costly and potentially danger-

Fig. 1. Different uses of snapdragons including (**A**) dwarf varieties used for pot culture; (**B**) medium varieties used for landscape borders; and (**C**) tall varieties used for cut flowers.

*Corresponding author. Email: lianzhaoyuan@ufl.edu

Fig. 2. The flower color change of snapdragon JI2 in the different temperatures of 25 °C and 15 °C.

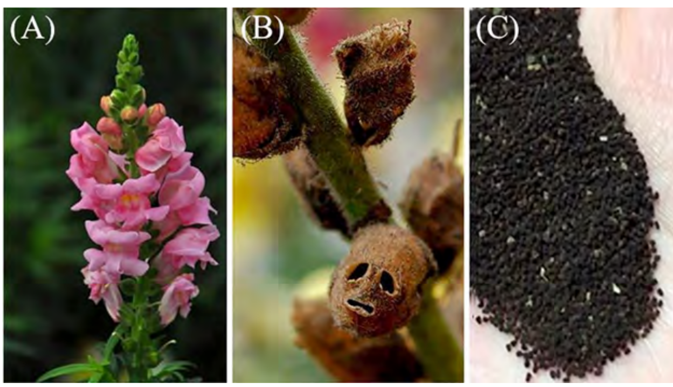

Fig. 3. Snapdragon reproductive stages of (**A**) flowers, (**B**) pods, and (**C**) seeds.

ous to use. Therefore, significant health and safety training must be completed prior to use. In addition, deleterious mutations are frequently created with chemical/physical mutagenesis, resulting in additional efforts of purifying through backcrossing to the wild type plants. Another key drawback of traditional mutagenesis is that it is extremely hard and time-consuming to develop molecular markers for utilizing these mutants as breeding materials since a great number of random mutations will be created in each genome and could be difficult to detect. In contrast to traditional mutagenesis, the Tam3 mutagenesis system is completely risk free. No specific chemicals and instruments are needed in the entire mutagenesis process. A cooler room with an adjustable temperature to 15 °C is the only required facility to activate Tam3 transportation for mutagenesis.

Non GMO varieties. Unlike other model plant species like Arabidopsis that heavily rely on T-DNA mutant libraries for functional characterization, mutant libraries created by random transpositions of temperature-dependent Tam3 fall outside the scope of USDA/EPA regulations. The availability of whole genome and rapid sequencing technology advancement make it simpler to identify and characterize transposon-induced mutations, which in return can be developed for molecular markers. More importantly, these mutants can be directly used as breeding lines for developing novel snapdragon varieties. Especially in Florida, the year-long high temperatures will strongly inhibit the activation of Tam3, so the new variety can maintain genetic stability under natural environments.

High mutation efficiency. The snapdragon JI2 Tam3 mutation system has proved to be very efficient, mainly due to two reasons. Firstly, a maximum of 35 copies are expected to be moveable among the total genomic 60 Tam3 copies (Kishima et al., 1999). Secondly, every pod of the snapdragon can produce hundreds of tiny seeds, and usually no more than ten pods are expected to be collected per plant (Fig. 3). Theoretically, every pod is an independent mutant unit, so just a few plants are sufficient to create an appropriate mutant population. The massive production of seeds will easily enable researchers to screen mutants with desirable phenotypes. This may be especially useful for screening abiotic stress tolerance mutation, in which a large number of seeds are required due to high mortality under various stress treatments.

A variety of mutants are shown in Fig. 4, all of which were produced from only five original wild-type snapdragon JI2 plants. Mutants with increased branching capacity may be valuable for nursery production of cut flowers, whereas the mutants with twisted branches and red-leaf mutants exhibited higher aesthetics values. Potential isolation of a heat tolerant mutant could significantly broaden the year-round use of snapdragon in Florida (Huo et al., 2018).

Photoperiod-dependent short-life cycles. Snapdragon usually requires 5–6 months from seeds to seedlings in the natural environment. However, its growth cycle period can be significantly

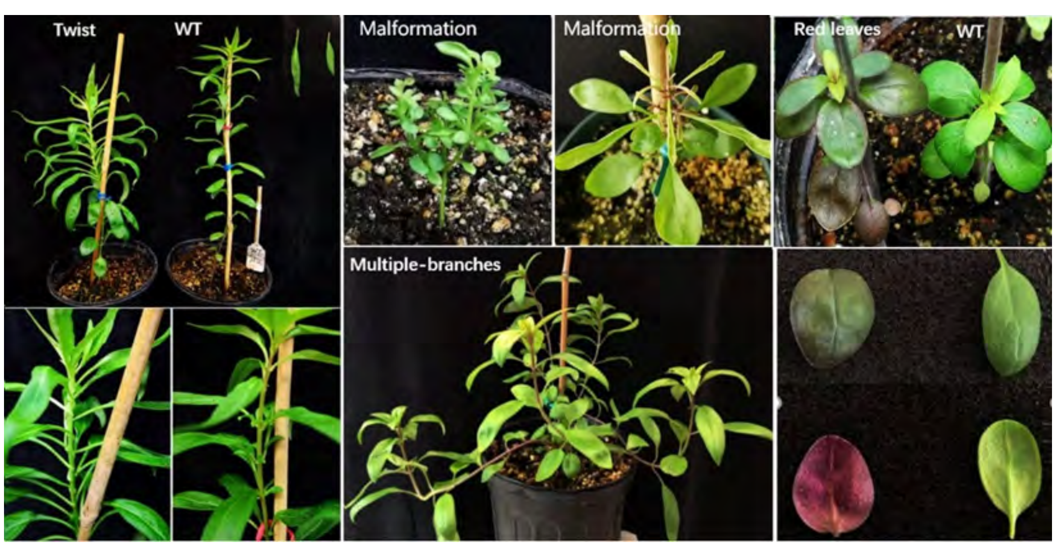

Fig. 4. Snapdragon mutants generated from Tam3 transposon transportation.

Fig. 5. Comparison of snapdragon growth and development under 24 h light and 16 h light/8 h dark photoperiodic treatments.

reduced to approximately 3.5 months when plants are grown in 22–24 hours daylight. This longer photoperiod can promote snapdragon flower bud differentiation, and significantly shorten and harden plant stems and accelerate seed production (Fig. 5).

Easy complementary testing. For functional characterization of candidate genes/mutations, one validation approach is to perform molecular complementation tests, in which a functional wild type gene will be expressed for rescuing the mutant phenotypes. This approach generally requires a complicated process including plasmid construction and genetic transformation, which is time-consuming and technically challenging. In our strategy, the mutants are cultivated at 15 °C again to reactivate Tam3 and allow it to "jump" out of the mutation locus for restoring the gene function and phenotype. This is a much simpler and straightforward process. The excision of Tam3 can be monitored by a general PCR with primers flanking the original insertion locus.

In summary, snapdragon offers endless potential for the plant breeder in developing new varieties. The many advantages to using the JI2 mutagenesis system include: operating convenience, a non-GMO alternative, photoperiod-dependent short-life cycles, and simpler complementary testing.

Literature Cited

Gilman, E.F., R.W. Klein, and G. Hansen. 2018. *Antirrhinum majus* Snapdragon. Extension Fact Sheet FPS-44. University of Florida, Gainesville, FL.

Hashida, S.N., T. Uchiyama, C. Martin, Y. Kishima, Y. Sano, and T. Mikami. 2006. The temperature-dependent change in methylation of the Antirrhinum transposon Tam3 is controlled by the activity of its transposase. Plant Cell 18(1):104–118. doi: 10.1105/tpc.105.037655.

Heqiang, H. and C. Jianjun. 2018. Planting and propagation of snapdragons in Florida. Extension Fact Sheet ENH-1285. University of Florida, Gainesville, FL.

Jackson, D., K. Roberts, and C. Martin. 1992. Temporal and spatial control of expression of anthocyanin biosynthetic genes in developing flowers of *Antirrhinummajus*. Plant J. 2(4):425–434.

Kishima, Y., S. Yamashita, C. Martin, and T. Mikami. 1999. Structural conservation of the transposon Tam3 family in *Antirrhinum majus* and estimation of the number of copies able to transpose. Plant Mol. Biol. 39(2):299–308.

USDA. 2015. Floriculture Crops. United States Department of Agriculture. 15 Dec. 2019. <https://downloads.usda.library.cornell.edu/usdaesmis/files/0p0966899/pz50gz655/8910jx14p/FlorCrop-04-26-2016.pdf>.

Proc. Fla. State Hort. Soc. 132:213–214. 2019.

Celebrating and Protecting Florida's Native Pollinators

Alicia Lamborn*[1] and Nicole Pinson[2]

[1]*University of Florida/IFAS, Baker County Extension, 1025 W. Macclenny Ave., Macclenny, FL 32063*

[2]*University of Florida/IFAS, Hillsborough County Extension, 5339 County Rd. 579, Seffner, FL 33584*

Additional index words: pollinator habitat conservation, science education

Pollinator education programs in Baker and Hillsborough Counties are focused on getting residents to think beyond the honeybee. With more than 4000 species of bees, 750 species of butterflies, and thousands of species of wasps, flies, and beetles acting as pollinators for 75% of flowering plant species in the United States, it is important for Florida residents to understand the role these pollinators play, why pollinators are in need of our help, and how we can help lessen pollinator population decline. With human beings reliant on pollinators to produce 35% of the world's food crops, it is also important to understand why a diverse population of pollinators is necessary for a healthy environment, agriculture industry, and economy. It is especially imperative since over-reliance on a single species of pollinator such as the western honeybee (*Apis mellifera*) could lead to critical food shortages in the future.

Pollinator populations have been declining worldwide due to several factors, including habitat loss, pesticide use, invasive species, and disease. While a recent University of Florida study has shown that most people who learn about the problems facing pollinators are willing to help, many are still unsure how they can help. The primary objectives for the pollinator education programs are to increase awareness of the variety of pollinator species that reside in Florida and encourage conservation of pollinators and habitat. Highlighting common pollinator species, their nesting habits, and other beneficial aspects of their life cycle—such as acting as biological controls for agronomic pests—teaches understanding of and appreciation for the role of pollinators. Conservation is improved when people learn about important pollinator forage plants including so-called "weeds," ways to manage pests that do not involve pesticides, techniques for avoiding pesticide exposure to bees and other pollinators, and providing pollinator nesting habitats such as bare ground that is not tilled and cavities made from wood and bamboo.

Methods

In both Baker and Hillsborough Counties, pollinator gardens demonstrate these concepts using plant material, signage, and sample bee nesting sites. In some cases, pollinator talks have been held entirely in the garden setting. In Hillsborough County, 175 adults and 2897 youth learned about pollinators and their importance to agriculture during 2016–19. Students, chaperones, and teachers learn that pollinators include many animals, such as beetles, moths, birds, bats, bees, wasps, and flies through a variety of interactive teaching methods. Students use art to create beneficial insect necklaces. Garden signs with pollinator trivia are placed throughout the learning and teaching garden for children, parents, and other visitors to discover. Garden plants labeled as "good bug blends" help residents learn about plants that attract beneficial insects and thrive in Florida. Garden walks provide microscopes, magnifying lenses, and identification decks for children and adults to learn about beneficial insects and the critical services they provide to ecosystems and to our food supply. These hands-on opportunities enable workshop participants to process what they learn through fun, experiential activities that take place at their schools, the Extension office, park, natural area, or demonstration garden. In Baker County, pollinator talks and demonstrations have been presented to 384 adults and 589 youth during 2016–19. Teaching materials have included colorful posters, pamphlets and brochures, sample bee nest boxes called "bee houses," and occasionally live butterflies. Additionally, pollinator workshops that include a lecture followed by a hands-on activity have been conducted for 20 adults and 24 youth. The lecture portion spends an equal amount of time addressing the objectives of increasing awareness and promoting conservation. The make and take activity that follows allows participants to build and stuff a bee house for immediate use in their home landscape. A pollinator garden that features a variety of pollinator plants and "good weeds," educational signage, and a large "bee hotel" is also used for experiential learning and observation.

Results and Discussion

As a result of these individual efforts, a Pollinator Power! Curriculum was co-authored by the agents. The curriculum includes lesson plans targeted for grades 5 to 8; however, the curriculum and lesson plans may be adapted for learners of other ages and even for adults. Examples of these lesson plans include: What are Pollinators? ; Beneficial vs. Pest Insects; Researching Native Bee Pollinators; Pollinator Safari; Be a Bee; What Does a Beekeeper Do? ; Plan a Virtual Garden; Communicating the Importance of Pollinators; and Citizen Science Projects. The easy-to-follow

*Corresponding author. Email: alamborn@ufl.edu

curriculum provides student learning objectives for each lesson, approximate timeframes and materials needed for activities, online references, and University of Florida/Institute of Food and Agricultural Sciences (UF/IFAS) resources. In addition to a flash drive with the *Pollinator Power!* lesson plans and printable resources, the curriculum also includes a *Pollinator Power! Safari Kit* which contains: three insect jars, a butterfly net, one magnifier, a set of insect rubbing plates, a set of flower rubbing plates, and a UF/IFAS Extension drawstring bag. Pollinator concepts are explored through digital literacy, real-world inquiry, outdoor learning labs, hands-on insect scouting, and by use of writing, marketing, and communication skills. The curriculum provides lesson plans to teach pollinator concepts to youth, adults, and Extension audiences. It also gives tips to create garden habitat, increase pollinator diversity, minimize risks to beneficial insects, and monitor and study pollinator populations.

The curriculum was produced by the UF/IFAS Communications, reviewed by a State Extension Specialist and middle school science teacher, and made available through the UF/IFAS Extension Bookstore.

Proc. Fla. State Hort. Soc. 132:215–216. 2019.

The Tree Steward Volunteer Pruning Program in Jacksonville, Florida

Lawrence Figart*

Urban Forestry Extension Agent, University of Florida/IFAS, Duval County Extension, 1010 N. McDuff Ave., Jacksonville, FL, 32254.

Additional index words. Master Gardener, training, Duval, IFAS

The objective of the Duval County Tree Steward Program is to train and equip a group of Master Gardener Volunteers whose mission is to improve and restore the tree canopy in local parks. The Duval County Urban Forestry Extension Agent recruits, trains, and retains an active group of Master Gardener Volunteers to become Tree Steward Volunteers. This training is held every other year and includes 25 hours of advanced training in subjects such as pruning, identification, selection, and maintenance of trees. Since 2015, sixty-one volunteers have been trained to properly prune trees by removing the less desirable of co-dominant stems, improving structure, removing rubbing and crossing limbs, and increasing park user safety by keeping limbs above eye level along sidewalks. The trees pruned are typically less than 20 feet tall. The Duval County Tree Stewards donated over 2218 hours of time pruning trees during 76 work projects in City of Jacksonville parks. Over 2000 trees have been properly pruned by removing structural defects, therefore improving public safety and increasing the likelihood that the trees will grow structurally sound. In an annual survey of tree steward volunteers, 97% feel more confident pruning younger trees, 76% feel more active by working outside, 76% enjoy the social aspect of working together, 97% enjoy learning about trees, and 93% feel they are making a difference improving the urban forest. The Tree Steward model used in Duval County provides new opportunities for volunteer service and could be adapted for use in other counties.

The City of Jacksonville operates the largest urban park system in the United States, with more than 337 locations covering more than 80,000 acres. Because of budget limitations parks are often underfunded and necessary maintenance cannot be routinely scheduled. Hence newly planted and/or younger park trees grow without being pruned and structural defects such as included bark and co-dominant stems are going unchecked. Trees should be structurally pruned at a young age while defects can still be corrected. Trees that are structurally pruned while they are young are safer, more wind resistant, live longer and require less maintenance later. Properly pruned trees in city parks also serve as good examples for the public to replicate in their landscapes.

The Duval County Tree Steward program was created in 2014 with the help of a United States Forest Service Urban and Community Forestry Grant. This grant provided $2228.00 and was locally matched by the extension agent teaching time used as in-kind services. The Duval County Tree Stewards program is loosely based on the Virginia Tree Stewards program sponsored by the Virginia Urban Forestry Council.

Materials and Methods

The Duval County Urban Forestry Extension Agent recruits, trains, and retains an active group of Master Gardener volunteers to become Tree Steward Volunteers. This training is held every two years and includes 25 hours of advanced training. Subjects taught include tree biology, tree benefits, tree soil relations, water relationships, construction around trees, tree planting, pruning, first aid, tree selection, right tree right place, invasive plants and tree pests. There are two hands-on pruning practicums prior to program graduation to ensure that the trainees have mastered pruning techniques such as natural target pruning, and reduction pruning. Following graduation, the Tree Steward Trainees are able to participate in the twice a month pruning workdays.

Results and Discussion

Since 2014, sixty-one volunteers have been trained in the Duval County Tree Steward Program. Volunteer that have gone through the program are able to properly prune trees by removing the less desirable of co-dominant stems, improving structure, removing rubbing and crossing limbs. By raising the canopy and removing undergrowth around trees they are able to increase park user safety by improving visibility and reducing pedestrian/ tree conflicts along sidewalks. The trees pruned are newly planted trees and existing trees typically less than 20 feet tall. The volunteers should be able to reach the limbs to be pruned with pole saw extensions and are not allowed to climb the tree or use ladders.

During the period of 2015–19, the Duval County Tree Stewards have donated over 2218 hours of time pruning during 76 work projects in City of Jacksonville parks. Over 2900 trees have been properly pruned by removing structural defects, thereby improving public safety and increasing the likelihood that the trees will grow structurally sound. Using the National

*Corresponding author. Email: lfigart@coj.net

Tree Benefits Calculator, the trees maintained by the Duval County Tree Stewards provide $81,200 in ecosystem services annually. As they grow another 5 years, the ecosystem services value grows to $148,000. The estimated dollar value of Tree Steward volunteer time in 2014–18 is $51,296 in value for the City of Jacksonville.

In an annual survey of tree steward volunteers:

- 97% feel more confident pruning younger trees
- 76% feel more active by working outside
- 76% enjoy the social aspect of working together
- 93% feel they are making a difference improving the urban forest.

Conclusion

The tree steward model used in Duval County provides new opportunities for volunteer service and could be easily adapted for use in other counties, or cities.

Proc. Fla. State Hort. Soc. 132:217–218. 2019.

Lethal Viral Necrosis, the New Nightmare for Saint Augustinegrass 'Floratam'

Henry Mayer*[1], Laurie Albrecht[2], Romina Gazis[3], Phil Harmon[4], and Jane E. Polston[4]

[1]*University of Florida/IFAS, Miami Dade County Extension, 18710 SW 288 St, Homestead, FL 33030*

[2]*University of Florida/IFAS, Palm Beach County Extension, 559 N Military Trail, West Palm Beach, FL 33415*

[3]*University of Florida, Tropical Research and Education Center, 18905 SW 280th Street, Homestead, FL 33031*

[4]*University of Florida, Department of Plant Pathology, 1453 Fifield Hall, P.O. Box 110680 Gainesville, FL 32611*

Additional index words. lethal viral necrosis, Stenotaphrum secundatum, sugarcane mosaic virus.

Lethal viral necrosis (LVN) of 'Floratam' St. Augustinegrass (*Stenotaphrum secundatum*) was first reported in 2013 when laboratory tests confirmed an outbreak that was associated with the Sugarcane mosaic virus (SCMV) in Pinellas County, Florida; later tests identified occurrences in Palm Beach County in 2014. Since then, the disease has spread to 12 Florida counties, including Broward and Miami-Dade. Prior to 2013 and since the 1960s, SCMV had been known to cause mosaic disease of St. Augustinegrass in rural Palm Beach County and other sugarcane producing areas of Florida. LVN is distinguished from mosaic disease by the severe symptoms that include death of infected 'Floratam', which had not been reported prior to 2013. Recent research has associated LVN disease with a second virus and cannot be prevented or cured by fungicide or other chemical means. It is only known to be fatal to 'Floratam' St. Augustinegrass, which is the most widely planted residential turfgrass in Florida. Infected 'Floratam' usually dies within three years or less of detection. Other cultivars of St. Augustinegrass as well as other species of turfgrass may harbor the viruses, but are not killed. Zoysiagrass is not a host of SCMV. The only long-term solution for infected 'Floratam' areas is to re-sod with resistant turfgrass species or other cultivars of St. Augustinegrass. Economic losses in Palm Beach County already are severe and are expected to rise as the disease continues to spread throughout the county.

Mosaic disease caused by Sugarcane mosaic virus (SCMV) of St. Augustinegrass (*Stenotaphrum secundatum*) was first reported in the 1960s in sugarcane producing areas of Florida (rural Palm Beach County). Over the last 50 years, differences in susceptibility among St. Augustinegrass cultivars were well-known, but no variety had been reported to die from this disorder. In 2013 and 2014 outbreaks of mosaic occurred in Pinellas and Palm Beach Counties that resulted in death of the 'Floratam' variety of St. Augustinegrass. Later investigation associated these outbreaks with SCMV and a second virus. The name lethal viral necrosis (LVN) was used after this point to refer to the disease of 'Floratam' St. Augustinegrass associated with two viruses that leads to death of affected lawns. As of Dec. 2019, turf samples with LVN have been confirmed in 12 Florida Counties (UF/IFAS Extension Plant Diagnostic Center).

Early symptoms of LVN include a mosaic pattern with yellow streaks running between the veins on an otherwise green blade. The leaf becomes necrotic (turns brown and dies) in the fall (September and October) when temperatures drop to around 65 °F and progress into spring. The symptoms may become difficult to see during the warmer and wetter months when turf is growing vigorously. Unfortunately, 'Floratam' in lawns that appear to recover in summer will die the following fall and tend to worsen and spread over a period of 1–3 years.

*Corresponding author. Email: hmayer@ufl.edu

Discussion

Laboratory and greenhouse tests suggest that lethal viral necrosis is caused by a combination of two viruses. One of the viruses was just recently described from Bermuda grass (*Cynodon dactylon*) and called Bermuda grass latent virus, BGLV) and causes no known symptoms on Bermuda grass. The second virus (SCMV) belongs to the potyvirus family and is commonly found in sugarcane-producing areas around Lake Okeechobee, even though it is currently not a concern for sugarcane growers. Although the two viruses have been associated with samples from Pinellas as well as Palm Beach Counties, additional research is needed to establish that both viruses are required. No working diagnostic test currently exists for BGLV.

The most severe and deadly symptoms have been observed on 'Floratam' St. Augustinegrass, which unfortunately is the most popular turf used in Florida. Where 'Floratam' sod was used to replace lawns that died in 2013, the lawns died again in 2014 and 2015. The cultivars Palmetto and Bitterblue used in these communities in Pinellas County developed a mild mosaic symptom and tested positive for SCMV, but did not die, and provided an acceptable lawn. The SCMV virus has been detected in weedy grasses in diseased lawns including *Cynodon* (Bermuda grass), and *Digitaria* (crabgrass). Ornamental *Pennisetum* grasses (ornamental fountaingrasses) have been reported as hosts, but have not been found in Florida lawns to date. At least one weedy grass in the genus *Paspalum* was reported to be a host of SCMV, but bahiagrass and seashore paspalum are not thought to be hosts at this time. Grasses in the genus *Zoysia* spp. (Zoysiagrass) are not known to be hosts of SCMV. In general, plant host resistance is the best management tool for viral diseases.

The most common way of spreading the disease is mechanical. Lawn equipment such as lawn mowers and string trimmers containing lawn clippings infected with LVN in the sap can spread the diseases. For that reason, it is recommended to mow the suspect property last!

Once the sap and clippings completely dry out, they no longer harbor the virus because viruses do not survive for long periods of time outside the plant tissue, so good sanitation is very important. Cleaning, or at a minimum, drying and blowing off equipment before leaving one site to travel to another site may help prevent disease spread. In areas where the disease is known to occur, potentially contaminated equipment parts should be cleaned and sprayed down using a suitable commercial sanitizer such as: PineSol (The Clorox Co., Oakland, CA), potassium peroxymonosulfate and sodium chloride (Virkon S, DuPont Animal Health Solutions, Wilmington, DE), quaternary ammonia, Physan 20 (Maril Product, Inc., Tustin, CA), or Lysol® (Reckitt Benckiser, NJ) according to the manufacturers' instructions and in accordance with all local rules and regulations. Aphids are known to transmit the virus as well, but it is not known how important aphids are for disease spread.

Pesticides cannot stop development or spread of this viral disease. Also, there are no known agronomic inputs that homeowners or lawn care companies can use to cure a lawn once it has become infected. One potential complicating factor is that lawns with the virus may also suffer from fungal disease including take-all root rot (TARR). It is not known what impact the fungal disease may have on the severity of the viral disease.

Research is underway by the University of Florida to characterize genetic differences within 'Floratam' and 'Bitterblue' St. Augustinegrass varieties. Additionally, molecular markers are being developed to help accurately identify St. Augustinegrass varieties. Currently, the genetics of older varieties like 'Bitterblue', are not well understood. This will help improve the ability to get the varieties of St. Augustinegrass accurately identified, in order to test for resistance to the disease within these and other commercially available varieties.

Proc. Fla. State Hort. Soc. 132:219. 2019.

—Scientific Note—

Demonstration Landscape Overhaul: How to Create Habitat, Foster Partnerships, and Inspire Volunteers

Tina McIntyre*, Kaydie McCormick, and Hannah Wooten

University of Florida/IFAS, Seminole County Extension, 250 W. County Home Rd., Sanford, FL 32773

Additional index words. Extension, Florida–Friendly garden

The University of Florida/IFAS Seminole County Extension Florida–Friendly Demonstration Landscape required an overhaul and new management approach after neglect and the harsh summer months. The Florida–Friendly Landscaping Agent started in July of 2018 and brought 10 years of experience in working in the environmental and horticultural realm. The first step was to assess the existing landscapes at the Extension office, which included the Florida–Friendly Landscaping Demonstration Landscape, The Herb Garden, the Live Honey Bee Hive and the Ground Cover Bed. The Florida–Friendly Agent worked together with the Residential Horticulture Agent and the Sustainable Agriculture Agent to devise goals for the landscape demonstration areas at the Extension office. They identified a need for more color, structure, better educational signage, and the need for a more welcoming sense of place. Individual plants were targeted to be retained, while other areas were identified to expand the landscaped area.

The next step was to identify existing partnerships, resources, and strategies. Fostering new relationships with Seminole County Greenways and Natural Lands brought in an in-kind donation of a hand-made arbor and a bench that enables the landscaped areas to feel more welcoming. Direct communication with local landscape and land managers through the planning process introduced the potential of cultivating Florida–Friendly Landscape beyond one demonstration garden. The Agents are currently working with them to restore a 100-acre golf course with Florida–Friendly landscaping. Their partnership has also created opportunities to work with the business that manages all county landscape contracts, BrightView. Working with BrightView, the Agents were able to remove major trees and shrubs, add color, diversity, perennials, natives, and most importantly irrigation to the site.

Master Gardener Volunteers have been an integral part of the expansion and remodeling of the demonstration landscapes. The ground cover bed and the herb garden were done exclusively by Master Gardener Volunteer labor and expertise. A work day on Valentine's Day in Feb. 2019 called "Fall in Love with FFL" inspired Master Gardener Volunteers to come help weed, plant, water and mulch many of the sites. A Master Gardener Volunteer who owns a landscape design business donated the use of his expertise, employees, and equipment to the construction of both the groundcover bed and the herb bed. Master Gardener Volunteers are also in charge of much of the ongoing maintenance of the beds and have organized "Weeding Before the Meeting" events that occur once a month before their monthly meeting.

The Seminole County Beekeepers Association has a contracted partnership with the Sustainable Agriculture Extension Agent. They utilize the Extension Auditorium for their monthly meetings and keep bee hives on Extension property for educational purposes. In Spring 2019, native plants were purchased to install in close proximity of the hives. This partnership brings access to a local audience easily, volunteer hours, access to materials, expertise and networking opportunities.

Similarly the Florida-Friendly Landscaping Extension Agent has a partnership with the Cuplet Fern Chapter of the Florida Native Plant Society. The group meets monthly in the Extension Auditorium and has a plot on the Extension grounds. Society members are required to do monthly maintenance and submit hours to the Extension Agent. This partnership offers much to both entities.

New signage was designed and installed on site. Signs explore the nine Florida–Friendly principles including right plant, right place, water efficiently, fertilize appropriately, mulch, attract wildlife, manage yard pests responsibly, recycle, reduce stormwater runoff, and protect the waterfront. A sign with the Florida–Friendly logo was installed close to the nearby road to identify what the garden is to through traffic moving through the area and people waiting at the local bus stop. QR code signage was also installed and linked to various Electronic Data Information Source (EDIS) publications.

With this overhaul the educational potential of the gardens have been exponentially enhanced. Incorporating partners into the herb garden, ground cover bed, Florida–Friendly Landscaping, and apiary demonstration gardens has been a valuable addition to the University of Florida/IFAS Extension program in Seminole County. Involving the community partners committed to the Extension mission creates opportunities to plant more sustainable landscapes and reach new audiences.

*Corresponding author. Email: k.mcintyre@ufl.edu

Proc. Fla. State Hort. Soc. 132:220. 2019.

—Scientific Note—

Tradeshow Attendee Objectives, Booth Visit Likelihood, and Visual Attention: A Tropical Plant Industry Exposition Case Study

Alicia Rihn[1] and Hayk Khachatryan*[2]

[1]*Food and Resource Economics Department, University of Florida, P.O. Box 110240, Gainesville, FL 32611*

[2]*Mid-Florida Research and Education Center, University of Florida, 2725 Binion Rd., Apopka, FL 32703*

Additional index words. networking, ordered logit model, survey

This case study investigated the relationship between tradeshow attendees' objectives for the tradeshow and how that related to their booth visit likelihood and visual attention to the booth. Seventeen tradeshow booth images from the 2017 Tropical Plant International Exposition (TPIE) were shown to 30 tradeshow attendees while their eye movements were recorded. While viewing the images, participants rated their likelihood of visiting the booth (1 = very unlikely; 7 = very likely). A survey instrument was used to collect objective and demographic information. An ordered logit model was used to analyze the data.

Participants indicated their primary objective at the tradeshow was to network and visit, followed by obtain new product information/ideas, and attend educational sessions indicating that the participants were peripheral market actors rather than purchasers/sellers (Tafesse and Skallerud, 2017). Participants reported viewing the total booth the most, followed by the products, and then the brand/logo. The increased visual attention to the total booth and products was not surprising given the larger area committed to those items and that the products frequently consisted of brightly colored plants. These results show that attendees who were there to check out the competition, visit and network, or make contracts were more likely to visit the booths than those that were there for "other" reasons. Conversely, attendees who were there to gather ideas were less likely to visit the booths. Older participants with higher incomes were more likely to visit the booths. Results also suggest that greater visual attention to the total booth increases booth visit likelihood which demonstrates that having an "eye catching" display is important. Conversely, additional visual attention to the brand/logo decreased visit likelihood.

Future studies could use similar methods to investigate the relationship between tradeshow attendees' objectives, visual attention, and behavior. The addition of different industries or tradeshows targeting end consumers instead of industry clientele would be of interest for marketers and retailers. Subsequent studies could benefit by using a larger sample from more geographically diverse locations to test robustness.

Literature Cited

Tafesse, W., and K. Skallerud. 2017. A systematic review of the trade show marketing literature: 1980–2014. Industrial Marketing Mgt. 6318–30.

*Corresponding author. Email: hayk@ufl.edu

Proc. Fla. State Hort. Soc. 132:221. 2019.

—Scientific Note—

Assessing the Relationship Between Green Industry Consumers' Shopping Styles and In-store Promotions Effectiveness

Alicia Rihn[1] and Hayk Khachatryan*[2]

[1]*Food and Resource Economics Department, University of Florida, P.O. Box 110240, Gainesville, FL 32611*

[2]*Mid-Florida Research and Education Center, University of Florida, 2725 Binion Rd., Apopka, FL 32703*

Additional index words. coupons, impulse buyers, planners, price seekers;,print ads

This study explored consumers' shopping behavior for plants and how different type of promotions influenced their choices to visit garden centers, purchase specific plants, and the number of plants purchased. An online survey was combined with ordered logit models to analyze the results by shopping behavior type and promotion strategy. A total of 1680 Florida homeowners completed the survey and three distinct shopping behavior types were identified, including planners, impulse purchasers, and price seekers.

Participants frequently belonged to more than one shopping behavior type with planners occurring the most frequently (63% of the sample), followed by impulse buyers (52%), and price seekers (50%). Using 7-point Likert scales, participants indicated the effectiveness of promotions prompting them to visit garden centers (1 = very ineffective; 7 = very effective). For planners, printed ads, radio/TV ads, educational programs, outside signage, previous experience, and loyalty programs were more effective at encouraging them to visit a garden center than online advertisements. Impulse buyers were encouraged to visit garden centers by print ads, online ads, in-store events, in-store information, outside signage, previous experiences, in-store events, word of mouth advertising, and loyalty programs when compared to online advertisements. Conversely, price seekers were encouraged to visit garden centers by print ads, coupons/financial incentives, in-store events, and outside signage while they were discouraged by experience-oriented promotions. Participants also indicated how different promotions influence their purchase likelihood for different plants.

Planners responded positively to ads that were available ahead of time (e.g. print, radio/TV, tags/labels, recommendations, and branding); however, financial incentives and demonstrations decreased their purchase likelihood. Impulse buyers responded positively to in-store promotions (e.g. demonstrations, sales, and decorative packaging) but negatively to radio/TV ads, coupons, events, recommendations, and brands. Not surprisingly, price seekers responded positively to financial incentives but negatively to promotions that are often associated with premiums (e.g., brands).

Respondents indicated how different price promotions impacted the number of plants purchased. In this instance, all promotions positively impacted number of plants selected; however, planners responded most positively to coupons, impulse buyers to BOGO and slash through sales, and price seekers to slash through sales.

Future research could expand on these results in several directions, including using real in-store data or conducting an in-depth analysis of a specific type of promotion (e.g. price promotions, etc.) and how that influences ornamental plant purchases. Each of these suggestions could aid in developing a deeper understanding of how different promotions impact consumers' behavior in garden centers.

*Corresponding author. Email: hayk@ufl.edu

Proc. Fla. State Hort. Soc. 132:222. 2019.

—Scientific Note—

How Visual Attention Affects Consumer Preference for Product Labels: An Eye Tracking Study

Xuan Wei, Hayk Khachatryan*, and Alicia Rihn

Mid-Florida Research and Education Center, University of Florida, 2725 Binion Rd., Apopka, FL 32703

Additional index words. neonicotinoids, ornamental plants, plant labeling, visual attention, willingness to pay

This study combined non-hypothetical second-price auction experiments and eye tracking technology to explore the relationship between Floridian consumers' preferences for horticultural product labels communicating the use of neonicotinoid insecticides in ornamental plant production. In a total of 15 sessions of second-price auctions, 52 participants from central Florida viewed 28 garden center products (14 annual bedding plants, followed by 14 perennial plants) on computer screens and submitted bids for each plant. Eye tracking equipment recorded their eye movements throughout the bidding process.

Participants' willingness to pay (WTP) was estimated, employing a random effects tobit model (Lusk et al., 2004). Results showed that participants valued labels disclosing the absence of neonicotinoids. They were willing to pay a price premium of $0.20 to $0.29 for neonicotinoid-free text and $0.39 to $0.53 for the neonicotinoid-free logo relative to plants labeled with the "Protected from Neonicotinoids" phrase. However, the respondents did not differentiate between the different texts communicating the *presence* of neonicotinoids (i.e., "Treated with Neonicotinoids" vs. "Protected from Neonicotinoids"). Given consumers' positive response to products free of neonicotinoids, policymakers may consider a prudent policy approach by encouraging voluntary disclosure of the absence of neonicotinoids on labels. Our results confirmed visual attention patterns are useful in explaining consumer purchase decisions and WTP for plants. We found knowledgeable participants paid greater attention to neonicotinoid labels, while not knowledgeable participants paid more visual attention to the image of the (entire) product. The first and last gaze cascade effect (Shimojo et al., 2003) was confirmed by a significant negative impact of participants' first fixation to labels disclosing the presence of neonicotinoids on their bid values. A first fixation on neonicotinoid labels decreased participants' WTP for labels disclosing the presence of neonicotinoids (i.e., treated with neonicotinoid), but had no impact on their WTP for labels disclosing the absence of neonicotinoids (i.e., neonicotinoid-free text or logo). We also found evidence of an asymmetric impact of attribute focus on consumers' WTP. While increased visual attention (measured by relative total fixation counts) on neonicotinoid labels reduced bids for labels treated with neonicotinoid by 91 cents, there was no effect on participants' bids for neonicotinoid-free text and the neonicotinoid-free logo. Even though consumers may have visually attended to both positive (i.e., absence of neonicotinoids) and negative information (presence of neonicotinoids), it was the negative information that carried through to the final decision. Consumer's focus on a particular attribute may not necessarily alter his/her bid value and WTP.

Future studies may expand this research and compare consumers' preferences for neonicotinoids-free labeling with other eco-labels or sustainable practices (e.g., pesticide-free, organic, naturally produced, and GMO-free).

Literature Cited

Lusk, J.L., T. Feldkamp, and T.C. Schroeder. 2004. Experimental auction procedure: Impact on valuation of quality differentiated goods. Am. J. Agric. Econ. 86(2):389–405.

Shimojo, S., C. Simion, E. Shimojo, and C. Scheier. 2003. Gaze bias both reflects and influences preference. Nat. Neurosci. 6:1317–1322.

*Corresponding author. Email: hayk@ufl.edu

Proc. Fla. State Hort. Soc. 132:223. 2019.

—Scientific Note—

Preferences for Florida-Friendly Residential Landscapes: The Interplay between Monetary Incentives and Environmental Attitudes

Xumin Zhang and Hayk Khachatryan*

[1]*Mid-Florida Research and Education Center, University of Florida, 2725 Binion Rd., Apopka, FL 32703*

Additional index words. environmental concern; latent class logit model, rebate

This study investigated the link between homeowners' self-reported environmental concerns (EC), rebate incentives, and preferences for Florida-Friendly Landscapes (FFL). Specifically, it tested whether the EC scale predicts homeowners' engagement in pro-environmental landscaping practices. We also investigated the effects of the interplay between environmental attitudes and rebate incentives on homeowners' landscape preferences. We used the discrete choice experiment method with eight choice scenarios to simulate different residential landscape designs. A series of landscape images and attribute tables was displayed as choice sets on a panel of eight 75-inch digital screens for participants making landscape choice. A total of 132 participants successfully completed the experiment and were included in the analysis.

Results revealed that the study participants preferred most FFL attributes. However, special attention should be paid to the designs with 100% non-turfgrass coverage, which was found to be the least preferred landscape design. Because study participants preferred low maintenance landscapes, it is necessary to disentangle the link between the proportion of non-turfgrass area and maintenance requirements. The main misperception among the study participants was that the designs with more plants necessarily require a higher level of maintenance. In addition, environmental attitudes are significant predictors for segmenting homeowners into distinct groups. Participants' average EC score was 3.81 (out of 5). Incorporating the EC score in the latent class mixed logit model, two latent classes were identified as environment-oriented homeowners (latent class 1) and turfgrass-oriented homeowners (latent class 2), based upon homeowners' heterogeneous preferences. Seventy-one percent of study participants were assigned to the environment-oriented group, and 29% were assigned to the turfgrass-oriented group. The environment-oriented group preferred all the landscape design attributes and can be positively influenced by rebate incentives, while the turfgrass-oriented group preferred only the traditional turfgrass and low maintenance requirement attributes. The two segments can be significantly differentiated by study participants' environmental attitudes, age groups, landscape practices, and FFL related knowledge. Participants in the environment-oriented group had high EC scores, were less likely to be younger age groups (e.g., millennials age group), preferred DIY landscaping, and were aware of the FFL program. In contrast, study participants in the turfgrass-oriented group had lower and insignificant EC scores and were more likely to be in the younger age group, preferred hiring landscaping service providers (as opposed to DIY), and were less aware of the FFL program. Results also revealed that the effect of rebate incentives was substantively different between the two groups. The effect of the interplay between environmental attitudes and rebate incentives was more significant and positive for the environment-oriented group than the turfgrass-oriented group. These results provide resource conservation policymakers and industry stakeholders with practical information for designing and developing effective programs.

These findings provide evidence that using rebate incentives to promote FFL without incorporating homeowners' environmental attitudes and related segmentation can be less effective and even misleading. Since rebate incentives are a significant monetary outlay, future research could focus on mechanisms for incentive programs that incorporate environmental attitudes.

*Corresponding author. Email: hayk@ufl.edu

Proc. Fla. State Hort. Soc. 132:224 2019.

—Scientific Note—

What Eco-label Format is the Most Effective in the Green Industry?

Alicia Rihn, Hayk Khachatryan*, and Xuan Wei

Mid-Florida Research and Education Center, University of Florida, 2725 Binion Rd., Apopka, FL 32703

Additional index words. experimental auction, heirloom, industry eco-label, non-GMO

A non-hypothetical second-price auction was paired with eye tracking technology to assess the relationship between Floridian homeowners' preferences, valuation, and visual attention to eco-labels presented as either logos or text on fruit-producing plants. A total of 82 people participated in the study, of which, 53 respondents had their eye movements recorded. Each respondent submitted bids on 14 plants displaying eco-labels with different formats.

Participant's willingness-to-pay (WTP) for each product and label was estimated using random effects tobit models. Three different eco-labels were represented using a logo, text, or absent option. The labels included an industry eco-label, non-GMO eco-label, and heirloom eco-label. Participants indicated they were most knowledgeable about the non-GMO and heirloom eco-labels and least knowledgeable about the industry eco-label. The non-GMO eco-label logo and heirloom eco-label logo captured more visual attention than their text counterparts. Conversely, the industry eco-label logo captured less visual attention than the text counterpart. However, the industry eco-label text version included informative text because researchers anticipated that participants were less familiar with the eco-label. The informative text indicated that the industry eco-label symbolized environmentally friendly production, fair labor practices, and product quality. Given respondents' low knowledge level and the additional informative text, one would expect the industry eco-label text version to capture more visual attention than the logo version. In general, the presence of eco-labels improved participants' WTP when compared to plants without the eco-labels. Participants were willing to pay \$0.72 to \$0.80 more for fruit-producing plants with the industry eco-label logo and \$1.07 to \$1.16 more to the industry eco-label text version when compared to plants without the industry eco-label. The non-GMO eco-label logo generated a premium of \$1.11 to \$1.12 while the text version generated a premium of \$0.63 to \$0.76 compared to no non-GMO label. The heirloom eco-label logo did not generate a premium; however, the text version generated a \$0.73 to \$0.87 premium compared to no label. Great visual attention to the industry eco-label logo resulted in a premium of \$1.40 while visual attention to the industry eco-label text version and non-GMO eco-label text resulted in discounts of –\$0.38 and –\$1.16 respectively. These results indicate there is potential to use eco-labeling strategies to differentiate products and generate additional value for fruit-producing plants.

Future studies can address additional questions about eco-labeling, branding, valuation, and visual attention. For instance, the inclusion of different, well-known eco-labels (e.g., organic) and/or brands could aid in further understanding consumers' decision-making processes for ornamental plants. Furthermore, the inclusion of participants in different geographical areas (rather than just residents of Florida) could assist in testing the robustness of the results.

*Corresponding author. Email: hayk@ufl.edu

Proc. Fla. State Hort. Soc. 132:225. 2019.

Evaluation of Light Quality and Light Levels for In Vitro Production of Ornamental Bananas

Cassandre Feuille* and Wagner Vendrame

Environmental Horticulture Department,
University of Florida Tropical Research and Education Center,
18905 SW 280th St., Homestead, FL 33031

Additional index words. banana, chlorophyll content, leaf anatomy, light sources, light-emitting diodes (LEDs), micropropagation, plant growth and development, stomata

Light emitting diodes (LEDs) have become very popular in agriculture, particularly for the production of horticultural crops, such as vegetables, fruits, and ornamentals. LEDs represent an alternative lighting source to regular fluorescent bulbs, which not only increase the quality of in vitro plantlets, but also minimize the per plant production costs. In addition, LEDs can provide selective light intensity and quality, which makes them suitable for commercial micropropagation. The development of a suitable protocol for the commercial micropropagation of banana using traditional in vitro techniques that address low energy inputs is essential for the improvement of banana production, particularly in countries that have limited or unreliable power supply, such as Haiti. The main objective of this study was to evaluate the effects of light quality and light levels for in vitro production of ornamental bananas.

Materials and Methods

Two ornamental banana varieties were selected for this study; *Musa* 'Little Prince' and *Musa* 'Truly Tiny'. Three different light sources were evaluated, including: 1) fluorescent light bulbs (control) with intensity of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$; 2) low intensity blue and red LEDs (90 $\mu mol\ m^{-2}\ s^{-1}$); and 3) high intensity white LEDs (116 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Light quality distribution showed peak emissions of 100% red (660 nm)/20% blue (440 nm) for high intensity LED, 100% red (660 nm)/10% blue (440 nm) for low intensity LED and a wider distribution of the spectrum for fluorescent lights, ranging from 30% at 440 nm, 25% at 490 nm, 100% at 540 nm, 30% at 590 nm, 80% at 620 nm, and 10% at 710 nm.

Banana in vitro growth and development were evaluated, including plant size, stem diameter, fresh and dry weight of shoots and roots, number of leaves, number and length of roots, chlorophyll content, and stomata size and number. In addition, leaf anatomy was evaluated, including the thickness of the abaxial surface epidermis, adaxial epidermis, abaxial hypodermis, adaxial hypodermis, palisade parenchyma, and spongy parenchyma.

Results and Discussion

Although no significant differences in leaf anatomy were observed among treatments, cells of the palisade parenchyma were more defined and showed an elliptical shape under LED lights, while they were not well defined under fluorescent lights. The general morphology of plantlets was very homogeneous under all light treatments evaluated, and the number of leaves was similar among treatments. Significant increases in the length of shoots and roots, and biomass of plantlets were observed under the higher intensity LEDs (116 $\mu mol \cdot m^{-2} \cdot s^{-1}$), but they were not significantly different from the lower intensity LEDs (90 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

Values for growth and development and chlorophyll content were significantly higher for banana plants under LEDs as compared to fluorescent lights. For the fresh and dry weight of shoots and roots, number of leaves, number and length of roots there were no significant difference between treatments. Chlorophyll content was significantly greater under both LED light conditions as compared to fluorescent lights. Stomata size and number were higher for banana plants under fluorescent lamps. Most of the stomata under fluorescent light had guard cells round shaped and wide open while the stomata under LEDs were ellipsoid shaped and remained closed. This suggests that bananas produced under fluorescent light probably lost their ability to open and close the stomata, that is to regulate transpiration. Banana in vitro plants grown under high and low intensity LEDs had the smallest number and size of stomata whereas those under fluorescent light had the largest number and biggest stomata size.

Our results indicate that shoot length and biomass could be improved by controlling light quality and intensity. However, the effects of light quality and intensity related to banana plant in vitro growth and development in general, including the other parameters evaluated could be expanded and fully investigated in future studies. Furthermore, although the number of shoots per plantlet produced in vitro (multiplication) were not considered during this particular study, some of the plantlets grown under the LED lights produced multiple shoots, while no additional shoots formed from plants grown under fluorescent lights (control). Therefore, in vitro multiplication as affected by LED light intensity and quality is another study of interest for the future.

*Corresponding author. Email: c.feuille@ufl.edu

Proc. Fla. State Hort. Soc. 132:226. 2019.

—Scientific Note—

Educating At-risk Students about Gardening and Life with Master Gardener Volunteers

Wayne H. Hobbs*

University of Florida/IFAS, Clay County Extension, 2463 State Rd. 16 West, Green Cove Springs, FL 32043

Additional index words. healthy lifestyles, horticulture

The University of Florida/IFAS Clay County Extension (UF/IFAS Extension)has been working extensively with Bannerman Learning Center, which conducts an alternative program for students with intellectual disabilities, behavior issues, and different educational needs, in Green Cove Springs, FL, for almost 20 years and this partnership continues to expand.

This program originally started with a group of Master Gardeners and a plan to develop an unused section of the school grounds for a garden to educate the students about gardening and food production. This area is now developed with a student-built greenhouse, gazebo, succulent garden, fish pond, herb garden, several ornamental areas, and a vegetable garden. However, in 2017 volunteers and UF/IFAS Extension faculty went to work to expand the scope and impact of this long-established project.

Extension volunteers met with faculty to discuss needs and goals for this program and the decision was made to include the school as part of a grant with the AETNA Foundation. The goal became to improve the overall health of students by educating them about horticulture and healthy lifestyles and the garden was updated to include hydroponics systems and new vegetable garden areas. Through this process, all in-ground vegetable gardens were replaced with raised beds with drip irrigation and three different types of hydroponic systems were installed by students. This allowed access to produce for students as well as train them on the use of current horticultural technologies. Further expansion is planned in Spring 2019 through the installation of an orchard through a grant with the Whole Foods Foundation.

Over the last three years, Master Gardener volunteers donated 791 hours working with youth and have put in an additional 606 hours so far this year. In doing so over 4500 educational interactions were given to students. Eight hundred pounds of produce has also been grown and given to students.

The successful partnership between faculty, volunteers, and school staff has allowed for this project to better meet the needs of the students and establish plans and goals to make it viable for years to come.

*Corresponding author. Email: whhobbs@ufl.edu

Proc. Fla. State Hort. Soc. 132:227. 2019.

—Scientific Note—

Master Educators: Effectiveness of a Pilot Advanced Master Gardener Volunteer Educator Training to Improve Skills and Increase Confidence

Wayne H. Hobbs*

University of Florida/IFAS, Clay County Extension, 2463 State Rd. 16 West, Green Cove Springs, FL 32043

Additional index words. education, gardening, volunteer

The University of Florida/IFAS Extension Master Gardener Volunteer program in Clay County is robust, with 78 active members educating over 12,000 people each year through workshops, plant clinics, and other educational outreach events. However, with Clay County rapidly expanding in population, it is necessary to build the skills of new and current Master Gardeners to reach our audience as people access information in new ways. Furthermore, many Master Gardener volunteers are hesitant to interact with the public as they do not have the experience or training to plan and implement workshops, answer and research homeowner questions, or create articles and other written works.

To address this need a series of courses was developed and implemented where volunteers actively develop their educational skills through both in-class and experiential learning. This course has been completed by nine volunteers, all of whom shared that they gained new knowledge and skills and now feel more confident as a Master Gardener. Also, since March 1, 2019, participants have completed three written creative works, taught eight new workshops, and volunteered 44 hours in educational plant clinics. This course was very well received and another series is planned for later in the year 2019 to expand its reach to other volunteers and increase its impact.

*Corresponding author. Email: whhobbs@ufl.edu

Proc. Fla. State Hort. Soc. 132:228–232. 2019.

A Master Gardener Survey: Promoting Pollinator-friendly Plants Through Education and Outreach

Heather Kalaman[1], Gary W. Knox[2], Sandra B. Wilson[1], and Wendy Wilber[3]

Additional index words. **Florida gardening, insect identification, native bees, plant identification, pollinator conservation**

Summary. **As land-use patterns change over time, some pollinating insects continue to decline both in abundance and diversity. This is due, in part, to reductions in floral resources that provide sufficient nectar and pollen. Our overall goal is to help increase the use of plants that enhance pollinator health by providing research-based information that is easily accessible to the public. To assess the most successful mode of sharing this information, a survey was distributed to more than 4000 Master Gardener (MG) volunteers of Florida. The objectives of our survey were to gauge both knowledge and interest in common pollinators, common pollinator-friendly floral resources, and a favored means of accessing material about additional pollinator-friendly plants for landscape use. With a response rate of just over 18%, results showed that there is a clear interest among Florida MGs in learning more about pollinators and pollinator-friendly plants with face-to-face classes followed by a website as the preferred modes of accessing educational materials on this topic. Respondents on average were extremely interested in learning more about pollinator plants [mean of 4.41 out of 5.0 (SD = 0.89)], with greatest interest in butterflies/moths (Lepidoptera), followed by bees (Hymenoptera), birds (Aves), bats (Chiroptera), and beetles (Coleoptera). Overall, MG participants felt more confident ($P < 0.0001$) in their knowledge of pollinator-friendly plants (mean 3.24 out of 5.0) than pollinator insects (mean 3.01 out of 5.0). When tested, 88.5% were able to correctly identify black-eyed susan (*Rudbeckia hirta*), with 70.1% correctly identifying spotted beebalm (*Monarda punctata*). Variations were observed in tested knowledge of pollinating insects, with 90.2% correctly identifying a zebra longwing (*Heliconius charithonia*) and only 32.6% correctly identifying a striped-sweat bee (*Agapostemon splendens*). These results revealed that MGs perceived themselves to be fairly knowledgeable about both pollinator plants and pollinating insects, yet their tested knowledge ranged widely depending on the actual plant and pollinator type. This suggests an emphasis be given for future MG training focused on diverse plant and pollinator species, preferably in a face-to-face environment. Results also show that additional resources regarding pollinator-friendly plants, as well as identification material on pollinating insects, are both desired and valued by our Florida MG community.**

The significant contributions made by pollinating insects to the prosperity and health of our many ecosystems are well known among both the public and the scientific community (Novacek, 2008; Smith, 2016). Not only are the pollination services provided by these insects fundamental for the proliferation of some of our most prized cultivated crops, but nearly 90% of all wild plants depend on insects to survive and flourish (Ashman et al., 2004; Hoshiba and Sasaki, 2008). Nonetheless, as our land-use patterns have been altered over time, some pollinating insects have continued to decline both in abundance and diversity due, in part, to reductions in floral resources that provide sufficient nectar and pollen (Foley et al., 2005). One possible way to help mitigate these threats is to increase the use of plants that enhance pollinator health by generating research-based information that is easily attainable by the public. In a recent survey, 46% of consumers purchased pollinator-friendly plants for their home landscape, noting their enthusiasm for both aiding in pollinator health and their attractive landscape qualities (Campbell et al., 2017). Information regarding gardening and landscape use plants is available in many formats from phone-based applications, websites, webinars, and face-to-face classes (Varlamoff et al., 2002). Depending on the demographics of the community, various sources are relied on more heavily than others. Earlier studies have shown, for example, that information from Internet sources was less commonly acquired by participants over age 61 years than by younger survey participants. Notably, when these survey participants were asked what the most important attribute was in accessing and retaining gardening information, convenience and interaction ranked the highest (Meyer and Foord, 2008).

Creating opportunities for the public to conveniently access educational materials on pollinators and pollinator-friendly plants with an interactive tone can be accomplished in a variety of ways. Through encouragement and employment of enthusiast community members and apprentice naturalists, enormous strides can be made toward the sustainability of our pollinators. For example, volunteer-based citizen science groups have been invaluable contributors to an array of data scientists rely on every day due to the widespread use of mobile computing, large-scale and free computational power, and the personal satisfaction gained from participating in ongoing research (Bonney et al., 2009; Silvertown, 2009). Remarkably, data collected through an online database for bird observations (eBird; Cornell University, Ithaca, NY) has been used in at least 90 peer-reviewed articles and book chapters on a wide range of ornithological topics (Bonney et al., 2014). Surely, the most effective tool for public acknowledgment and comprehension of scientific information is through cooperation. Therefore, the objectives of our survey were to gauge overall MG desire to learn more about pollinators and pollinator-friendly plants, to assess both perceived and tested knowledge of common pollinators and their associated floral preferences, and to determine the preferred means of accessing educational material about landscape use plants that will aid in supporting our pollinating insect communities.

Materials and methods

Survey. A survey questionnaire was constructed to anonymously assess the interest and knowledge Florida MGs have related to pollinator plants. The resulting survey (approved by the University of Florida Institutional Review Board, ID number 201802622) comprised 16 questions consisting of free response, image-based identification, Likert scale, and multiple choice. Questions focused on 1) gauging overall desire to learn more about pollinators and their preferred floral resources, 2) assessing both perceived and tested knowledge of common pollinators and common pollinator-friendly plants of Florida, and 3) ascertaining the preferred means of accessing educational material about floral resources that will aid in supporting pollinating insect communities (Table 1).

Survey distribution. The survey was designed and implemented using an online software platform (Qualtrics XM; Qualtrics, Provo, UT). This delivery format was employed as online surveys have proven more effective than standard mail surveys due to their affordability, convenience, speed and timeliness (Evans and Mathur, 2005). The survey was distributed by the Florida Statewide MG Volunteer Program Coordinator, using an e-mail list-serve address representing all 4000 registered MGs. MG volunteers were our target audience for this study because they have historically and consistently displayed great interest in growing and learning about landscape-use plants. As a volunteer-driven organization working closely with the University of Florida's Institute of Food and Agricultural Sciences Extension Service (UF/IFAS), they work diligently not only to expand their own knowledge of horticulture but also to share that knowledge with their communities. The survey link was accompanied with a letter introducing members of the project, a mission statement, instructions for further survey involvement, and a message of gratitude for participation because studies have shown personalization and salient acknowledgment contribute to better response rates (Schaefer and Dillman, 1998). Individual responses were recorded and remained anonymous throughout the course of survey data collection because assured anonymity tends to lend itself to more sincere or honest answers (Stanton, 1998). To avoid some potential weaknesses of online surveys, such as a respondent's lack of online experience or expertise and technological variations (Evans and Mathur, 2005), the survey remained open for 6 weeks, and all configuration issues and inquiries were answered accordingly.

The mean and standard deviation of survey responses were calculated using Qualtrics XM. When appropriate, means were compared using a paired Wilcoxon signed-rank test implemented in SAS (version 9.2; SAS Institute, Cary, NC) at $P \leq 0.05$.

Table 1. Sixteen questions included within the online survey instrument emailed to 4000 Master Gardeners in Florida to gauge both knowledge and interest in common pollinators, common pollinator-friendly floral resources, and a favored means of accessing material about additional pollinator-friendly plants for landscape use.

1. How confident are you in your ability to identify different pollinators? (0–5 scale)
2. Do you feel more interested in specific types of pollinators? (choose all that apply)
3. How familiar are you with pollinator-friendly plants? (0–5 scale)
4. How interested are you in learning more about pollinator-friendly plants? (0–5 scale)
5. Do you currently grow any pollinator-friendly plants? (yes or no)
6. How many different species of pollinator-friendly plants do you grow? (selected category)
7. Do you currently use any nature-oriented phone applications or websites? (yes or no)
8. What, if any, nature-oriented phone applications or websites do you typically use? (choose all that apply)
9. If you answered "Other," what phone application or website do you commonly use? (open answer)
10. What is it about this phone application or website that you enjoy the most? (choose all that apply)
11. If you answered "Other," why do you enjoy this phone application or website? (open answer)
12. What would be the most successful mode of learning more about pollinators and pollinator-friendly plants? (open answer)
13. Name this pollinator-friendly plant (image). (multiple choice)
14. Name this pollinator-friendly plant (image). (multiple choice)
15. Name this pollinator (image). (multiple choice)
16. Name this pollinator (image). (multiple choice)

Results and discussion

Survey response. The online survey was initiated by 970 participants, with 731 successfully completing all 16 questions (18.3% response rate, 75.4% completion rate).

Overall desire to learn more about pollinators and pollinators plants. The aim of the first portion of the survey was to assess overall interest in insect and non-insect pollinators, as well as the desire among our MG participants to learn more about pollinator-friendly resources. When asked to select each of the pollinators they felt most interested in [butterflies and moths (Lepidoptera), bees (Hymenoptera), birds (Aves), bats (Chiroptera), and beetles (Coleoptera)], "butterflies and moths" received the greatest number of responses (821), followed by "bees" (729), "birds" (628), "bats" (326), and "beetles'" (209) (Fig. 1). Participants were then asked how interested they were in learning more about pollinator-friendly plants for landscape use on a Likert scale ranging from 0 to 5 (0 = not at all interested, 5 = extremely interested). With 797 respondents, results showed a mean of 4.41 out of a possible of 5 (SD = 0.89) (Table 2). Results from this portion of our survey

Received for publication 17 July 2019. Accepted for publication 17 Dec. 2019.

Published online 24 January 2020.

[1]Institute of Food and Agricultural Sciences Extension Service (IFAS), Department of Environmental Horticulture, University of Florida, P.O. Box 110675, Gainesville, FL 32611

[2]IFAS, Department of Environmental Horticulture, North Florida Research and Education Center, University of Florida, 155 Research Road, Quincy, FL 32351

[3]IFAS, Center for Landscape Conservation and Ecology, University of Florida, P.O. Box 110675, Gainesville, FL 32611

We gratefully acknowledge the U.S. Department of Agriculture, National Institute of Food and Agriculture, Crop Protection and Pest Management Extension Implementation Project for funding this project and James Cooley for statistical consultation of survey results.

This research was presented as a poster and paper proceeding at the Florida State Horticultural Society Annual Meeting, 9–11 June 2019, Orlando, FL.

H.K. is the corresponding author. E-mail: hkalaman@ufl.edu.

https://doi.org/10.21273/HORTTECH04460-19

indicated that there is a strong desire by MG volunteers to learn more about pollinator-friendly plants, and that of the five pollinator choices listed, MGs are most interested in butterflies/moths and bees. Of interest to note is that 95.4% reported that they already grow pollinator-friendly plants. When asked how many species they grow, 78.1% reported growing fewer than 20 species and 21.9% reported growing more than 20 species (Fig. 2). Studies have shown that an increase in the diversity of floral species planted plays a significant role in both pollinator abundance and diversification, due to prolonged seasonal resource value and availability of a wide range of pollen and nectar sources (Potts et al., 2003; Williams et al., 2015).

Perceived and tested knowledge. The second objective of the survey was to gauge MGs perceived knowledge of both common insect pollinators and popular pollinator-friendly floral resources and to test that perceived knowledge. When asked how familiar they were with pollinator-friendly plants on a Likert scale from 0 to 5 (0 = not at all knowledgeable, 5 = extremely knowledgeable), the mean response was 3.29, showing a slightly above moderate familiarity. When asked how confident they were in their ability to identify different pollinators, the mean was 3.01 (731 respondents), slightly lower than reported for pollinator-friendly plants (Table 2).

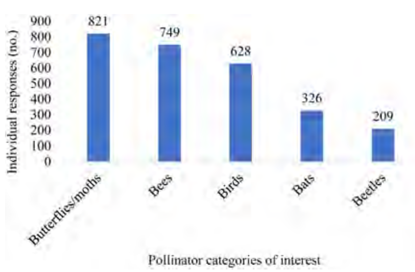

Fig. 1. Individual Florida Master Gardener responses when asked to select each of the pollinators types they felt most interested in from five choices: butterflies/moths, bees, birds, beetles, and bats.

When tested on plants and insect pollinators, on average MGs performed higher in their tested knowledge of plants compared with insects (Table 2). For example, when shown a photo of a commonly advertised pollinator plant, spotted beebalm (*Monarda punctata*), 587 of 838 respondents (70.1%) were able to correctly identify an image of this floral resource. Similarly, when shown a photo of a black-eyed susan (*Rudbeckia hirta*), 744 of 841 respondents (88.5%) were able to correctly identify this plant. MGs tested knowledge of two common pollinating insects varied depending on the image provided. When shown an image of the Florida state butterfly, the zebra longwing (*Heliconius charithonia*), 731 of 833 respondents (90.2%) were able to correctly identify it (Table 2). Yet when shown an image of a Florida native striped-sweat bee (*Agapostemon splendens*), 270 of 829 respondents (32.6%) were able to correctly identify this bee pollinator.

In addition to providing information on which plants best serve as beneficial floral resources to our pollinating insect communities, educational materials to aid in the identification and conservation of our native bees here in the United States is crucial. Growing interest and concern for insect pollinators, particularly bees, is evident through recent studies that have shown that 99% of surveyed respondents believed bees were critically important in their ecosystem services (Wilson et al., 2017). However, the ability to distinguish bee types is less common. For example, Wilson et al. (2017), found that these same survey respondents were largely only able to successfully identify honeybees (*Apis mellifera*) and bumble bees (*Bombus* sp.) compared with other bee species. This is not surprising with recent bee campaigns associated with the extensive losses in honeybee communities due to various pests, pathogens, climatic changes, and other stressors (Pettis and Delaplane, 2010). With more than 4000 currently described species of native bees in North America, Florida is home to more than 300 species, 29 of which are entirely endemic to the state (Michner, 2000; Pascarella et al., 1999). Resources that aid in the identification of native bee communities will help in educating consumers about the diversity of existing bee species.

Preferred mode of learning. The last portion of our survey was designed to determine the most successful means of both developing and distributing educational materials about pollinating insects and pollinator-friendly plants for landscape use. When MGs were asked to identify their preferred mode of learning about pollinators and pollinator-friendly plants, a face-to-face class was preferred (36.1%), followed by an online website (21.6%) (Fig. 3). Preference for a face-to-face class may be influenced by the fact that the median age range of a MG volunteer is 65 and the majority are retired, addressing the possibility of technological apprehension as well as the probable unrestricted free time to dedicate to in-person classes (Dorn et al., 2018; Relf and McDaniel, 1994; Vines et al., 2016). It should be noted that information technology–based training programs still serve as effective educational resources as they have shown to have fewer missed participants, can be more cost effective, and produce similar levels of mastery of material as face-to-face training

Table 2. Master Gardener participants survey results showing overall interest in learning more about pollinator-friendly plants and their perceived and tested knowledge of identifying pollinators and pollinator-friendly plants.[z]

	Perceived knowledge [mean ± SD (0–5 scale)][y]	Tested knowledge [mean (% correct response)][x]	Interest to learn more [mean ± SD (0–5 scale)][w]
Pollinator-friendly plants			4.41 ± 0.89
Black-eyed susan		88.5	
Spotted beebalm		70.1	
Pollinators	3.01 ± 1.21		
Zebra longwing		90.2	
Striped-sweat bee		32.6	

[z]Means were significantly different based on Wilcoxon signed-rank *t* test at $P \leq 0.05$.
[y]0 = not knowledgeable, 5 = extremely knowledgeable.
[x]Multiple-choice format.
[w]0 = not at all interested, 5 = extremely interested.

programs (Lim et al., 2007) In fact, when asked whether they currently use any nature-oriented phone applications or websites, 532 of 859 respondents (61.9%) answered "yes" (data not presented).

When asked to select the nature-oriented phone applications or websites they most frequently use from a list of five, respondents most frequently chose Facebook Groups [Facebook, Menlo Park, CA (21%)], followed by National Audubon Society [Manhattan, NY (18.9%)], and the phone application PlantNet [Paris, France (10.5%)] (data not presented). The most common reasons for use of these websites or phone applications were that they were easy to use, free, and interactive. We additionally allowed for an open answer option for this question ("other"), completed by 37.9% of respondents. It is of interest to note that of these open answer responses, many respondents (44.3%) answered "UF/IFAS" and/or "EDIS" (Electronic Data Information Sources of UF/IFAS Extension) resources, followed by the phone application PictureThis [Glority, Hangzhou, China (9.8%)], Florida Native Plant Society [Winter Park, FL (5.7%)], and the phone application PlantSnap [PlantSnap, Telluride, CO (4.7%)] (data not presented). Participants stated they use these other resources because they are research based, reviewed by the scientific community, and state specific.

In summary, results from our survey revealed a strong MG interest in learning more about pollinator-friendly plants, as well as specific types of pollinators. Although a significant number of Florida MG volunteers acknowledged that they already grow several pollinator-friendly plants in their landscape, providing additional research-based materials on pollinator-friendly plants and pollinating insects will allow for both expansion of MG knowledge on this topic and the dispersal of that knowledge to larger audiences with the overall goal of supporting pollinating insect communities. Differences in perceived and tested knowledge of both pollinator-friendly plants and insect pollinators also suggest an emphasis to be given in developing targeted extension programs to meet these needs. By providing additional identification materials to our MG volunteers on important pollinating insects such as Florida native bee communities, we can try to alleviate some of the hardship these organisms face as a result of our changing landscape patterns.

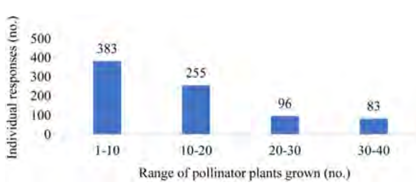

Fig. 2. Individual Florida Master Gardener responses when asked how many pollinator-friendly plants they currently grow in their home garden/ landscape.

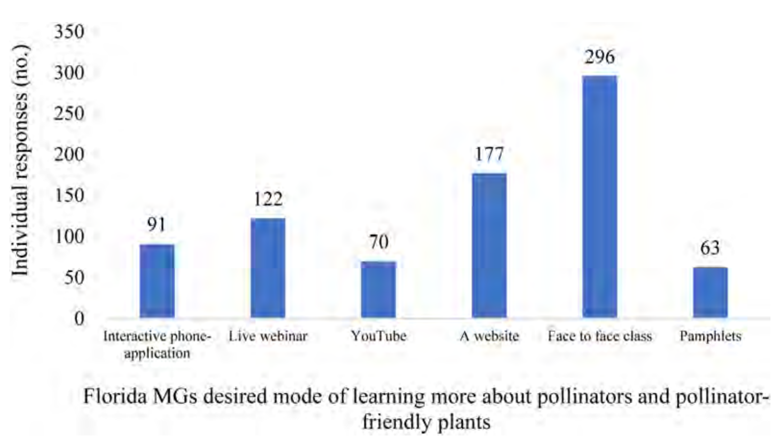

Fig. 3. Individual Florida Master Gardener (MG) survey responses when asked their preferred mode of learning more about pollinators and pollinator plants from six choices: interactive phone-application, live webinar, a YouTube video sharing platform (YouTube, San Bruno, CA), a website, face-to-face class, and pamphlets.

Literature cited

Ashman, T.L., T. Knight, J. Steets, P. Amarasekare, M. Burd, D. Campbell, M. Dudash, M. Johnston, S. Mazer, R. Mitchell, M. Morgan, and W. Wilson. 2004. Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. Ecology 85:2409–2421.

Bonney, R., C. Cooper, J. Dickinson, S. Kelling, T. Phillips, K. Rosenberg, and J. Shirk. 2009. Citizen science: A developing tool for expanding science knowledge and scientific literacy. Bioscience 59:977–984.

Bonney, R., J. Shirk, T. Phillips, A. Wiggins, H. Ballard, A. Miller-Rushing, and J. Parrish. 2014. Next steps for citizen science. Science 342:1436–1437.

Campbell, B., H. Khachatryan, and A. Rihn. 2017. Pollinator-friendly plants: Reasons for and barriers to purchase. HortTechnology 27:831–839.

Dorn, S.T., M.G. Newberry, III, E.M. Bauske, and S.V. Pannisi. 2018. Extension master gardeners of the 21st century: Educated, prosperous, and committed. HortTechnology 28:218–229.

Evans, J. and A. Mathur. 2005. The value of online surveys. Internet Res. 15:195–219.

Foley, J.A., R. DeFries, G. Asner, C. Barford, G. Bonan, S. Carpenter, F.S. Chaplin, M. Coe, G. Daily, H. Gibbs, J. Helkowski, T. Holloway, E. Howard, C. Kucharik, C. Monfreda, J. Patz, C. Prentice, N. Ramankutty, and P. Snyder. 2005. Global consequences of land use. Science 309: 570–574.

Hoshiba, H. and M. Sasaki. 2008. Perspectives of multi-modal contribution of honeybee resources to our life. Entomol. Res. 38:15–21.

Lim, H., S. Lee, and K. Nam. 2007. Validating e-learning factors affecting training effectiveness. Intl. J. Inf. Mgt. 27:22–35.

Meyer, M. and K. Foord. 2008. Consumer preferences and perceptions of gardening information. HortTechnology 18:162–167.

Michner, C.D. 2000. Bees of the world. 2nd ed. Johns Hopkins Univ. Press, Baltimore, MD.

Novacek, M. 2008. Engaging the public in biodiversity issues. Proc. Natl. Acad. Sci. USA 105:11571–11578.

Pascarella, J., K. Waddington, and P. Neal. 1999. The bee fauna (Hymentoptera: Apoidea) of Everglades National Park, Florida and adjacent areas: Distribution, phenology, and biogeography. J. Kans. Entomol. Soc. 72:32–45.

Pettis, J. and K. Delaplane. 2010. Coordinated responses to honeybee decline in the USA. Apidologie 41:256–263.

Potts, S., B. Vulliamy, A. Dafni, G. Ne'eman, and P. Willmer. 2003. Linking bees and flowers: How do floral communities structure pollinator communities. Ecology 84:2628–2642.

Relf, D. and A. McDaniel. 1994. Assessing master gardeners' priorities. HortTechnology 4:181–184.

Schaefer, R. and D.A. Dillman. 1998. Development of a standard email methodology: Results of an experiment. Public Opin. Q. 62:378–397.

Silvertown, J. 2009. A new dawn for citizen science. Trends Ecol. Evol. 24:467–471.

Smith, T. 2016. Honeybees: The queens of mass media, despite minority rule among insect pollinators. Insect Conserv. Divers. 9:384–390.

Stanton, J.M. 1998. An empirical assessment of data collection using the internet. Person. Psychol. 51:709–726.

Varlamoff, S., W. Florkowski, J. Latimer, S.K. Braman, and J. Jordan. 2002. Homeowners and their choice of information sources about gardening. J. Ext. 40(3): 3FEA7. 15 May 2019. <https://www.joe.org/joe/2002june/a7.php>.

Vines, K.A., K. Jeannette, E. Eubanks, M. Lawrence, and R. Radharishna. 2016. Extension master gardener social media needs: A national study. J. Ext. 54(2): 2FEA5. 7 Nov 2019. <https://joe.org/joe/2016april/a5.php>.

Williams, N., K. Ward, N. Pope, R. Isaacs, J. Wilson, E. May, J. Ellis, J. Daniels, A. Pence, K. Ullmann, and J. Peters. 2015. Native wildflower plantings support wild bee abundance and diversity in agricultural landscapes across the United States. Ecol. Appl. 25:2119–2131.

Wilson, J., M. Forister, and O. Carril. 2017. Interest exceeds understanding in public support of bee conservation. Front. Ecol. Environ. 15:460–466.

This paper was presented during the 2019 FSHS Annual Meeting and originally published in HortTechnology [HortTechnology 30(2):163–167. 2020. https://doi.org/10.21273/HORTTECH04460-19]. The paper is included in this Proceedings as a reprint (with permission).

Proc. Fla. State Hort. Soc. 132:233–237. 2019.

Morphological and Cytological Comparisons of Eight Varieties of Trailing Lantana (*Lantana montevidensis*) Grown in Florida

Carlee Steppe and Sandra B. Wilson
Department of Environmental Horticulture, University of Florida, Institute of Food and Agricultural Sciences, P.O. Box 110675, Gainesville, FL 32611

Zhanao Deng and Keri Druffel
Department of Environmental Horticulture, University of Florida, Institute of Food and Agricultural Sciences; and Gulf Coast Research and Education Center, 14625 County Road 672, Wimauma, FL 33598

Gary W. Knox
Department of Environmental Horticulture, University of Florida, Institute of Food and Agricultural Sciences; and North Florida Research and Education Center, 155 Research Road, Quincy, FL 32351

Additional index words. invasive plants, ploidy, pollen stainability, tetraploid, triploid

***Abstract*. Trailing lantana (*Lantana montevidensis*) is a popular low-growing ornamental plant valued for its heat and drought tolerance and continuous purple or white flowering throughout much of the year. Recently, trailing lantana was predicted to be invasive by the University of Florida/Institute of Food and Agricultural Sciences (UF-IFAS) Assessment of Non-Native Plants in Florida, and therefore not recommended for use. All cultivars fall under this designation unless proven otherwise. Eight trailing lantana varieties were obtained from wholesale growers and naturalized populations found in Texas and Australia. Plants were propagated vegetatively, finished in 4-inch pots, and planted under field conditions to determine morphological and cytological differences among varieties. Australian trailing lantana differed morphologically from the other varieties in its smaller habit, leaves (which had serrate-crenate leaf margins, and fewer appressed hairs), heavy fruiting, and cold sensitivity (observational reduced growth and flowering during winter months). Nuclear DNA content analysis suggests that Australian trailing lantana is likely a tetraploid and all other varieties evaluated were likely triploids with high levels of sterility. Pollen stainability of Australian trailing lantana was moderately high (58.83%), whereas pollen production was rarely observed in all other varieties. Results support that there are two forms of trailing lantana, the U.S. varieties distinguished by their leaf and flower morphology, ploidy level, and the absence of fruit and viable pollen.**

Received for publication 1 Aug. 2019. Accepted for publication 11 Sept. 2019.
We gratefully acknowledge funding support from the U.S. Department of Agriculture's (USDA) Agricultural Marketing Service and Florida Department of Agriculture and Consumer Services Specialty Crop Block Grant Program, Florida Fish and Wildlife Conservation Commission, and Florida Nursery and Growers and Landscape Association. The contents of this publication are solely the responsibility of the authors and do not necessarily reflect the official view of the USDA. We thank Mark Kann, Chris Harchick, Bart Schutzman, Krishna Bhattarai, Julia Rycyna, and Jackson Jablonski for technical assistance; and Megan Thomas and Adam Black for plant collection assistance.
This paper is based on a presentation given during the Annual Meeting of the Florida State Horticultural Society, which was held 9–11 June 2018, in Orlando, FL.
C.S. is the corresponding author. E-mail: csteppe@ufl.edu.

Trailing lantana (*Lantana montevidensis*) is a low-growing woody shrub native to tropical areas of South America. As early as 1825, the species was described by German botanist Curt Polycarp Joachim Sprengel as *Lippia montevidensis.* This plant has also been described as *Lippia sellowiana* or *Lantana sellowiana* to honor German botanist Friedrich Sellow (or Sello). Finally, in 1904, it was reclassified as *Lantana montevidensis* by the Swiss botanist John Isaac Briquet and this name remains today (International Plant Names Index, 2015). The genus refers to the original Latin name for *Viburnum* "*Viburnum lantana*," having a similar inflorescence structure. The specific epithet is derived from Montevideo, Uruguay, where the plant was first found (Johnson, 2007).

Trailing lantana is listed as an invasive exotic in many subtropical ecosystems from Hawaii and Australia to the southeastern United States. In Australia, due to its rapid expansion and colonization of natural lands and improved pastures, trailing lantana is a restricted invasive prohibited for use by the Queensland Biosecurity Act of 2014 (Day et al., 2003; Johnson, 2007; Munir, 1996; O'Donnell, 2002). In the United States, it has escaped cultivation in seven states, including Alabama, California, Florida, Georgia, Hawaii, Louisiana, and Texas [U.S. Department of Agriculture, National Resources Conservation Service (USDA, NRCS), 2019]. In Florida, herbarium vouchers document its escape in 18 counties (Wunderlin et al., 2019). To date, trailing lantana has not been listed as a Category I or II invasive plant by the Florida Exotic Pest Plant Council (FLEPPC, 2019). However, based on a predictive test, the UF/IFAS Assessment of Non-Native Plants in Florida's Natural Areas does not recommend its use in northern, central, or southern Florida, with results concluding that it has high invasion risk (UF/IFAS Assessment, 2019). All cultivars fall under this conclusion unless proven otherwise and exempted from the ruling.

Trailing lantana is characterized by having a horizontal growth habit, with branches rooting at the nodes. Leaves are opposite, broadly ovate, and strongly aromatic when crushed. Umbel inflorescences, each containing ≈30 florets, are borne on long axillary peduncles ≈30 mm in length. If present, blackberry-like fruit is drupe, each having one seed and up to two embryos. In Australia, Johnson (2007) reported two forms (cultivated and wild) of trailing lantana differing in their ability to produce fruit. The Australian weedy variety was described as having purple flowers with white throats, and the ornamental nonweedy variety (cultivated garden form) was described as having either lilac petals with white to yellow throats, or white petals with yellow throats. Although the lavender variety is more popular in the trade, there is a white-flowered form *L. montevidensis* forma *albiflora* that was collected from the wild in Brazil as early as 1944 (IPNI, 2015; San Marcos Growers, 2019). Similar to the lavender variety, the white form flowers year-round and is drought tolerant and adapted to full sun or part shade in USDA cold hardiness zones 8 to 10 (USDA, 2012).

Henderson (1969) reported that the two forms of trailing lantana in Australia also differed dramatically in pollen viability and ploidy level/chromosome number. The weedy form had ≈65% pollen stainability and was a tetraploid with $2n = 4x = 48$ chromosomes, whereas the garden form had extremely low pollen stainability (less than 6%) and was a triploid with $2n = 3x = 36$ chromosomes. In India, only the triploid trailing lantana was noticed (Raghavan and Arora, 1960).

Although the introduction of new trailing lantana varieties is insignificant compared with *L. camara,* there has been some selection of the species with named patents (Google Patents, 2019). In 1990, Monrovia Nursery introduced White Lightnin® trailing lantana (*L. montevidensis* 'Monma') and Lavender Swirl® trailing lantana (*L. montevidensis* 'Monswee') that are still popular today. In 2007, Proven Winners released

Table 1. Varieties of trailing lantana (*Lantana montevidensis*) evaluated for morphological and cytological comparisons. The source of plant material and contact information are shown.

Source/variety	Contact/company	Additional information provided if available
American Farms (AF) lavender trailing lantana	Justin Orion, American Farms, Naples, FL	Unrooted cuttings produced from Dummen's ORO farm in Guatemala
Australia (AUS) trailing lantana	Megan Thomas, Queensland Herbarium, Brisbane Botanic Gardens	Naturalized location in Queensland Australia, Permit USDA APHIS P37-17-01621
Costa Farms (CF) lavender trailing lantana	Purchased through distributer, Lowe's, Gainesville, FL	Costa Farms, Miami, FL
Hatchett Creek Farms lavender trailing lantana (HCTL)	George Griffith, Hatchett Creek Nurseries, Gainesville, FL	Mother plants obtained 20+ years ago
Hatchett Creek Farms white trailing lantana (HCTW)	George Griffith, Hatchett Creek Farms, Gainesville, FL	Mother plants obtained 20+ years ago
Luscious® grape trailing lantana	Jim Putman, New Products Manager, Proven Winners (PW)	'Robpwpur' is a product of a planned breeding program developed by plant breeder, Robert J. Roberson. It was discovered in July 2002 as a naturally occurring branch mutation of *Lantana montevidensis* 'Alba'. Base of leaf blade is cuneate rather than cordate (trailing purple). Has denser pubescence on lower side of the leaf blade and darker violet main color on the upper side of the flower. Application date: 2007 (Google Patents, 2019)
Riverview Farms (RF) purple trailing lantana	Rick Brown, Riverview Farms, Seffner, FL	Quality Cuttings, Mexico
Texas (TX) lavender trailing lantana	Adam Black, Peckerwood Garden, Northwest of Houston, TX	Collected from a naturalized, abandoned, unmaintained, overgrown area where it has existed in a large patch (≈12.2 m long × ≈3.7 m wide) for decades

Table 2. Morphological comparison of eight trailing lantana varieties. Texas and Australia plants were obtained from natural populations. All other varieties were obtained from different nurseries as indicated in Table 1. Plants were grown from unrooted cuttings, finished in 4-inch pots, and planted under the same conditions. Leaf and flower morphology data were collected from opposite stems of the third or fourth node of each plant (n = 6) 20 weeks after planting. Means followed by different letters within each column are significant at $P \leq 0.05$ as determined by Tukey's range test.

Source/variety of trailing lantana	Leaf blade length (cm)	Peduncle length (mm)	Corolla tube length (mm)	Inflorescence diam (mm)	Flowers/inflorescence (no.)	Teeth/side of leaf (no.)
American Farms	46.79 AB	36.15 A	11.53 AB	32.92 AB	20.17 AB	20.15 B
Australia	29.35 C	28.16 A	9.10 D	20.74 D	12.20 B	12.90 A
Costa Farms	45.50 AB	29.68 A	11.76 A	30.95 B	20.40 AB	19.83 B
Hatchett Creek Farms white	42.04 B	33.33 A	12.63 A	28.16 BC	24.40 A	19.55 B
Hatchett Creek Farms lavender	52.31 A	33.01 A	10.68 BC	33.05 AB	25.33 A	18.45 B
Proven Winners Luscious® grape	48.20 AB	29.65 A	11.57 AB	36.68 A	23.17 A	18.65 B
Riverview Farms	52.35 A	28.42 A	10.77 BC	28.67 BC	23.83 A	20.50 B
Texas	45.22 AB	29.63 A	10.67 C	24.64 CD	23.83 A	19.05 B

Fig. 1. Representative comparison of U.S. cultivated (top) and Australian naturalized (bottom) trailing lantana. Note differences among leaf serrations, flower number, fruiting and form. Field picture taken 20 weeks after planting. Leaf photo credit B. Schutzman.

Luscious® grape trailing lantana (*L. montevidensis* 'Robpwpur') and it has since received prestigious awards as a top performer from plant trials at Kansas State University, University of Georgia, Texas A&M University, Longwood Gardens, North Carolina State University, and University of Delaware. In 2013, Lord Brooks trailing lantana (*L. montevidensis* 'Lord Brooks') was patented as a new variety distinguished by its darker-colored purple flowers. Unnamed white and lavender trailing varieties are also commonly available from nurseries throughout the United States. To our knowledge, all U.S. varieties are propagated vegetatively, as fruit is extremely rare or absent altogether. Among the known trailing lantana introductions in the United States, none have been reported from planned breeding programs for plant morphology, female sterility, pollen stainability, and/or ploidy level, such as with *Lantana camara* (Czarnecki et al., 2012; Deng et al., 2017).

The objective of this study was to assess morphological and cytological differences among eight trailing lantana varieties collected from different growers and a naturalized area in Texas and Australia. Information in these aspects was much needed to determine the invasive potential and suitability for continued commercial production and landscape use in the United States.

Materials and Methods

Plant material. Eight sources of trailing lantana were identified for use in this study, as described in Table 1. Four of the plant

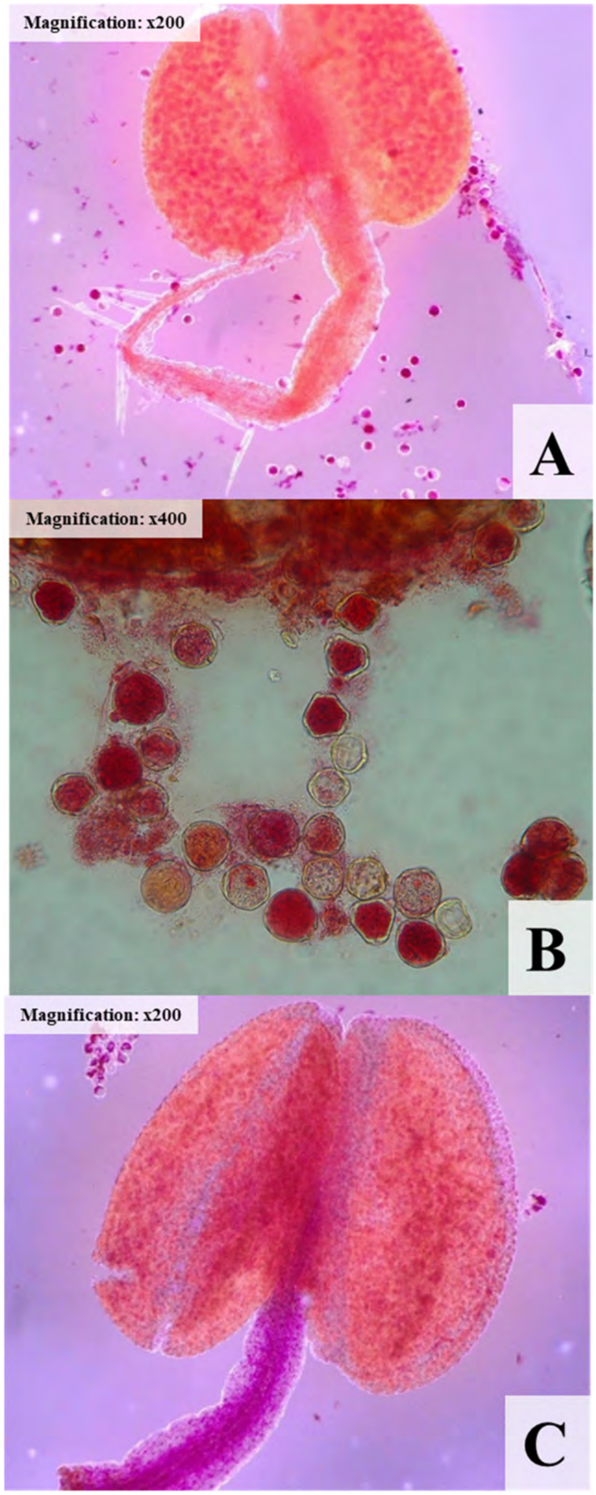

Fig. 2. Anther sac (**A**) and stainable pollen grains (**B**) of the Australian trailing lantana and Luscious® grape trailing lantana (**C**) stained with acetocarmine overnight and observed under a bright field microscope at ×200 (**A** and **C**) or ×400 (**B**) magnification.

sources were nurseries based in Florida (Hatchett Creek Farms, Gainesville; Costa Farms, Miami; Riverview Farms, Riverview; and American Farms, Naples); one was a commercial breeding company (Proven Winners, Sycamore, IL), one was a naturalized area in Houston, TX; and one was a naturalized area in Queensland, Australia. Under Permit P37-17-01621 [USDA Animal and Plant Health Inspection Service (APHIS)], vegetative plant material from Australia was shipped overnight from the Queensland Herbarium to Gainesville, FL. The Australian form was fruiting at the time of collection, and the U.S. varieties were reported to have never fruited on site. Plants were propagated at the UF/IFAS Gulf Coast Research and Education Center in Wimauma, FL. Cuttings each with 3 to 5 nodes were dipped in 2000 ppm indole-3-butyric acid talc and rooted under mist. After 4 weeks, rooted cuttings were finished in 4-inch pots filled with Fafard 2P soilless medium (Sun Gro Horticulture, Agawam, MA) and installed under full-sun conditions in north central Florida (Citra, FL; USDA cold hardiness zone 9a) on 16 July 2018. Fields were fumigated at least 1 month before planting. Plants were placed 6.0 ft on center in raised beds covered with white on black polypropylene plastic. Plants were drip irrigated 3 to 5 d per week as needed; topdressed with 9 g (0.5 tablespoon) of 12-month 15N–3.9P–10K Osmocote Plus (Scotts Co., Marysville, OH), and fertigated twice a month (15N–0P_2O_5–15K_2O; JR Peters Inc., Allentown, PA).

Plant morphology and fruit production. Leaf and flower morphology were assessed from plants of each variety grown under the same conditions and of the same age. Measurements included leaf blade length, peduncle length, corolla tube length, inflorescence diameter, number of flowers per umbel, and number of serrations per lamina, as described by Sanders (1987). For each of six plant replicates, two measurements were taken from opposite leaves 3 to 4 nodes from the apex and averaged.

Pollen stainability. Pollen stainability has been used as an indicator of lantana's male fertility (or sterility) and hybridization potential (Czarnecki et al., 2014; Deng et al., 2017; Henderson, 1969). To determine pollen stainability, anthers were collected from field-grown plants of each variety and stained in a 1.5-mL microcentrifuge tube containing 50 µL of acetocarmine (4%) (TCI America, Portland, OR) overnight. Slides were prepared the following day by pulling the anther sacs out of the tubes with a toothpick and placing on a slide in a drop of the acetocarmine stain. Anther sacs were broken with a dental probe and swirled to release the pollen grains from the anther sacs. Then 5 to 10 µL of 2% acetocarmine/glycerol (1:1) was added, a coverslip placed, and the preparation sealed with fingernail polish. The slides were observed and photographed under a bright field microscope (Olympus BH-2, Center Valley, PA) at ×200 and ×400 magnification. Plump, round, uniformly stained pollen grains were considered stainable, whereas misshaped, nonstained, or unevenly light-stained pollen grains were counted as non-stainable. For each variety, more than 600 pollen grains (if produced and available) were examined from four replicate samples.

Nuclear DNA content and ploidy analysis. Over the past two decades, flow cytometry has become a reliable and quick approach for determining plant ploidy levels (Bohanec, 2003). This approach has been widely used to determine the ploidy level of *Lantana camara* varieties (Czarnecki et al., 2012, 2014; Deng et al., 2017). In this study, the ploidy level of trailing lantana varieties was determined using a CyFlow Cube 6 flow

Table 3. Nuclear DNA content, ploidy level, pollen production and quantity in anthers, and percent pollen stainability of eight trailing lantana varieties.

Variety or source of trailing lantana	Nuclear DNA content ±SD (pg/2C)	Ploidy level	Pollen production and quantity	Pollen stainability ±SD (%)
American Farms	2.80 ± 0.06	Triploid	No to little, nonstainable	—[z]
Australia	3.98 ± 0.03	Tetraploid	Numerous, most were stainable	58.83 ± 3.92
Costa Farms	2.91 ± 0.04	Triploid	Little, misshaped, and nonstainable	—
Hatchett Creek Farms lavender	2.85 ± 0.05	Triploid	No	—
Hatchett Creek Farms white	2.80 ± 0.10	Triploid	No	—
Proven Winners	2.84 ± 0.09	Triploid	No	—
Riverview Farms	2.85 ± 0.04	Triploid	No to little, nonstainable	—
Texas	2.83 ± 0.01	Triploid	No	—

[z]Indicates pollen sacs were empty and stainable pollen grains were not observed.

cytometer (Sysmex; Partec GmbH, Münster, Germany) that was equipped with laser lights (488 nm blue and 638 nm red) and could allow the determination of absolute nuclear DNA contents.

The nuclear DNA content of each variety was determined using with the procedure described by Doležel et al. (2007) and modified by Cao et al. (2014). Tomato (*Solanum lycopersicum* 'Stupické polní rané') with a nuclear DNA content of 1.69 pg/2C, was selected as the internal reference for use in this study. Approximately 40 mg of fresh leaf tissues was cochopped with a similar amount of tomato leaf tissues in a petri dish in 1 mL of cold HB01 lysis buffer (Doležel et al., 2007). The homogenate containing lantana and tomato nuclei was filtered through a nylon mesh (50-µm pore size) into a sample loading tube with 50 µL of a stock solution containing DNA fluorochrome propidium iodide (1 $mg{\cdot}mL^{-1}$; Sigma-Aldrich, St. Louis, MO) and RNase (1 $mg{\cdot}mL^{-1}$; Sigma-Aldrich). The collected suspension containing lantana and tomato nuclei was then analyzed on the flow cytometer. At least three runs were performed for each accession, and a minimum of 3000 particles were counted in each run. The nuclear DNA content of trailing lantana samples was calculated according to Doležel et al. (2007): sample nuclear DNA content (pg/2C) = internal reference nuclear DNA content (1.69) × (mean fluorescence value of sample / mean fluorescence value of internal reference). The ploidy level of the trailing lantana samples was determined by comparing their nuclear DNA contents with another white trailing lantana variety (from a local wholesale nursery in Tampa, FL) whose somatic chromosomes in root tip cells were counted previously (R. Qian and Z. Deng, unpublished data).

Experimental design and data analysis. Eight varieties of trailing lantana were randomized and replicated in six plant plots. A one-way analysis of variance was conducted using JMP, Version 13 (SAS Institute Inc., Cary, NC). Significant means were separated by a Tukey's honestly significant difference at $P \leq 0.05$.

Results and Discussion

Plant morphology. Leaf and flower morphology were generally similar among U.S. varieties, with the exception that Hatchett Creek Farms' and Riverview Farms' lavender trailing lantana had longer leaf blades, Costa Farms' lavender and Hatchett Creek Farms' white trailing lantana had longer corolla tubes, and Luscious® grape trailing lantana had slightly larger inflorescence diameter compared with most other varieties (Table 2). Observationally, U.S. varieties were also very similar in landscape appearance, habit, and flowering (S. Wilson, unpublished data).

The Australian trailing lantana was morphologically distinct compared with the U.S. varieties (Fig. 1). Leaves were 54% to 78% smaller, with 43% to 59% fewer leaf serrations (Table 2), distinct serrate-crenate margins, and fewer appressed hairs (Fig. 1). Flowers were smaller in inflorescence diameter, with fewer flowers per inflorescence, and shorter corolla tube lengths (Table 2). Both the Australian and U.S. varieties had lavender petals with occasional white corolla tubes and yellow eyes, but this was more pronounced with the U.S. varieties than the Australian variety (Fig. 1).

Fruit production. Fruit production was not observed on any trailing lantana varieties from the United States. Plants of the Australian trailing lantana produced abundant fruit, on average 7.7 drupes per flower cluster (Fig. 1). The distinction of two types of trailing lantana (one fruiting and one nonfruiting) is consistent with previous literature (Johnson, 2007). Personal observations from nursery suppliers of the U.S. varieties used in this study (Table 1) reliably report plants do not fruit.

Pollen stainability. Well developed, stainable pollen grains were readily observed in the anther sacs of the Australian trailing lantana plants (Fig. 2A and B). The average pollen stainability was 58.83% (Table 3). Anther sacs of all U.S. trailing lantana plants rarely contained pollen grains or stainable pollen grains (Fig. 2C). This is consistent with the report by Czarnecki et al. (2014) and indicates high levels of male sterility in the U.S. trailing lantana varieties tested in this study.

Nuclear DNA content and ploidy analysis. Ploidy level is one of the most important factors in determining lantana pollen stainability/male sterility (Czarnecki et al., 2014) and fruit/seed production (Czarnecki, 2011). In this study, the average nuclear DNA content of Australian trailing lantana plants was determined to be 3.98 pg/2C, using tomato as the internal reference (Table 3). All U.S. varieties had the nuclear DNA content ranging from 2.80 to 2.85 pg/2C (Table 3). This study represents the first report of the nuclear DNA content of trailing lantana. By comparing the nuclear DNA content of these varieties with that of a triploid lavender trailing lantana with $2n = 3x = 36$, it was determined that the Australian trailing lantana is a tetraploid and all the U.S. varieties are triploids.

Conclusion

Results from this study provide evidence that the U.S. trailing lantana varieties are morphologically and cytologically distinct from the Australian weedy form of trailing lantana. The Australian weedy form is a tetraploid and highly fertile in terms of male (pollen) and female fertility, and the U.S. varieties examined in this study are triploids and highly male and female sterile. To our knowledge, the Australian variety is not grown in the United States. Measures should be taken to prevent its introduction. The U.S. varieties are highly ornamental and meet the general criteria for sustainable landscaping (drought tolerant, maintenance free, year-round flowering for pollinators). Additional studies are warranted to evaluate U.S. varieties in multiple growing areas for an extended amount of time in the presence or absence of the Australian variety in preparation of Infraspecific Taxon Protocol Requests (UF-IFAS, 2019) for potential recommended use in Florida.

Literature Cited

Bohanec, B. 2003. Ploidy determination using flow cytometry, p. 397–403. In: M. Maluszynski, K.J. Kasha, B.P. Forster, and I. Szarejko (eds.). Doubled haploid production in crop plants. Springer Publishing, Dordrecht.

Cao, Z., Z. Deng, and M. McLaughlin. 2014. Interspecific genome size and chromosome number variation sheds new light on species classification and evolution of Caladium (Araceae). J. Amer. Soc. Hort. Sci. 49:449–459.

Czarnecki, D.M. 2011. Genetic sterilization and reproductive biology of *Lantana camara*. University of Florida, Gainesville, PhD Diss.

Czarnecki, D.M., S.B. Wilson, G.W. Knox, R. Freyre, and Z. Deng. 2012. UF-T3 and UF-T4: Two sterile *Lantana camara* cultivars. HortScience 47:132–137.

Czarnecki, D.M., A.J. Hershberger, C.D. Robacker, D.G. Clark, and Z. Deng. 2014. Ploidy levels and pollen stainability of *Lantana camara* cultivars and breeding lines. HortScience 49:1271–1276.

Day, M., C.J. Wiley, J. Playford, and M.P. Zalucki. 2003. Lantana: Current management status and future prospects. Austral. Ctr. Intl. Agr. Res. Canberra, Australia.

Deng, Z., S.B. Wilson, X. Ying, and D.M. Czarnecki, II. 2017. Infertile *Lantana camara* cultivars UF-1011-2 and UF-1013A-2A. HortScience 52:652–657.

Doležel, J., J. Greilhuber, and J. Suda. 2007. Estimation of nuclear DNA content in plants using flow cytometry. Nat. Protoc. 2:2233–2244.

Florida Exotic Pest Plant Council. 2019. Florida Exotic Pest Plant Council's 2019 list of invasive species. 20 May 2019. <https://www.fleppc.org/list/list.htm>.

Google Patents. 2019. Lantana plant named 'Robpwpur'. 20 May 2019. <https://patents.google.com/patent/USPP19357P2/en?q='Robpwpur'&oq='Robpwpur'+>.

Henderson, R.J.F. 1969. A cytological study of *Lantana montevidensis* (Spreng.) Briq. in Queensland, 1–4. Contributions from the Queensland Herbarium, No. 3. Queensland Herbarium.

International Plant Names Index (IPNI). 2015. *Lantana montevidensis*. 20 May 2019. <htpp://www.ipni.org>.

Johnson, S.B. 2007. Review of the declaration of lantana species in New South Wales. Dept. Primary Ind., Orange NSW, Australia.

Munir, A.A. 1996. A taxonomic review of *Lantana camara* L. and *L. montevidensis* (Spreng.) Briq. (Verbenaceae) in Australia. J. Adel. Bot. Gard. 17:1–27.

O'Donnell, C. 2002. The creeping lantana handbook. A guide to ecology, control and management. Queensland Dept. Primary Ind., Natl. Resources and Mines, Brisbane, Australia.

Raghavan, R.S. and C.M. Arora. 1960. Morphological and cytological studies in the genus *Lantana* L. Bull. Bot. Surv. India 2:299–303.

San Marcos Growers. 2019. *Lantana montevidensis* 'Alba'. 20 May 2019. <https://www.smgrowers.com/products/plants/plantdisplay.asp?plant_id=925>.

Sanders, R.W. 1987. Identity of *Lantana depressa* and *L. ovatifolia* (Verbenaceae) of Florida and the Bahamas. Syst. Bot. 12:44–60.

University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS). Assessment of Non-Native Plants in Florida's Natural Areas. 2019. *Lantana montevidensis*. 20 May 2018. <https://assessment.ifas.ufl.edu/assessments/lantana-montevidensis/>.

U.S. Department of Agriculture, Agricultural Research Service. 2012. Plant hardiness zone map. 20 May 2019. <https://planthardiness.ars.usda.gov>.

U.S. Department of Agriculture, National Resources Conservation Service. 2019. The PLANTS database. Natl. Plant Data Team, Greensboro, NC. 20 May 2019. <htpp://plants.usda.gov>.

Wunderlin, R.P., B.F. Hansen, A.R. Franck, and F.B. Essig. 2019. Atlas of Florida plants. Inst. Systemic Bot., Univ. South Florida. 20 May 2019. <http://florida.plantatlas.usf.edu>.

This paper was presented during the 2019 FSHS Annual Meeting and originally published in *HortScience* [HORTSCIENCE 54(12):2134–2138. 2019. https://doi.org/10.21273/HORTSCI14443-19]. The paper is included in this Proceedings as a reprint (with permission).

Proc. Fla. State Hort. Soc. 132:238. 2019.

How Can We All Get Along? The Common Goals for the Diversity of Topics Relevant to the Florida State Horticultural Society's New Agroecology and Natural Resources Section

Zachary T. Brym*

Tropical Research and Education Center, University of Florida/IFAS, Agronomy Department, 18905 SW 280th St., Homestead, FL 33031

Additional index words. food systems, environment, productive, sustainable

The new Florida State Horticultural Society's Agroecology and Natural Resource Section provides a forum to link the shared goals of agricultural and natural land management.

Objectives

Agricultural and natural systems are inextricably connected. The renaming of the Natural Resources Section to the Agroecology and Natural Resources Section (ANR) is an attempt to bring that conversation to the Florida State Horticultural Society (FSHS). The Agroecology and Natural Resources Section will facilitate a program of research, extension, and professional contributors that can speak to the shared goals of agriculture and natural resource management.

Agroecology provides a framework that links the shared goals of agricultural and natural area management: sustainable production, resource conservation, and social responsibility. Well managed agricultural systems, or "agroecosystems", can be productive and buffer impacts of human consumption on natural areas. Natural systems conservation, restoration, and enhancement are also essential to maintain suitable habitats for native flora and fauna and to provide critical ecosystem services transferred to agroecosystems.

The agroecosystem concept structures goal-oriented management plans that consider the environmental and ecological characteristics of a farm and the surrounding area. Agroecosystems are complex and challenging to manage and require a multidisciplinary understanding from specialties such as horticultural sciences, agronomy, economics, ecology, engineering, entomology, natural resource management, soil and water sciences, and sociology.

Findings

The Agroecology and Natural Resources Section inaugural session promoted a cohesive discussion and diverse presentations on management strategies for agriculture and natural resources through shared goals to maintain productivity, ecosystem health, and social responsibility. A facilitated discussion identified keywords associated with the core topics included in the section: Agroecology, Natural Resources, and Food Systems (Table 1). The terms "environment" and "money" were mentioned for each of the core topics. "Diversity," "productive," and "sustainable" are foundational principles of agroecology that were mentioned in at least two of the core topics discussed. System resilience is another foundational principle of agroecology, which was not mentioned directly but can be associated with long-term outlooks of the terms "quality," "regenerative," and "security". The social aspect of these core topics is also important to mention appearing as "aesthetic", "choice", and "responsible". Overall, participants demonstrated appreciation for the shared goals of the section's core topics. Presenters pointed out linkages to this discussion often throughout the day and offered ways their work fit within the goals of the FSHS Agroecology and Natural Resources Section.

Future Directions

The FSHS ANR had a strong showing of presentations and participation during its inaugural session. The intent of the morning discussion was to motivate future involvement in the section and a greater understanding of the relevant topics and purpose of the program. The section may continue to facilitate cohesion and linkages among presentations in future meetings through collaborative and interactive opportunities within the program.

Table 1. Words associated with the FSHS Agroecology and Natural Resources key topics.

Agroecology			
Agricultural	Ecological	Holistic	Organic
Productive	Regenerative	Responsible	Sustainable
Animals	Environment	Farms	Food
Money	Natural Areas	Plants	Resources
Society	Soil & Water	Systems	
Natural Resources			
Beautiful	Biodiverse	Dynamic	Limited
Native	Political	Renewable	Shared
Aesthetic	Animals	Conservation	Environment
Green	Habitat	Money	Plants
Quality	Recreation	Soil, Air & Water	Wild
Food Systems			
Available	Cyclical	Interactive	Local
Organic	Productive	Sustainable	
Access	Choice	Distance	Diversity
Environment	Inputs	Logistics	Market
Money	Safety	Security	Waste

*Corresponding author. Email: brymz@ufl.edu

Proc. Fla. State Hort. Soc. 132:239–240. 2019.

Using Quantum™ on Leatherleaf Fern (*Rumohra adiantiformis*) as an IPM Soil Conditioning Tactic to Stimulate Growth

Karen Stauderman*[1] and David J. Norman[2]

[1]*University of Florida/IFAS Extension, Volusia County, 3100 East New York Ave., DeLand, FL 32724*

[2]*University of Florida/IFAS Mid-Florida Research and Education Center, 2725 Binion Rd., Apopka, FL 32703*

Additional index words. total leaf area, fern anthracnose, *Colletotrichum acutatum*

SUMMARY. This field trial evaluated leatherleaf blade growth using Quantum Growth™ products. The manufacturer claims that frond production is increased by 20% with 50% less irrigation and fertilizer, resulting in heartier plants with resistance to pathogens. Our hypothesis was that combining both Quantum Light™ and Quantum VSC™ would enhance growth of leatherleaf fern (*Rumohra adiantiformis*) and provide protection against fern anthracnose. A fernery in Pierson, FL was the site for this study. Treatments were set up in randomized complete-block design with two rates (high, standard) and untreated (control). The trial was replicated within the fernery. An initial spray application containing Quantum Light™ 2.5 oz/gal + Quantum VSC™ 2.5 oz/gal treated the high rate plots. A spray using Quantum Light™ 2 oz/gal + Quantum VSC™ 2 oz/galtreated the standard rate plots. Three weekly sprays followed by a monthly spray over two months was applied using Quantum Light™ 1 oz/gal + Quantum VSC™ 1 oz/gal on the standard plots and Quantum Light™ 1.25 oz/gal + Quantum VSC™ 1.25 oz/gal to the high plots. At 60 and 90 days, fronds were harvested from each block and separated into both 30 cm (12 inch) and 60 cm (25 inch) heights. Total leaf area was calculated and blades were statistically compared by using analysis of variance (ANOVA) and least significant difference (LSD) procedures at 90% and 95% confidence interval (CI). There was no statistical difference in leaf area harvested within each plot, between each treatment nor between replications. The data did not indicate growth acceleration of the fronds under these specifications during this time span.

The purpose of this field trial was to evaluate the effects of growth from Quantum Growth™ (Ecological Laboratories, Inc., Cape Coral, FL) (EPA bio-pesticide label 3920798), a blend of photosynthetic and heterotrophic culture of three bacteria species, on leatherleaf fern (*Rumohra adiantiformis*). Quantum™ works to sequester atmospheric nitrogen and carbon dioxide converting and storing them as sugars and proteins for the plant while forming a symbiotic relationship with the plant. It also serves to restore impaired plant and soil back to health. The manufacturer claims that root mass and frond production are increased by 20% with 50% less irrigation and 50% less fertilizer, allowing the plant to absorb more nutrients and water. The plants are heartier and are more resistant to pathogens. Our hypothesis was that together, Quantum Light™ and Quantum VSC™ would enhance growth of leatherleaf fern and reduce the occurrence of disease incidence of fern anthracnose (*Colletotrichum acutatum*) outbreak.

Materials and Methods

A previously mowed fernery, under saran, in Pierson, FL was selected for this study. The test was replicated at two sites (Orange and Green as designated by the color of the field flags) within the fernery. Treatments were set up in randomized complete-block design with two differing rates and a control check (A-C), comprising 4 plots (1–4) per treatment.

In Sept. 2018, the initial application combined 2 oz Quantum Light™ + 2 oz Quantum VSC™ per gallon for the standard rate and 2.5 oz Quantum Light™ + 2.5 oz Quantum VSC™ per gallon for the high rate. The control check was unsprayed. Applications were administered with a 3-gallon backpack sprayer to treat 4-10' × 10' plots per treatment (Fig. 1).

Following the initial field treatment, treatment plots were sprayed weekly for three weeks then monthly for two additional months (November and December) using a reduced rate of Quantum™. Standard treatments contained a tank mix of 1 oz Quantum Light™ + 1 oz Quantum VSC™ per gallon and a tank mix of 1.25 oz Quantum Light™ + 1.25 oz Quantum VSC™ per gallon delivered the high rate.

Results and Discussion

At 60 and 90 days, after treatment, plots were scored for overall percent frond coverage (% coverage) with new growth. Fern fronds were then harvested from each block and separated into both medium (30 cm) and tall (60 cm) frond sizes, similar to what is mimicked at time of sale. Length and width of the leaf blades were measured of each frond from which total leaf area (L

*Corresponding author. Email: kstauderman@ufl.edu

Fig. 1. Applying Quantum™ spray treatments to leatherleaf fern (*Rumohra adiantiformis*) plots.

× W) was calculated. A total of fifteen (15) fronds measuring 30 cm (12 inches) in height and twenty-five (25) fronds measuring 60 cm (25 inches) in height was harvested from each treatment plot in the following months of November and December. Additionally, each leaf blade was given a numerical score based on a University of Florida/IFAS Disease Incidence key for percent leaf area with anthracnose damage (% DI).

The data compared treatments using ANOVA and LSD statistical procedures at 90% (P = 0.1) and 95% (P = 0.05) confidence levels. It was found that there was no statistical difference within replications. The products had no significant effect on frond size in the limited time (4 months) of this test. There was no significant difference in disease incidence within the Orange replication. However, in the Pink replication, the standard rate had significantly higher disease incidence. Yet, it was random based on uneven disease distribution within the test plots. This preliminary field trial did not find Quantum™ products to significantly enhance blade and frond growth nor improved disease incidence from the methods performed in this trial. Future assays might consider higher rates of Quantum™ and/or extension in time in the field trial for further assessment.

Proc. Fla. State Hort. Soc. 132:241–244. 2019.

Herbicide Efficacy on the Small Floating Weeds Redroot Floater and Feathered Mosquitofern

Lyn A. Gettys*, Kyle L. Thayer, Joseph W. Sigmon, Ian J. Markovich, and Mohsen Tootoonchi

University of Florida/IFAS Fort Lauderdale Research and Education Center, 3205 College Ave., Davie, FL 33314

Additional index words. nonnative plants, invasive, aquarium plants, water garden plants

Redroot floater (*Phyllanthus fluitans* Benth. ex Müll. Arg.) and feathered mosquitofern (*Azolla pinnata* R. Brown) are relatively recent invaders in Florida's waters. Both species are small (< 3 cm) obligate aquatic plants that float on the surface of the water with their roots dangling in the water column. We conducted greenhouse experiments in mesocosms to evaluate the efficacy of 13 foliar-applied aquatic herbicides alone and in combination for a total of 35 treatments. Plants were treated once and grown out for 6 weeks after treatment, then scored for visual quality and destructively harvested to determine dry biomass. Most herbicides provided effective control (reduction in visual quality and biomass of at least 90% compared to untreated controls) of redroot floater and feathered mosquitofern. Both species were susceptible to any mix that included diquat, and the majority of herbicides evaluated also performed well alone. These findings suggest that lackluster results after field management of these species are not due to herbicide tolerance, but are likely the result of other factors. Both species tolerate shoreline stranding, and stranded plants can escape herbicide treatments by persisting in wet soil along the banks of a treated area. Once floating populations have been killed as a result of herbicide treatment, stranded plants may flush back into the water, where they can quickly re-populate the treated area (which is now nutrient-enriched from the dying plants) with greatly reduced intraspecific competition. Therefore, it is critically important that all plants targeted for treatment – including those stranded along shorelines – actually receive herbicide applications.

Florida's waters are routinely invaded by new exotic plants. Although the invasion pathways for these species are varied, many introduced aquatic plants arrive in Florida's waters after escaping cultivation in water gardens and aquariums. Two relatively recent floating invaders are redroot floater (*Phyllanthus fluitans* Benth. ex Müll. Arg.) and feathered mosquitofern (*Azolla pinnata* R. Brown).

Redroot floater, also called floating spurge, is a South American dicot in the Euphorbiaceae (USDA–NRCS, 2019a) or the Phyllanthaceae (USDA–APHIS, 2017) and was first reported in a canal attached to the Peace River west of Fort Ogden (Desoto County, FL) in 2010 (Sowinski, 2011). This species is an attractive free-floating ornamental plant with round, concave leaves that are up to 2 cm long and attached to the reddish stems in a distichous manner. There is a lack of information available regarding seed production and viability, but redroot floater reproduces easily via fragmentation (Wilder and Sowinski, 2010). It is considered established in the Peace River (Sowinski, 2017) and has not been found in other U.S. states (USDA–NRCS, 2019a). However, redroot floater is naturalized in Mexico (Steinmann, 2002) and its potential range includes the southeastern United States as far north as North Carolina and west into Texas (USDA–APHIS, 2017). Despite being a "high-risk" species, with the likelihood of it becoming a major or minor invader being 54% and 44%, respectively (USDA–APHIS, 2017), redroot floater is not a prohibited plant or noxious weed and is readily available through the aquarium and water garden trade.

Feathered mosquitofern is an Australian fern that was formerly placed in the Salviniaceae but is now in the family Azollaceae (Madeira et al., 2013, Smith et al., 2006). It was first collected in Florida's waters near Jupiter (Palm Beach County, FL) in 2007 (Bodle, 2008) and is now in at least four Florida counties (Wunderlin et al., 2019), North Carolina (USDA–NRCS, 2019b), and possibly Arizona and Louisiana (Pfingsten et al., 2019). Like other members of the genus *Azolla*, feathered mosquitofern has very small (~1 mm) velvety leaves that are attached to the branch in an alternate manner. The upper lobe of each leaf hosts the nitrogen-fixing cyanobacterium *Anabaena azollae*, which has resulted in this and other species of *Azolla* being used in rice cultivation. Feathered mosquitofern is roughly deltoid or triangular in shape and is often larger (1.5–3 cm long) than other members of the genus (Jacono, 2016). The feathered mosquitofern is a Federal Noxious Weed (USDA–NRCS PPQ, 2012).

Despite their very different botanical classifications, these small floating species share a number of characteristics. For example, both species form dense mats via vegetative means and cause ecosystem harm by blocking light and oxygen penetration into the water column. They also interfere with recreational activities such as boating, fishing, and swimming. In addition, both feathered mosquitofern and redroot floater appear to be able to

This research was supported by the Florida Agricultural Experiment Station, the US Department of Agriculture National Institute of Food and Agriculture (HATCH project FLA-FTL-005682), and the Florida Fish and Wildlife Conservation Commission Invasive Plant Management Section. Mention of a trademark, proprietary product or vendor does not constitute a guarantee or warranty of the product and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

*Corresponding author email: lgettys@ufl.edu

survive out of the water on damp soil along canals, ponds, and other aquatic systems.

Control efforts thus far have not resulted in eradication. Both species continue to persist and form nascent populations in waters connected to invaded aquatic systems. Although the current distribution of both species is primarily southern Florida, future range expansion is likely. It is critical that we identify control methods to manage feathered mosquitofern and redroot floater, so the goal of these experiments was to evaluate the efficacy of aquatic herbicides on these small, weedy floating plants.

Materials and Methods

Both species were field-collected from existing populations in southern Florida and transported to a greenhouse at the University of Florida IFAS Fort Lauderdale Research and Education Center in Davie, FL. 68-L high density polyethlene (HDPE) mesocosms were filled with well water, then plants were placed on the water surface and cultured until surface coverage was 80% or greater. Each mesocosm was stocked with a single species to avoid competition and to accommodate different growth rates. Only foliar (surface-applied) herbicide applications were evaluated in these experiments and are shown in Table 1. All treatments were applied once and utilized an appropriate adjuvant (Breeze, WinField Solutions, St. Paul, MN) at 0.5% v/v to aid penetration through the waxy cuticles of these plants. Treatments were applied using a handheld sprayer in a diluent volume equal to ca. 65 gallons per acre (ca. 10 mL of solution per mesocosm); an untreated control treatment (water spray only) was included in these experiments. Four replicates (mesocosms) were prepared for each herbicide treatment. Redroot floater plants were treated in mid-Oct. 2018 and feathered mosquitofern plants were treated in early Feb. 2019.

All plants were monitored for 6 weeks after treatment (WAT), then scored for visual quality using a numerical scale of 0–10, where 0 = dead; 5 = fair quality, somewhat attractive form and color, little to no chlorosis or necrosis; and 10 = excellent quality, perfect condition, healthy and robust. All live biomass was then subjected to a destructive harvest; plant material was rinsed to remove algae and other debris, and then placed in a forced-air oven at 65 °C for 2 weeks before weighing to obtain dry biomass. Visual quality and dry biomass data were subjected to analysis of variance and LSD separation of means (SAS Software Version 9.3, SAS Institute, Cary, NC) to determine differences in biomass compared to untreated controls.

Results and Discussion

Mean dry biomass of redroot floater plants in untreated control treatment mesocosms was 6.16 g and visual quality was 6.75. Most (29 of 35) herbicide treatments reduced biomass and visual quality of redroot floater by at least 90% compared to untreated controls (Fig. 1a). Treatments that resulted in a 75 to 85% reduction in biomass compared to untreated controls were topramezone at 8 oz/ac (visual quality 3.25); glyphosate at 48 oz/ac (visual quality 3.0); and carfentrazone at 8.6 or 13.5 oz/ac (visual quality 4.75 and 4.25, respectively). Redroot floater treated with fluridone at 7.7 oz/ac had a biomass and visual quality reduction of 52% compared to untreated controls. Plants treated with 3.9 oz/ac of fluridone had a visual quality rating of 7 and accumulated biomass that was almost double that of the untreated control plants.

Mean dry biomass of feathered mosquitofern plants in untreated control treatment mesocosms was 21.43 g and visual quality was 9.5. As with redroot floater, most (31 of 35) herbicide treatments reduced biomass and visual quality of feathered mosquitofern by at least 90% compared to untreated controls (Fig. 1b). Treatments that resulted in a 72 to 88% reduction in biomass compared to untreated controls were 2,4-D at 64 oz/ac (visual quality 8.75) and fluridone at 3.9 or 7.7 oz/ac (visual quality 8.25 and 6.0, respectively). Florpyrauxifen-benzyl had little effect on feathered mosquitofern and only reduced biomass by 47% compared to untreated control plants (visual quality 7.75).

Based on these results, it seems clear that most herbicides labeled for aquatic use provide effective control (biomass reduction of at least 90% compared to untreated controls) of redroot floater and feathered mosquitofern. Both species were susceptible to any mix that included diquat, and the majority of herbicides evaluated also performed well alone. Products that were not effective (failed to reduce biomass and visual quality by at least 90%) on redroot floater were fluridone at 3.9 or 7.7 oz/ac, carfentrazone at 6.8 or 13.5 oz/ac, topramezone at 8 oz/ac, and glyphosate at 48 oz/ac. Products that were not effective on feathered mosquitofern were fluridone at 3.9 or 7.7 oz/ac, 2,4-D at 64 oz/ac, and florpyrauxifen-benzyl at 1.5 oz/ac. These findings suggest that lackluster results after field management of these species are

Table 1. Herbicides and combinations evaluated for efficacy on redroot floater (*Phyllanthus fluitans* Benth. ex Müll. Arg.) and the feathered mosquitofern (*Azolla pinnata* R. Brown).

Active ingredient	Brand name	Rate(s) (oz/acre)	Source
2,4-D	WEEDestroy	64	Nufarm Americas
Triclopyr	Garlon 3A	256	Dow AgroSciences LLC
Flumioxazin	Clipper	6*, 12*	Valent USA Corp.
Carfentrazone	Stingray	6.75*, 13.5*	SePRO Corp.
Penoxsulam	Galleon SC	2.8, 5.6	SePRO Corp.
Bispyribac	Tradewind	1*, 2*	Valent USA Corp.
Imazamox	Clearcast	64**, 128**	SePRO Corp.
Imazapyr	Ecomazapyr 2SL	16*, 32*	Alligare LLC
Fluridone	Alligare Fluridone	3.85, 7.7	Alligare LLC
Topramezone	Oasis	8*, 16*	SePRO Corp.
Glyphosate	Refuge	48, 96	Syngenta Crop Protection LLC
Diquat	Tribune	128, 256	Syngenta Crop Protection LLC
Florpyrauxifen-benzyl	ProcellaCOR	1.5	SePRO Corp.

*Evaluated alone and in combination with 128 oz diquat.

**Evaluated alone and in combination with 32 oz glyphosate.

not due to herbicide tolerance, but are likely the result of other factors. For example, although redroot floater and feathered mosquitofern are floating plants, both are able to survive out of the water and are tolerant of shoreline stranding. Stranded plants are able to escape herbicide treatments by persisting in wet soil along the banks of a treated area. Once floating populations have been killed as a result of herbicide treatment, stranded plants may flush back into the water, where they can quickly re-populate the treated area (which is now nutrient-enriched from the dying plants) with greatly reduced intraspecific competition. These experiments highlight the importance of ensuring that all plants targeted for treatment—including those stranded along shorelines—actually

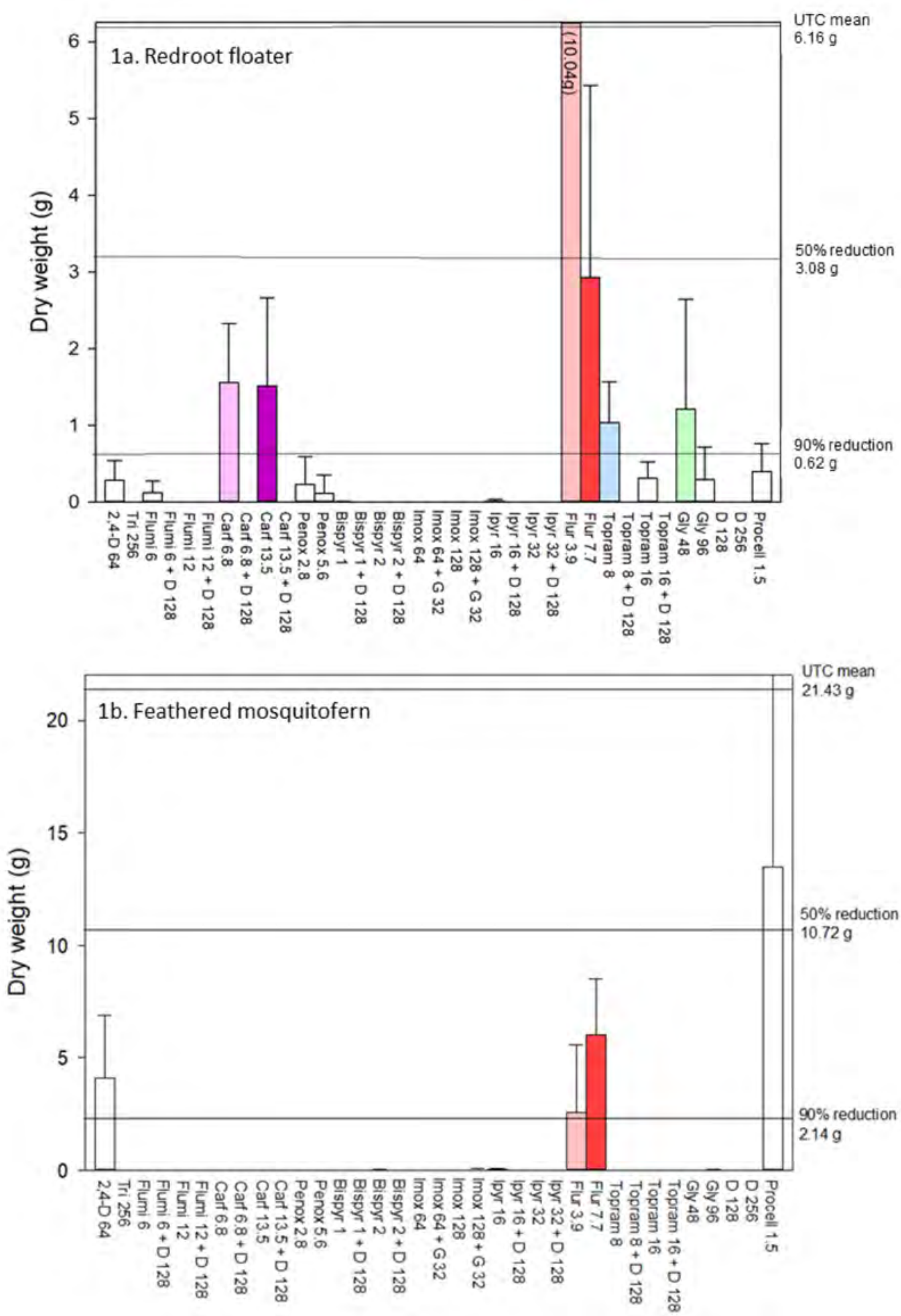

Fig. 1. Dry weights of redroot floater (*Phyllanthus fluitans* Benth. ex Müll. Arg.) **(1a)** and feathered mosquitofern (*Azolla pinnata* R. Brown) **(1b)** 6 weeks after a single foliar treatment with aquatic herbicides. Bars are the mean of four replicates (mesocosms) of each treatment and error bars represent one standard deviation from the mean. The upper horizontal rule represents the mean dry weight of untreated (UTC) plants. Center and lower horizontal rules represent a 50% and 90% reduction in dry weight compared to untreated control plants. 2,4-D = 2,4-D; Tri = triclopyr; Flumi = flumioxazin; D = diquat; Carf = carfentrazone; Penox = penoxsulam; Bispyr = bispyribac; Imox = imazamox; Ipyr = imazapyr; Flur = fluridone; Topram = topramezone; Gly = glyphosate; Procell = florpyrauxifen-benzyl

receive herbicide applications. Failure to do so will allow some plants to escape treatments and thus may facilitate quick recolonization of previously treated areas.

Literature Cited

Bodle, M. 2008. Feathered mosquito fern (*Azolla pinnata* R. Br.) comes to Florida. Aquatics 30(2):4–9.

Jacono, C. 2016. Taking a second look at floating ferns. Aquatics 38(2):18–23.

Madeira, P.T., T.D. Center, J.A. Coetzee, R.W. Pemberton, M.F. Purcell, and M.P. Hill. 2013. Identity and origins of introduced and native *Azolla* species in Florida. Aquatic Botany 111:9–15.

Pfingsten, I.A., D.D. Thayer and V. Howard. 2019. Fact sheet: *Azolla pinnata* R. Brown. U.S. Geological Survey Nonindigenous Aquatic Species Database, Gainesville, FL. Revision date 24 June 2016. Accessed online 11 Oct. 2019. <https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=2745>

Smith, A.R., K.M. Pryer, E. Schuettpelz, P. Korall, H. Schneider, and P.G. Wolf. 2006. A classification for extant ferns. Taxon 55(3):705–731.

Sowinski, M.P. 2011. Red root floater *Phyllanthus fluitans* (Euphorbiaceae): Another aquatic invader for Florida. Aquatics 33(3):7–10.

Sowinski, M.P. 2017. Red root floater update. Aquatics 39(1):5–7.

Steinmann, V.W. 2002. Diversidad y endemismo de la familia Euphorbiaceae en Mexico. Acta Botanica Mexicana 61:61–93.

USDA–APHIS (United States Department of Agriculture Animal and Plant Health Inspection Service). 2017. Weed risk assessment for *Phyllanthus fluitans* Benth. ex Müll. Arg. (Phyllanthaceae)—Red root floater. Plant Epidemiology and Risk Analysis Laboratory, Center for Plant Health Science and Technology, Plant Protection and Quarantine, Animal and Plant Health Inspection Service, United States Department of Agriculture, Raleigh, NC 27606. Accessed online 10 Oct. 2019. <https://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/wra/phyllanthus-fluitans.pdf>

USDA–APHIS PPQ (United States Department of Agriculture Animal and Plant Health Inspection Service Plant Protection and Quarantine). 2012. Federal noxious weed list (1 Feb. 2012). Accessed online 22 Sept. 2019. <https://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/weedlist.pdf>

USDA–NRCS (United States Department of Agriculture Natural Resources Conservation Service). 2019a. PLANTS profile for *Phyllanthus fluitans* Benth. ex Müll. Arg.: Floating spurge. The PLANTS Database, National Plant Data Team, Greensboro, NC 27401. Accessed online 12 Nov. 2019. <https://plants.sc.egov.usda.gov/core/profile?symbol=PHFL10>

USDA–NRCS (United States Department of Agriculture Natural Resources Conservation Service). 2019b. PLANTS profile for *Azolla pinnata* R. Br.: Feathered mosquitofern. The PLANTS Database, National Plant Data Team, Greensboro, NC 27401. Accessed online 17 Dec. 2019. <https://plants.sc.egov.usda.gov/core/profile?symbol=AZPI>

Wilder, G.J. and M.P. Sowinski. 2010. *Phyllanthus fluitans* Benth. (Euphorbiaceae): A newly reported invasive species in Florida. Wildland Weeds (Fall 2010):14–15.

Wunderlin, R.P., B.F. Hansen, A.R. Franck, and F.B. Essig. 2019. Atlas of Florida plants species page: *Azolla pinnata*. [S.M. Landry and K.N. Campbell (application development), USF Water Institute.] Institute for Systematic Botany, University of South Florida, Tampa. Accessed online 6 Dec. 2019. <http://florida.plantatlas.usf.edu/Plant.aspx?id=4278>

Proc. Fla. State Hort. Soc. 132:245–249. 2019.

Magnitude of the Cultivated Flora of Florida

Alan R. Franck*[1] and Arian Farid[2]

[1]*Dept. of Biological Sciences, International Center for Tropical Botany, Florida International University, 11200 SW 8th St., Miami, FL 33199*

[2]*Herbarium, Department of Cell, Molecular, and Microbiology, ISA 2015, University of South Florida, Tampa, FL, 33620, USA*

Additional index words. botanical, garden, herbarium, iNaturalist, native, naturalized, species

Florida is an estimated 37 million acres in size, of which approximately 12% is composed of loblolly and slash pine plantations, 6% bahiagrass pasture, 1% orange groves, 1% sugarcane plantings, and 0.4% bermudagrass golf courses. Here we set out to estimate the diversity of the cultivated flora. Compiling information from herbaria, botanic gardens, and iNaturalist, we estimate 7468 species of land plants are known only from cultivation (not native and not naturalized) in Florida, but perhaps only around 500 of these cultivated-only species are relatively common in cultivation. An estimated 200 native and 300 naturalized species are relatively common in cultivation. Summing the native (~3200 species), naturalized (1500), and cultivated (7500) flora gives an estimate of 12,200 species of land plants occurring in Florida in recent time.

The study of the flora of an area usually makes the distinction between wild and cultivated plants. Wild plants occur primarily in natural areas and include the indigenous (native) species and the non-native naturalized species that reproduce and spread in the region. Cultivated plants generally occur in heavily altered areas and rely on more intensive human management for establishment and persistence.

The wild plants of Florida consist of about 4700 species of native or naturalized land plants (Embryophyta) (Wunderlin et al., 2019). About 10 million acres of conservation land (excluding perennial open water; FNAI 2019) help to preserve natural areas that support these wild plant species (see also Pearlstine et al., 2002), but these wild plants also occur in privately owned timberland, rangeland, perennial open water, natural areas fragments, ruderal areas, and in cultivated areas as weeds. Here we focus on the cultivated flora of Florida, especially pine plantations, pasture, crops, lawns, urban trees, and ornamentals (Fig. 1).

There are an estimated 11 million acres of private timberland in Florida (Hodges et al. 2017), of which about 4.4 million acres are cultivated pine plantations (Guldin and Wigley 1998; Zhang and Polyakov 2010), the vast majority consisting of *Pinus elliottii* and *P. taeda* (Landers et al., 1995). Planted pasture covers approximately 4 million acres (NRI 2015; USDA 2019) and, of this, over 2 million acres consist of *Paspalum notatum* pasture (Chambliss and Sollenberger 1991; Vendramini 2016). In the past few decades, pasture had covered as much as 5 million acres (NRI 2015).

We are grateful to Kent Perkins (FLAS), Brett Jestrow (FTG), Bruce Holst (MSBG), and Shawn McCourt (MSBG) for their kind assistance. Many thanks to Javier Francisco-Ortega (FIU) for his review of the manuscript.

Corresponding author; email: afranck@fiu.edu

Approximately 2.8 million acres are devoted to cropland in Florida (USDA 2019). In 2017, the 15 largest crops, by acreage, were *Citrus ×aurantium* (oranges; 422,421 acres), *Saccharum officinarum* (sugarcane; 386,428), *Arachis hypogaea* (peanuts; 186,803), *Gossypium hirsutum* (cotton; 98,569), *Zea mays* (corn for grain, silage, or greenchop; 62,717), *Citrus ×aurantium* (grapefruit; 40,248), *Solanum tuberosum* (potatoes; 30,378), *Solanum lycopersicum* (open-grown tomatoes; 29,136), *Zea mays* (sweet corn; 28,403), *Phaseolus vulgaris* (green beans; 27,823), *Cucumis sativus* (cucumbers; 26,222), *Citrullus lanatus* (watermelons;

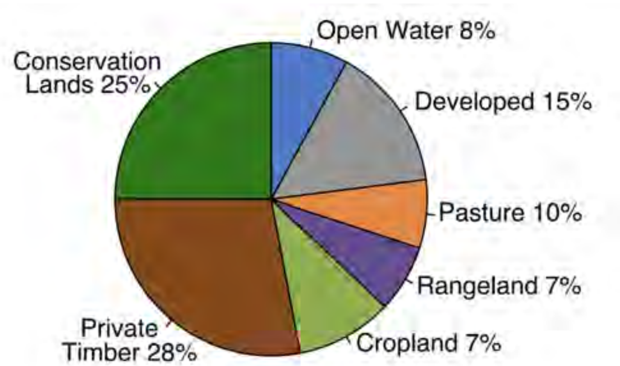

Fig. 1. Estimates of land use in Florida. Conservation lands here represent only the government-owned portion (9.5 million acres) and not the 0.85 million acres of privately owned conservation land. Land use totals add up to 38.1 million acres, exceeding the 37.5 million acres of surface area in Florida. This is due to the heterogeneity of sources (NRI 2015; Hodges et al. 2017; FNAI 2019) which have probably caused double-counting for some acres. Since forest land is 13.2 million acres in NRI (2015) and 17.2 million acres in Hodges et al. (2017) of which 66% is privately owned, land acreage coverage of 0.6 acres was subtracted from private timber to bring land use total to 37.5 million acres. An additional discrepancy is the pasture and rangeland in the NRI (2015) data is much higher than the USDA (2019) data.

22,071), *Oryza sativa* (rice; 18,422), *Glycine max* (soybeans; 14,376), and *Capsicum annuum* (peppers; 11,739). Acreages of oranges and grapefruits were approximately 666,000 and 118,000 (Hodges et al. 2001), respectively, in 2000, and have continually declined since (Court et al., 2017).

Stenotaphrum secundatum is a dominant lawn grass in Florida, comprising over half of the sod grass, followed by *Paspalum notatum* (Satterthwaite et al., 2009). In 2000, golf courses had about 147,000 acres of turf, primarily (93%) composed of *Cynodon dactylon* (Haydu and Hodges 2002).

A comprehensive view of the plant species cultivated in Florida is lacking, especially concerning the ornamental species and urban trees which are important floristic components of the 5.5 million acres of developed/urban areas in Florida (NRI 2015). Here we attempt to inventory the plant species cultivated in Florida using herbarium records, botanic garden lists, and iNaturalist (inaturalist.org). With some quantitative data, we roughly estimate their abundance in Florida.

Materials and Methods

Documented occurrences of cultivated plants were compiled into one list from herbaria, iNaturalist, and botanic gardens. Cultivated specimens of the FLAS (standard abbreviation for the University of Florida herbarium) and USF (University of South Florida) herbaria were downloaded 31 May 2019. Cultivated observations in iNaturalist were downloaded 15 June 2019. The species richness of select botanical gardens were consulted, Fairchild Tropical Botanic Garden (FTG 2016), Marie Selby Botanical Gardens (MSBG; B. Holst and S. McCourt, pers. comm.), and Montgomery Botanical Center (MBC 2016). The compiled list was reviewed to find and match synonyms to minimize duplicate listings.

Only records identified to species were analyzed (except for *Bougainvillea* spp.); infraspecific taxa were not considered. Species identifications of the cultivated bougainvilleas are erratic and inconsistent; therefore they were considered one species for convenience and any records identified to genus were included. Review of the records of the cultivated bougainvilleas suggest they are primarily identifiable as *Bougainvillea ×buttiana* and less often as *B. glabra*; all cultivated bougainvilleas were called *B.* cf. *×buttiana* in the dataset for convenience. The records given as *Lantana camara* appear more likely to be *L. strigocamara*, and were referred to as *L.* cf. *strigocamara*. The records of *Epipremnum pinnatum* appear to be *E. aureum* and were thusly named. The records given as *R. indicum* in iNaturalist appear to be *R. pulchrum* or *R. simsii*; they were all named as *R.* cf. *simsii* for convenience. The iNaturalist records of *Handroanthus chrysanthus* seem more likely to be *H. chrysotrichus* and were named thusly as *H. chrysotrichus*. The iNaturalist records of *Aristolochia littoralis* are more likely *A. elegans*, and were so named in the dataset.

Records in iNaturalist found to be plant products and not plants actively being cultivated were removed (e.g. *Abies fraseri* as a cut Christmas tree). Since cultivated observations in iNaturalist receive less scrutiny, all species with 1–3 observations (1389 species) were reviewed and identifications were added if there was disagreement. Of these, 249 species were determined either to be misidentifications or, less commonly, to be wild (not cultivated) observations, such as weeds in cultivated settings or wild plants clearly in natural settings; as a result, 313 observations and 249 species were removed from the initial iNaturalist dataset. The common name "sago palm" caused four observations of cycads to be misidentified as *Metroxylon sagu* (Arecaceae), which was also removed from the iNaturalist dataset.

For the top five most abundant species in herbaria (based on specimen sheets), records were reviewed to remove extra counts resulting from multi-sheet collections or duplicate specimens. The remainder of the records in each herbarium was not corrected for multi-sheet collections or duplicates.

To roughly estimate abundances of urban tree species, published analyses of urban tree studies from across the state were evaluated to identify the trees most abundant in each study and across the studies. The seven studies consulted were north Florida (Duryea 2007a), south Florida (Duryea 2007b), the panhandle (Escobedo et al., 2009a), Gainesville (Escobedo et al., 2009b),

Table 1. Top five most abundant cultivated plant species observations in Florida, as seen in iNaturalist in seven categories, with the number of observations in parentheses, as of 15 June 2019. FLEPPC = Florida Exotic Pest Plant Council. Cultivated-only refers to species known only from cultivation in Florida, neither native nor naturalized. Although *Hamelia patens* had 61 observations, nine of them were of the non-native *H. patens* var. *glabra*.

	Top five most abundant cultivated plant species observations in Florida, as seen in iNaturalist Plant category in seven categories, with the number of observations in parentheses, as of 15 June 2019.				
Native	*Magnolia grandiflora* (191)	*Zamia integrifolia* (143)	*Sabal palmetto* (68)	*Quercus virginiana* (65)	*Roystonea regia* (60)
Naturalized	*Hibiscus rosa-sinensis* (291)	*Schefflera arboricola* (174)	*Codiaeum variegatum* (163)	*Plumbago auriculata* (147)	*Ixora coccinea* (140)
FLEPPC Cat. I	*Ruellia simplex* (79)	*Eugenia uniflora* (65)	*Bauhinia variegata* (44)	*Nephrolepis cordifolia* (39)	*Tradescantia spathacea* (38)
FLEPPC Cat. II	*Cocos nucifera* (57)	*Epipremnum aureum* (56)	*Begonia cucullata* (38)	*Platycerium bifurcatum* (31)	*Aristolochia elegans* (31)
Cultivated-only eudicots	*Bougainvillea cf. ×buttiana* (412)	*Rhododendron cf. simsii* (59)	*Adenium obesum* (57)	*Ceiba speciosa* (47)	*Gardenia jasminoides* (45)
Cultivated-only monocots	*Cordyline fruticosa* (168)	*Philodendron bipinnatifidum* (82)	*Strelitzia reginae* (78)	*Ananas comosus* (62)	*Dietes bicolor* (52)
Cultivated-only palms	*Bismarckia nobilis* (41)	*Phoenix canariensis* (35)	*Phoenix roebelenii* (32)	*Adonidia merrillii* (19)	*Phoenix dactylifera* (16)

Orlando (Ekpe et al., 2012), Tampa (Landry et al., 2018), and Miami (Escobedo et al., 2011). Urban trees are often planted or, if from wild propagules, managed in cultivated settings; thus for convenience they are treated as part of the cultivated flora.

To compute the rate of taxa added to the flora of Florida per year, we compared totals from 2003–2019. From the first comprehensive state-focused flora (Wunderlin 1998) to the second edition (Wunderlin and Hansen 2003), there was the largest increase in both native and non-native taxa, probably partly due to records that had been overlooked. From 2003–2019, rates were fairly constant, increasing from 2827 native and 1318 naturalized taxa (Wunderlin and Hansen 2003) to 2,895 native and 1,512 non-native taxa (Wunderlin et al. 2019). Taxa here are comprised of about 98% as species and 2% as a second or third infraspecific taxon of a species in the flora.

Results

A total of 12,355 observations from iNaturalist, 9938 specimens from FLAS, and 4683 specimens from USF were included. Combined from iNaturalist, FLAS, USF, and FTG, there were 684 cultivated native species, 786 cultivated naturalized non-native species, and 4886 species known only from cultivation in Florida. Of the species known only from cultivation, iNaturalist recorded 896 species, FLAS recorded 2444, FTG recorded1966, and USF recorded 1156. Of these species known only from cultivation, 382 were unique to iNaturalist, 1443 to FLAS, 1270 to FTG, and 623 to USF, meaning only 1168 species known only from cultivation were in at least two of these datasets.

The most commonly observed cultivated species in iNaturalist are given in Table 1. The most commonly observed cultivated species endemic to Florida in iNaturalist was *Illicium parviflorum* (10 observations). *Lagerstroemia indica*, one of the most commonly planted small trees and occasionally naturalized in Florida, had 134 iNaturalist observations. There were 391 palm species in the compiled dataset, almost all found at FTG. The most common cultivated specimens at FLAS were *Podocarpus macrophyllus* (27 collections), *Galphimia gracilis* (20), *Asystasia gangetica* (19), *Cnidoscolus aconitifolius* (24), *Loropetalum chinense* (17), and *Hibiscus rosa-sinensis* (17), and at USF were *Ruellia nudiflora* (107), *Ruellia caroliniensis* (43), *Calliandra haematocephala* (35), *Allamanda cathartica* (33), *Hibiscus rosa-sinensis* (29), and *Parkinsonia aculeata* (29). Also reported in 2018 were the eradication of 3409 plants of *Cannabis sativa* grown outdoors and 692 plants grown indoors (FDMEP 2019).

The total number of plant species in the Araceae (405 species), Bromeliaceae (1215), Gesneriaceae (157), Orchidaceae (1456), and fern group (320) cultivated at MSBG all exceeded that in the compiled dataset from iNaturalist, FLAS, FTG, and USF [Araceae (207); Bromeliaceae (154); Gesneriaceae (62); Orchidaceae (323); ferns (144)]. The total number of plant species in the cycad group (228) cultivated at MBC exceeded the number of species in the compiled dataset [Cycadaceae (13) and Zamiaceae (54)]. Considering these specialized collections at MSBG and MBC, then at least 2824 more species of plants are cultivated in Florida (i.e. 957 species in these groups from the compiled dataset subtracted from 3781 species in these groups at MSBG and MBC), which would bring the total number of species cultivated in Florida to 9178 species (i.e. 682 cultivated native species, 786 cultivated naturalized non-native species, and 4886 cultivated-only species from the compiled dataset plus 2824 more species from MSBG and MBC). From these groups there are 355 native and naturalized species (including named hybrids) in the Florida flora [Araceae (34); Bromeliaceae (22); Cycadaceae (1); ferns (178); Gesneriaceae (0); Orchidaceae (118); and Zamiaceae(2)] and 844 species from the compiled cultivated-only dataset [Araceae (192); Bromeliaceae (135); Cycadaceae (12); ferns (94); Gesneriaceae (62); Orchidaceae (297); and Zamiaceae (52)]. Allowing that MSBG and MBC could be cultivating all of Florida's 355 native and naturalized species in these groups and as well as all of the 844 species in the compiled cultivated-only dataset, then MSBG and MBC could have 2582 more species known only from cultivation in Florida (i.e. subtracting both the 355 native and naturalized species in these groups and 844 species in the compiled cultivated-only dataset from 3781 species from MSBG and MBC), bringing the total to 7468 species known only from cultivation in Florida (i.e. 2582 plus 4886).

Based on the urban tree literature in Florida, the most abundant urban trees are *Quercus virginiana* (occurring in all seven references, possibly including some *Q. geminata* which can be difficult to identify), *Pinus elliottii* (five references), and *Quercus laurifolia* s.lat. (four references, probably including *Q. hemisphaerica*; absent to rare generally in extreme southern Florida, such as Miami). A checklist of woody plants of relative horticultural or commercial importance that were cultivated in Florida included 1979 species, of which 239 were native species (Burch et al. 1988), but abundances were not given. This list (Burch et al. 1988) has not yet been transcribed and incorporated with the present dataset.

Based on rates from 2003–19 (Wunderlin and Hansen 2003; Wunderlin et al. 2019), about 4.3 native and 12.1 naturalized taxa have been added to the flora per year.

Discussion

Based on these data (from iNaturalist, FLAS, FTG, MBC, MSBG, and USF), about 7468 species of land plants (Embryophyta) are known only from cultivation (neither native nor naturalized) in Florida. Only 1168 species known only from cultivation were found in at least two datasets (from iNaturalist, FLAS, FTG, and USF). Even if found in two datasets, some of these 1168 species might only be found in one place such as FTG (e.g. *Caloncoba echinata* is in the FTG list and the only FLAS specimen is from FTG, and thus it shows up in two datasets). Additionally, there are very likely some misidentifications as well as unmatched synonyms in the dataset which could reduce the number of species. There are also likely species cultivated in Florida not found in the dataset. A previous estimate suggested about 25,000 species of plants were cultivated but not naturalized in Florida (Frank and McCoy 1995).

For species with two or more cultivated observations in iNaturalist, there were 423 species known only from cultivation, 217 native species, and 307 naturalized species. Considering this, perhaps approximately 500 species of plants known only from cultivation, 200 native species, and 300 naturalized species are relatively common in cultivation in Florida. Following this, perhaps the other 7000 species known only from cultivation (many known only from botanic gardens), 500 native species, and 500 naturalized species are relatively rare in cultivation in Florida. Thus, together, roughly, ~1000 species of plants might be somewhat commonly cultivated or available in Florida.

While the iNaturalist website is quite useful for studying the cultivated flora, it is optimized for wild organisms and is of limited utility for cultivated observations. By default the option "verifiable" is selected for searches in iNaturalist, which excludes cultivated plants in the results. Cultivated observations are considered not "verifiable" or ineligible for "research grade" (when more than 2/3rds of species-level identifications agree). By default uploaded observations are presumed wild unless the "captive" option is selected. The result is that cultivated observations receive less scrutiny from users and many cultivated observations are uploaded as wild organisms. Further, some species that can be locally common in cultivation, e.g. *Dimocarpus longan*, *Elaeagnus pungens*, or *Magnolia figo* are poorly represented in iNaturalist, each of these having only two cultivated observations. One of the top crops in Florida, *Saccharum officinarum*, had zero observations in iNaturalist for Florida. The user bias of iNaturalist favors more urban observations and more observations of attractive and conspicuous plants. For example, *Quercus virginiana* is the most common urban tree and very widely planted, yet it had only 65 cultivated observations in iNaturalist while *Magnolia grandiflora* (commonly planted but not as much as *Q. virginiana*) had 191.

There are similar biases towards urban locations and wild organisms in herbaria. Herbarium collections tend to favor representing the diversity of species over the abundance of a species. Cultivated occurrences of common native trees and common lawn grasses are relatively poorly represented in herbaria. For example, the native *Coccoloba uvifera* had a combined total of nine cultivated specimens in the FLAS and USF herbaria, while the non-native *Adenanthera pavonina* had 25 cultivated specimens, even though *C. uvifera* is much more commonly planted. For this example, iNaturalist is a better representation of abundance as it recorded 28 cultivated observations of *C. uvifera* and zero cultivated observations of *Adenanthera pavonina*.

The 7468 non-native, cultivated-only plant species in Florida serve ornamental, agricultural, research, educational, and ex-situ conservation purposes. While some of these are cultivated indoors (e.g. greenhouses), the majority are cultivated outside, thus having the increased potential for ecological interactions such as microbial associations, animal visitations, and propagule dispersal. The extent of these ecological interactions are relatively poorly known overall for the cultivated flora of Florida (e.g. Riddle and Mizell 2016). While local populations of native species are genetically unique and contribute some of the most vitally important ecosystem services (Pauchard et al. 2018), both the positive and negative impacts to ecosystems from the non-native cultivated flora bear consideration (Dale and Polasky 2007; Schlaepfer 2018a, 2018b; Pyšek et al. 2019). It is also interesting to consider the taxonomic diversity of propagule pressure from the cultivated-only flora, as the Florida flora on average adds about one non-native naturalized taxon per month, some being escapes from cultivation and others naturalizing without being cultivated.

Literature Cited

Burch, D., D.B. Ward, and D.W. Hall. 1988. Checklist of the Woody Cultivated Plants of Florida. Publication SP-33. Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL.

Chambliss, C.G. and L.E. Sollenberger. 1991. Bahiagrass: The foundation of cow-calf nutrition in Florida. Proc. 40th Florida Beef Cattle Shortcourse: 74–80.

Court, C.D., A.W. Hodges, M. Rahmani, and T.H. Spreen. 2017. Economic Contributions of the Florida Citrus Industry in 2015–16. Extension publication FE-1021. University of Florida, Gainesville, FL.

Dale, V.H. and S. Polasky. 2007. Measures of the effects of agricultural practices on ecosystem services. Ecological Economics 64:286–296.

Duryea, M.L., E. Kampf, and R.C. Littell. 2007a. Hurricanes and the urban forest: I. Effects on southeastern United State coastal plain tree species. Arboriculture & Urban Forestry 33:83–97.

Duryea, M.L., E. Kampf, R.C. Littell, and C.D. Rodríguez-Pedraza. 2007b. Hurricanes and the urban forest: II. Effects on tropical and subtropical tree species. Arboriculture & Urban Forestry 33:98–112.

Ekpe, E.K., E. Becker, J. Lab, R. Hinkle, F. Escobedo, and B. Iannone. 2012. Orlando, Florida's urban and community forests and their ecosystem services. Extension publication FOR-290. University of Florida, Gainesville, Fla.

Escobedo, F., S. Varela, C. Staudhammer, B. Thompson, and J. Jarratt. 2009a. Pensacola and southern Escambia County, Florida's urban forests. Extension publication FOR-231. University of Florida, Gainesville, FL.

Escobedo, F., J.A. Seitz, W. Zippere, and B. Iannone. 2009b. Gainesville Florida's urban tree cover. Extension publication FOR-215. University of Florida, Gainesville, FL.

Escobedo, F., J. Klein, M. Pace, H. Mayer, S. Varela, and B. Iannone. 2011. Miami-Dade County's urban forests and their ecosystem services. Extension publication FOR-285. University of Florida, Gainesville, FL.

FDMEP. 2019. Florida's Domestic Marijuana Eradication Program. 2018 Annual Report. Office of Agricultural Law Enforcement, Florida Department of Agriculture and Consumer Services and Drug Enforcement Administration, Tallahassee, FL.

FNAI. 2019. Summary of Florida Conservation Lands. Florida Natural Areas Inventory, Tallahassee, Fla.

Frank, J.H. and E.D. McCoy. 1995. Invasive adventive insects and other organisms in Florida. The Florida Entomologist 78:1–15.

FTG. 2016. 2016 Plant Names Catalog. <https://www.fairchildgarden.org/Portals/0/docs/Horticulture/2016%20Names%20Catalog%20botanical%20name.pdf?ver=2016-02-11-123625-480>

Guldin, J.M. and T.B. Wigley. 1998. Intensive management-Can the South really live without it? Transactions 63rd North American Wildlife and Natural Resource Conference: 362–375.

Haydu, J. and A. Hodges. 2002. Economic Impacts of the Florida Golf Course Industry. Economic Information Report EIR 02-4. IFAS, University of Florida, Gainesville, FL.

Haydu, J.J., L.N. Satterthwaite, and J.L. Cisar. 2002. An economic and agronomic profile of Florida's sod industry in 2000. Economic Information Report EIR 02-6. Food and Resource Economics Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL.

Hodges, A.W., C.D. Court, and M. Rahmani. 2017. Economic contributions of the forest industry and forest-based recreation in Florida in 2016. Ext. Pub. FE-1051. University of Florida, Gainesville, FL.

Hodges, A., E. Philippakos, D. Mulkey, T. Spreen, and R. Muraro. 2001. Economic Impact of Florida's Citrus Industry, 1999–2000. Economic Information Report 01-2. IFAS, University of Florida, Gainesville, FL.

Landers, J.L., D.H. Van Lear, and W.D. Boyer. 1995. The longleaf pine forests of the southeast: Requiem or renaissance. J. Forestry 93:39–44.

Landry, S., A. Koeser, R. Northrop, D. McLean, G. Donovan, M. Andreu, and D. Hilbert. 2018. City of Tampa Tree Canopy and Urban Forest Analysis 2016. Tampa, FL.

MBC. 2016. Living Plant Collection. 2016 Collection Statistics. <http://www.montgomerybotanical.org/Pages/Collection.htm>

NRI. 2015. Florida Land Use. National Resources Inventory, USDA. <http://www.nrcs.usda.gov/Internet/NRCS_RCA/reports/nri_fl.html>

Pauchard, A., L.A. Meyerson, S. Bacher, T.M. Blackburn, G. Brundu, M.W. Cadotte, F. Courchamp, F. Essl, P. Genovesi, S. Haider, N.D. Holmes, P.E. Hulme, J.M. Jeschke, J.L. Lockwood, A. Novoa, M.A. Nuñez, D.A. Peltzer, P. Pyšek, D.M. Richardson, D. Simberloff, K. Smith, B.W. van Wilgen, M. Vilà, J.R.U. Wilson, M. Winter, and R.D. Zenni. 2018. Biodiversity assessments: Origin matters. PLoS Biology 16(11): e2006686. https://doi.org/10.1371/journal.pbio.2006686

Pearlstine, L.G., S.E. Smith, L.A. Brandt, C.R. Allen, W.M. Kitchens, and J. Stenberg. 2002. Assessing state-wide biodiversity in the Florida Gap analysis project. J. Environ. Mgt. 66:127–144.

Pyšek, P., W. Dawson, F. Essl, H. Kreft, J. Pergl, H. Seebens, M. van Kleunen, P. Weigelt, and M. Winter. 2019. Contrasting patterns of

naturalized plant richness in the Americas: Numbers are higher in the North but expected to rise sharply in the South. Global Ecology and Biogeography 28:779–783.
Riddle, T.C. and R.F. Mizell. 2016. Use of crape myrtle, *Lagerstroemia* (Myrtales: Lythraceae), cultivars as a pollen source by native and non-native bees (Hymenoptera: Apidae) in Quincy, Florida. Florida Entomologist 99:38–46.
Satterthwaite, L.N., A.W. Hodges, J.J. Haydu, and J.L. Cisar. 2009. An Agronomic and Economic Profile of Florida's Sod Industry in 2007. Mid-Florida Research and Education Center, University of Florida, Apopka, FL.
Schlaepfer, M.A. 2018a. On the importance of monitoring and valuing all forms of biodiversity. PLoS Biology 16:e3000039
Schlaepfer, M.A. 2018b. Do non-native species contribute to biodiversity? PLoS Biology 16:e2005568.
USDA. 2019. 2017 Census of Agriculture. Florida. State and County Data. Volume 1. Geographic Area Series. Part 9. AC-17-A-9. National Agricultural Statistics Service, United States Department of Agriculture, Washington, D.C.
Vendramini, J. (ed.). 2016. Florida Forage Handbook. Ext. Pub. SS-AGR-97. University of Florida, Gainesville, FL.
Wunderlin, R.P. 1998. Guide to the Vascular Plants of Florida. University Press of Florida, Gainesville, FL.
Wunderlin, R.P. and B.F. Hansen. 2003. Guide to the Vascular Plants of Florida. University Press of Florida, Gainesville, FL.
Wunderlin, R.P., B.F. Hansen, A.R. Franck, and F.B. Essig. 2019. Atlas of Florida Plants. [S.M. Landry and K.N. Campbell (application development), USF Water Institute.] Institute for Systematic Botany, University of South Florida, Tampa, FL. <http://florida.plantatlas.usf.edu/>
Zhang, D. and M. Polyakov. 2010. The geographical distribution of plantation forests and land resources potentially available for pine plantations in the U.S. South. Biomass and Energy 34:1643–1654.

Proc. Fla. State Hort. Soc. 132:250–251. 2019.

Eumaeus atala Poey (Florida Atala Butterfly) Native Plant Society Field Days in St. Lucie County, Florida

Kenneth T. Gioeli*

University of Florida/IFAS, St Lucie County Extension, 8400 Picos Rd., Ste. 101, Fort Pierce, FL 34945

Additional index words. Endangered species, extinction, butterfly, atala, hairstreak, entomology

The Florida atala butterfly (*Eumaeus atala* Poey) is a beautiful, somewhat rare hairstreak butterfly characterized by satiny black wings featuring an iridescent turquois shimmer. It had been thought to be extinct due to overharvest of its host plant, *Zamia integrifolia* Linnaeus. f. (a.k.a. coontie). These beautiful butterflies are now found on coontie plants in localized colonies, primarily in South Florida. In Summer 2017, Annmarie Loveridge contacted the St. Lucie County Extension Office to report a significant abundance of these beautiful butterflies at the Fort Pierce Inlet State Park. Upon further study, it was learned that these butterflies emerge in synchronized patterns, which enabled the establishment of atala butterfly field days in 2017 and 2018. These events attracted media attention and public interest, enabling people to learn how to identify and conserve these rare butterflies.

Once thought to be extinct, the Florida atala butterfly was rediscovered and now has established populations in subtropical southeast Florida (Koi and Hall 2019). Populations of this butterfly were also discovered in coastal St. Lucie County with a large protected population at the Fort Pierce Inlet State Park. The Florida Natural Areas Inventory has listed this butterfly as S2—either very rare and local in Florida or found locally in a restricted range or vulnerable to extinction from other factors.

The Fort Pierce Inlet State Park is a unique environmental treasure in St. Lucie County that features beaches, dunes and a coastal hammock. It is 340 acres on the north side of the Fort Pierce Inlet. According to the U.S. Department of Agriculture Plant Hardiness Zone Map, the park is in zone 10a, which features an average annual extreme minimum temperature of 30 °F to 35 °F. The subtropical climate and abundance of the Florida atala host plant, *Zamia integrifolia*, has enabled this population to survive in this site.

Materials and Methods

In Summer 2017, resident native plant advocate Annmarie Loveridge contacted St. Lucie County Extension Agent Ken Gioeli to report the significant numbers of Florida atala butterflies at the Fort Pierce Inlet State Park. There were hundreds of atalas fluttering around the state park. Annmarie knew Ken had experience with these rare butterflies and that he had expertise addressing natural resource conservation issues. The unique abundance of these showy butterflies provided an opportunity for them to conduct public outreach on the conservation of Florida atala butterflies and their host-specific dependency on native *Zamia integrifolia* (Figs. 1, 2, and 3).

Results and Discussion

During Summer 2017 and 2018, atala viewing events were conducted for members of the native plant society, the general public and the media. Approximately one-hundred participants increased their knowledge of Florida atala butterflies and their unique lifecycle and history. While many participants wanted to help augment habitat for these butterflies in their own personal landscapes by planting *Zamia integrifolia*, Loveridge and Gioeli had to temper their enthusiasm. There may be natural or man-made barriers that would inhibit establishment of these colonies

Fig. 1. A Florida atala butterfly perches on a sea grape leaf at the Fort Pierce Inlet State Park. Photo credit: K. Gioeli.

Much thanks to Annmarie Loveridge for her commitment to conservation of Florida's native plants and animals. This project was made possible with the support of the management and staff of the Fort Pierce Inlet State Park.

*Corresponding author. Email: ktgioeli@ufl.edu

Fig. 2. Florida atala butterfly caterpillars are host-specific to *Zamia integrifolia* (a.k.a. coontie) which is found in the landscaping at the Fort Pierce Inlet State Park. Toxins in the coontie plant are ingested by the caterpillars making the caterpillars toxic to predators. Photo credit: K. Gioeli.

Fig. 3. Florida atala butterfly chrysalis aggregate on branches of the coontie plant at Fort Pierce Inlet State Park. Photo credit: K. Gioeli.

Fig. 4. Members of the Florida Native Plant Society observed atala butterflies and learned about their unique lifecycle during viewing events at the Fort Pierce Inlet State Park. Photo credit: D. Culbert.

such as the use of insecticides in urban areas and differences in climate (Fig. 4).

Because this unique natural phenomenon was brought to the attention of the management at the Fort Pierce Inlet State Park, park staff increased their knowledge about the dependency of atalas to their host plant. Educational signage has been authorized within state park boundaries so their employees remain aware of the need to conserve the host plant *Zamia integrifolia* for the survival of Florida's atala butterflies.

Literature Cited

Koi, S. and D.W. Hall. 2019. Atala Butterfly, Atala Hairstreak, Coontie Hairstreak, *Eumaeus atala* Poey 1832 (Insecta: Lepidoptera: Lycaenidae). Extension publication EENY-641. University of Florida, Gainesville, FL.

Proc. Fla. State Hort. Soc. 132:252–253. 2019.

Lessons for Florida from Southern Spain Horticulture Tour

Juanita Popenoe[1]*, Karen Stauderman[2], and Elizabeth Felter[3]

[1]*University of Florida/IFAS Lake County Extension, 1951 Woodlea Rd., Tavares, FL 32778*

[2]*University of Florida/IFAS Volusia County Extension, 3100 E. New York Ave., DeLand, FL 32724*

[3]*University of Florida/IFAS Central District Regional Specialized Extension Agent, 2725 Binion Rd., Apopka, FL 32703*

Additional index words. almond, *Carica papaya,* greenhouse, high tunnel, mango, *Mangifera indica,* olive, *Olea europaea,* papaya, protected agriculture, *Prunus dulcis,* sustainability

University of Florida/IFAS extension agents visited southern Spain in 2018 on a tour of horticultural industries. The tour began with a meeting of urban leaders, who presented an overview of the horticultural industry and spoke of their pride in the clean energy used in the greenhouses, boasting successful, sustainable and efficient greenhouses capitalizing on their abundant sun and wind resources. Southern Spain is on a progressive timetable to develop alternative water solutions to unforeseen drought conditions. More importantly, the agents visited citrus groves and olive orchards, vineyards, and both vegetables and tropical fruit grown in greenhouses, along with allied industries that have applications to Florida horticulture. All crops were grown using biological controls and intensive management that are achievable in Florida, including trellised mangos, papayas, and various vegetables under plastic. Growers all had small holdings and were members of co-ops to market their produce.

In 2018, a group of University of Florida/IFAS extension agents visited Southern Spain on a 10-day tour of horticultural industries. The horticultural areas visited in Andalusia province included Malaga for citrus, El Ejido for vegetables, Almeria for tropical fruits, Granada for almonds and olives and Seville for grapes.

El Ejido is located in the Almeria region of southern Spain and is known throughout the European Union for its innovative use of resources, investment in technology and sustainability. The visit began with a meeting hosted by urban leaders and growers that laid the groundwork for their current successful model. This appealed to the extension agents as a possible benefit and potential resource to assist Florida's agricultural industry. Local government promoted agriculture as a major asset to the region, unlike what is seen in Florida. The thin strip of beach frontage did have tourist hotels, but the area behind was covered with agricultural operations. There were some agritourism bed and breakfast type businesses in the grape, almond, and olive growing regions, but agriculture and tourism were found to be quite separate in most areas and both were valued. Urban sprawl was not seen, but greenhouse and agricultural sprawl was seen as growers moved to marginal land and trellised it with open fields or greenhouses.

Itinerary

Our group met with local authorities including the mayor of El Ejido, the Agriculture Councilor of the city, the Councilor of Tourism, Commerce and Trade, and a Professor of Applied Economics at the University of Almeria. The meeting also included two-time National Innovation award winner recipient, Lola Gómez Ferrón of Clisol Turismo Agricola, a nationally recognized greenhouse vegetable grower in the area.

The municipal leaders emphasized the importance of investing in agriculture by road improvement through a municipal road repair plan. Over 373 miles of rural roads and tracks enables farmers to travel and transport produce to the production facilities. They described how the system allowed El Ejido to be competitive in demanding international markets.

The mayor explained that 60% of the work force in El Ejido is employed in agriculture with tourism as a distant second. El Ejido, with a population 85,000, is the largest single vegetable production area in Spain with 8000 farmers with over 35,000 acres of greenhouses under cultivation, primarily devoted to producing peppers, tomatoes, zucchini, eggplant, watermelons, melons, and beans.

They presented an overview of the industry and spoke of their pride using clean electric energy in the greenhouses, boasting successful, sustainable and efficient greenhouses, and capitalizing on their abundant sun and wind resources. Southern Spain is on a progressive timetable to develop alternative water solutions to unforeseen drought conditions. They currently use cisterns to capture rainfall, then a complex filtering system that removes impurities and cycles water back through their operation. Some greenhouses are also equipped with onsite desalination systems in order to utilize saltwater.

*Corresponding author. Email: jpopenoe@ufl.edu

Observations

This region of Spain is hot, arid, and windy, yet greenhouses are updated with state-of-the-art ventilation that allows regulation of air flow and humidity. The plastic on the greenhouses is replaced every 3 years and fabricated and recycled at local facilities. Most of the greenhouses utilize hydroponic systems that drip feed chemical fertilizers into grow-bags to reduce possible leaching. Papaya and mango were grown in the ground in plastic structures, much like high tunnels, to protect against wind and cold damage at the research station trialing greenhouse systems on perennial fruit. Mangos were being trellised and papayas cut back after two years to keep the trees small enough to fit in the plastic greenhouses. These systems would be a perfect option for growers in central Florida who have to contend with possible cold damage.

Almeria's sea of white-roofed greenhouses are so vast that researchers from the University of Almeria have found that the white color of the plastic reflects sunlight into the atmosphere as if it were a mirror, slowing the warming of the earth's surface. While temperatures in the rest of Spain have climbed at rates above the world average, the local temperature has dropped an average of 0.3 °C every decade. In this way, the greenhouses at a local level offset the rising temperatures associated with global warming (Campra et al., 2008).

Citrus was also grown on small, family-owned 5–10 acre lots and growers formed cooperatives for packing and marketing. The main problems shown to us were Mediterranean fruit fly and voles. Fruit fly traps to monitor and attract and kill traps were seen in the orchards as well as mating disruptor pheromone traps. Dogs were trained to hunt and kill the voles that girdled the tree trunks. Irrigation systems were being changed from seepage to trickle to reduce water waste. The growers we visited claimed to be organic and used some local remedies for pest problems, including fermenting horsetail sedge to create a homoeopathic spray, and encouraging beneficial insects with refuges around the orchards.

Greenhouse growers also claimed to be organic with extensive use of beneficial insect releases and trapping of pests. Greenhouse vents were all covered with insect excluding screen. Greenhouses all had exclusion rooms at the entrance lined with yellow and blue sticky cards. Anyone entering the room was confined for several minutes to allow unwanted insects to be trapped before the interior door would open. Various forms of organic pest control were shown to us including pheromone traps, beneficial insects released, and wire twist pheromone mating disruptors. Bumble bees were brought in special boxes to pollinate crops as honeybees do not operate well under plastic. Luckily Florida does not have the leaf miner *Tuta absoluta* that has caused many problems in Spain and was a major focus of their pest management practices.

Olives and almonds had few pest problems and were widely planted in areas with no apparent irrigation, often on steep slopes that were cultivated to reduce weeds. Olive trees were trained to single or triple trunks leaning out from center to facilitate mechanical shaking harvesters. The almond grower admitted that harvesting his crop was not cost effective compared to California almonds, which were cheaper on the open market. The almond and olive grower's only inputs were fertilizer and pruning.

Upon our return, Carlos Mendez of Sotrafa Greenhouse Film, Almeria, Spain, contacted us to say he would be visiting Florida. We were able to arrange a meeting for him with BWI, a grower supply company in Apopka, FL. Sotrafa has been added to the list of greenhouse film suppliers.

The horticulture of southern Spain has many similarities to the current and potential horticulture of central Florida. Florida growers could learn from the techniques used in greenhouse construction, integrated pest management, tropical fruit production, and water management.

Literature Cited

Campra, P., M. Garcia, Y. Canton, and A. Palacios-Orueta. 2008. Surface temperature cooling trends negative radiative forcing due to land use change toward greenhouse farming in Southeastern Spain. J. Geophysical Res. 113. doi: 10.1029/2008JD009912, ISSN: 0360-1323.

Proc. Fla. State Hort. Soc. 132:254–256. 2019.

Urban Agriculture Initiatives in Orange County, Florida

Richard Tyson*, Caitlyn Glatting, and Liz Felter

University of Florida/IFAS Orange County Extension, 6021 S. Conway Rd., Orlando, FL 32812

Additional index words. urban farm, healthy lifestyle choices, agroecology

Urban agriculture has many definitions but generally involves producing and marketing local food in or near urban areas. Seeking solutions to enhance community revitalization, economic development, food security and greenspace, many local governments and food entrepreneurs in Orange County, FL, are focusing resources and planning initiatives around urban agriculture. These changes provide healthier lifestyle choices for local citizens. Successes include adoption of the Florida cottage food law and backyard chicken ordinances, legalizing front yard vegetable gardens and recognizing residential market gardens. Significant expansion of community gardens is underway at parks, community centers, schools, and other sites. A farm-to-school public/private partnership is expanding. Several private entrepreneurs were able to establish a commercial food hub, rooftop greenhouses, and indoor vertical hydroponics as well.

Significant changes to agricultural production and population dynamics in Orange County, Florida, over the last 40 years are creating support for a thriving local food movement among food entrepreneurs and residents interested in making healthy lifestyle choices. In 1978, commercial farmers in the county produced 75,560 acres of fruits and vegetables; that acreage has dropped dramatically to only 3308 acres today (USDA Census of Agriculture, 1978, 2017). Orange County has a population of 1,349,597 people and hosts 75 million tourist visitors annually according to the tourist bureau, Visit Orlando. If food imports into the county cease due to a national emergency, estimates are that food supplies in local warehouses and grocery stores would be depleted in about thirty days, based on interviews with suppliers. A comprehensive food production strategic plan for Orange County was created by the East Central Florida Regional Planning Council (ECFRPC) which can help guide future efforts to promote both the historic and a future culture which celebrates local food systems in the county (Nieves-Ruiz and Cassidy, 2016).

Opportunities of Urban Agriculture

The two most important reasons to support urban agriculture are food security for all citizens and economic development. Having healthy, nutritious food growing in our neighborhoods nourishes healthy lifestyles, which reduces long term health care costs. The local food economic multiplier effect contributes to community development. Every dollar spent on local food circulates throughout the economy when worker salaries pay for other items such as haircuts and clothing, while local farmers and food entrepreneurs use the sale to purchase more local products needed for the next crop or meal. The total economic impacts of local food purchases in Florida, including indirect multiplier effects calculated with a regional economic model, were estimated at 183,625 jobs and $10.47 billion in GDP (Hodges and Stevens, 2013). Widespread support for local and regional food systems is increasing across America and bringing significant economic benefits to many communities (Barham et al., 2012; Dumont et al., 2017; Martinez et al., 2010; Rife, 2013).

Challenges of Urban Agriculture

Most cities and counties do not have designated zoning rules for growing food in urban areas, so inquires by food entrepreneurs or residents wishing to start an operation usually results in a “not allowed” response by local governmental administrators. The experience of the authors has been that in order to get movement on these initiatives, city and county leadership at the highest levels need to support the plans and follow through with staff to see them implemented. Local governmental staff members generally do not have the power to make these types of changes on their own. The City of Orlando has taken a lead in this regard by starting several pilot programs to get initiatives quickly approved. They are also working on a framework for an urban agriculture ordinance plan that will allow areas in the city to be a designated use for urban farmers, such as vacant lots and indoor warehouse space.

Urban Farm and Garden Initiatives

Indoor and Rooftop Hydroponics. There are several Orange County food entrepreneurs who have started indoor vertical hydroponic systems, one being at the Marriott World Center Hotel (stacked bench bed design) and the other at the Orange County Convention Center (vertical towers). The first sells leafy salad crops and herbs to restaurants in the hotel and the second uses the same products for catering services at the Convention Center. In 2008, two state of the art greenhouses were built on top of the Roper Garden Building in the city of Winter Garden. One greenhouse is devoted to a tilapia/lettuce aquaponics operation and the other is for hydroponic vegetable production.

Florida Cottage Food Law. This law allowing small business cottage food startups in residential homes (FDACS, 2017) was adopted by Orange County Government and the City of Orlando in 2013. Residents use home kitchen appliances to produce breads, cookies, jellies, jams, or other food products that do not require refrigeration. No state license or inspection is required, but a

*Corresponding author. Email: rvt@ufl.edu.

local business license is required. You may sell only what you produce and sales must be person-to-person at farmers markets or community events or delivered directly to the consumer. Sales cannot exceed $50,000 annually from this home occupation. When this annual threshold is surpassed, it is expected that the business will move out of the home to a brick and mortar setting.

Fleet Farming. In 2013, the City of Orlando passed an ordinance that would allow for up to 60% of a residential front yard space to be put into vegetable gardens (Schlueb, 2013). In a grassroots effort to embrace the new policy, a group of local residents were inspired to establish a non-profit called Fleet Faming. Fleet Farming is a pedal-powered, urban farming initiative that converts underutilized front lawns to highly productive vegetable gardens. The program is primarily run by community volunteers who meet twice a month to build, maintain, harvest, and manage the residential plots, all via bicycles. The homeowner takes his or her share while the rest is harvested, processed, and sold at a nearby farmers market as well as to local restaurants. As of June 2019, Fleet Farming has converted over 70,000 square feet of lawns and harvested over 3,500 lb. of produce. In addition, recently signed Florida legislation (SB 82—Vegetable Gardens) removes most city and county restrictions on residential gardens and should open up many new possibilities for urban agriculture.

Residential Market Gardens. Residents can grow fruits, vegetables and herbs as a home occupation in unincorporated Orange County, FL, provided the produce is sold off-site, away from the residence. The home garden can become a market garden, boosting family income. Families should follow the same growing procedures recommended for home gardeners; just add more space for extra produce to sell. It must be sold whole, not processed, and obtaining a local business license is recommended to market at farmers markets and community events.

Back Yard Chicken Ordinances. The City of Orlando passed a back yard chicken pilot program in three of its five districts in 2013. With very few problems reported, the pilot program was passed as a city-wide ordinance in 2016. To qualify for the program, the ordinance requires applicants to attend a chicken care and safety certification class provided by the Orange County Cooperative Extension Service. Other cities in Orange County have also adopted similar ordinances. Chicken coop setbacks from the property line are usually 25 feet, and 3-4 egg laying hens (no roosters) are allowed. The certification class includes addressing concerns such as fly and rodent control, waste management, and Salmonella contamination.

Farmers Markets. With the increased demand for local food and consumer desire to connect with where their food is coming from, many are turning to local farmers markets. Orange County has over 15 different markets in diverse regions of the county. Farmers markets act as an opportunity for economic and community development. In an effort to address the widening food insecure population in Orange County, three markets have adopted the Fresh Access Bucks program. Fresh Access Bucks is a U.S. Department of Agriculture-funded nutrition incentive program that encourages SNAP recipients to redeem their benefits at farmers markets (Feeding Florida, 2020). Participating markets are able to double SNAP incentive up to $40 (20 food stamp dollars becomes 40) if spent on Florida produce, thus putting more money back in farmers pockets.

East End Market Food Hub. For many foodpreneurs looking to take the leap from vending at a farmers market to brick and mortar, it can be a large jump with many associated risks. Food halls can help to create a middle ground for such vendors looking to scale up with less risk. The East End Market, established in 2014, is Orlando's first local food and cultural hub of its kind. East End Market is home to one dozen individual merchants, a large event space, demonstration kitchen, incubator kitchen, offices, retail shops, and a world-class restaurant. It also hosts community food driven events that help to foster collaboration between residents, non-profits, and local government in order to move the needle forward in creating a more sustainable local food system.

Farm to School. Across the country, school districts are also getting involved in the food system. The Farm to School movement aims to help "students gain access to healthy, local food, as well as [provide] education opportunities such as school gardens, cooking lessons, and farm field trips," according to the National Farm to School Network. In Orange County, Florida there are over 70 active school garden programs where students learn how to grow their own fruits and vegetables and receive nutrition education to support healthy eating habits. School gardens can be impactful for many reasons. They have demonstrated to increase children's fruit and vegetable consumption and increase their knowledge of fruits and vegetables (Parmer et. al., 2009). This supports the school district's Farm to School initiative to procure Fresh from Florida items for school meals. In the 2017–18 school year, Orange County Public Schools purchased 3.4 million pounds of produce, and approximately 23% was grown locally in the state of Florida (Orange County Public Schools, 2018). Participating in a farm to school program can also reduce plate waste. "Students at schools with a Farm to School program ate 37% more vegetables and 11% more fruit than the average student consumed before their school adopted the program" (Kropp et. al., 2018).

Good Food Central Florida Food Policy Council. A discussion of urban agriculture initiatives in Orange County would not be complete without mentioning Good Food Central Florida. Formed in 2013 with the help of an initial grant from the Winter Park Health Foundation, the council has been the place where various public and private organizations, food entrepreneurs, and local food enthusiast have been able to come together and bounce ideas off each other. In addition, the council has worked to coordinate projects including grants, farm tours and partnering with the ECFRPC on the Orange County Food Production Strategic Plan to help smooth the adoption and expansion of local food system initiatives in the county. The latest council project, led by City of Orlando staff planners, is to help codify urban agriculture into a city ordinance by reviewing existing ordinances in other cities such as the one currently in place in Boston, Mass. (Boston Redevelopment Authority, 2014).

Conclusion

Significant progress has occurred over the last ten years to anchor urban local food initiatives into the communities, school system and economy of Orange County, FL. Continued education about the health and economic benefits of these initiatives to local communities helps focus private and public efforts to create a vibrant culture that celebrates local food systems. Networks help move projects forward due to the synergistic nature of multiple entities and people focused on the same goal of providing healthy, nutritious food to all the county's residents. The enthusiasm of the people within each private and public entity cannot be overlooked and has contributed greatly to the broad success to date of these initiatives. There is more to do, and as one municipality adopts an urban farming ordinance, others will follow!

Literature Cited

Barham, J., D. Tropp, K. Enterline, J. Farbman, J. Fisk, and S. Kiraly. 2012. Regional food hub resource guide. U.S. Dept. of Agriculture, Agricultural Marketing Service. Washington, DC. 83 p. Accessed 2 Apr. 2020 <http://www.ams.usda.gov/publications/content/regional-food-hub-resource-guide>

Boston Redevelopment Authority. 2014. Article 89 made easy: Urban agriculture zoning for the city of Boston. 54 p. Accessed 2 Apr. 2020. <http://www.bostonplans.org/planning/planning-initiatives/urban-agriculture-rezoning>

Dumont, A., D. Davis, J. Wascalus, T. Wilson, J. Barham, and D. Tropp. 2017. Harvesting opportunity: The power of regional food systems investments to transform communities. Federal Reserve Service. Accessed 2 Apr. 2020. <https://www.stlouisfed.org/~/media/files/pdfs/community-development/harvesting-opportunity/harvesting_opportunity.pdf?la=en>

Feeding Florida. 2020. Fresh access bucks initiative. Accessed 2 Apr. 2020. <https://www.feedingflorida.org/food-access/fresh-access-bucks>

Florida Department of Agriculture and Consumer Services. 2017. Cottage food operations. Division of Food Safety, Tallahassee, FL. Accessed 2 Apr. 2020. <https://www.fdacs.gov/Business-Services/Food-Establishments/Cottege-Foods>

Hodges, A. and T. Stevens. 2013. Local food systems in Florida: Consumer characteristics and economic development. Proc. Fla. State Hort. Soc. 126:338–345. Accessed 2 Apr. 2020. <https://journals/flvc.org/fshs/article/view/83771>

Kropp, J.D., S.J. Abarca-Orozco, G.D. Israel, D.C. Diehl, S. Galindo-Gonzalez, L.B. Headrick, and K.P. Shelnutt. 2018. A plate waste evaluation of the Farm to School Program. J. Nutrition Education and Behavior. 50(4):332–339.e1. <https://doi.org/10.1016/j.jneb.2017.10.005>

Martinez, S., M. Hand, M. Da Ora, S. Pollack, K. Ralston, T. Smith, S. Vogal., S. Clark, L. Lohr, S. Low, and C. Newman. 2010. Local food systems: Concepts, impacts and issues. U.S. Dept. of Agriculture/Economic Research Service, Washington D.C. Accessed 2 Apr. 2020. <http://www.ers.usda.gov/publications/pub-details/?pubid=46395>

Nieves-Ruiz, L. and M. Cassidy. 2016. Orange County food production strategic plan. East Central Florida Regional Planning Council, Orlando, FL. Accessed 2 Apr. 2020. <http://ftp.ecfrpc.org/Projects/Orange%20County%20Food%20Production%20Strategic%20Plan.pdf>

Orange County Public Schools. 2018. Sustainability impact report 2017–2018. Accessed 2 Apr. 2020. <https://ocps.net//cms/one.aspx?portalId=54703&pageId=98615>

Parmer, S.M., J. Salisbury-Glennon, D. Shannon, and B. Struempler. 2009. School gardens: An experiential learning approach for a nutrition education program to increase fruit and vegetable knowledge, preference, and consumption among second-grade students. J. Nutrition Education and Behavior 41(3):212–217. Accessed 2 Apr. 2020. <https://www.ncbi.nlm.nih.gov/pubmed/19411056>

Rife, J. 2013. Developing regional food hubs in central Florida. Proc. Fla. State Hort. Soc. 126:348–349. Accessed 2 Apr. 2020. <http://journals.flvc.org/fshs/article/view/83773>

Schlueb, M. 2013. Gardeners prevail in Orlando turf war: Veggies OK in front yard, too. Accessed 2 Apr. 2020. <http://www.orlandosentinel.com/news/os-xpm-2013-11-25-os-orlando-garden-landscape-rules-20131125-story.html>

United States Department of Agriculture, Census of Agriculture 1978. Historical archive: Florida. Accessed 2 Apr. 2020. <http://agcensus.mannlib.cornell.edu/AgCensus/censusParts.do?year=1978>

United States Department of Agriculture, Census of Agriculture 2017. Florida: County data. <https://www.nass.usda.gov/Publications/AgCensus/2017/index.php>

Proc. Fla. State Hort. Soc. 132:257. 2019.

—Scientific Note—

Using Soil Moisture Sensors to Conserve Water on Farms

Charles Barrett*

University of Florida, IFAS Extension, North Florida Research and Education Center Suwannee Valley, 7580 County Rd. 136, Live Oak, FL 32060

Additional index words: behavior change, best management practices, on-farm demonstrations

There were 130,231 irrigated acres in the Suwannee River Water Management District, in northern Florida, in 2016 and this number is expected to increase by nearly 40% over the next 20 years. Suwannee Valley row crop producers use overhead irrigation and rainfall to meet the 142 million gallons of water per day needed to cover the evapotranspiration demand of crops in an average year. Cost-share programs have been developed to encourage the adoption of water conservation best management practices (BMPs) and now more than 1000 soil moisture sensors (SMS) are in use in northern Florida. Growers need to understand the outputs from these devices and they would benefit from understanding how these devices can save capital and improve yield with properly managed irrigation and fertilizer. County agents and Florida Department of Agriculture and Consumer Services technicians have developed strong relationships and trust with growers leading growers to seek information from them. The objectives of this program were to help growers become familiar with SMS through on-farm demonstrations, understand the usefulness of SMS data in irrigation and nutrient management with one-on-one training, and to document behavior change when possible.

In 2018, 12 SMS were purchased and installed on 18 farms (6 reinstalls). Extension agents and technicians received basic training with SMS through 4 interactive workshops, which enabled them to educate growers on the use of SMS and provide data interpretation throughout the growing seasons. Extension agents and technicians followed up with growers about their SMS to quantify changes in behavior. As a result of the program, 15 growers tried SMS for the first time and eight growers adopted the BMP of using SMS for irrigation and nutrient management. Three growers did not spend much time with the data and were not inclined to use SMS in the future while the other five growers expressed a desire to try the sensors again before deciding if SMS were useful in their operations. Irrigation practices on one large corn farm were compared from before the use of soil moisture sensors to after implementing sensors. The results showed a savings of 1.5-inches of water on 4,000-acres or nearly 160 million gallons of water. This soil moisture sensor lending program has shown great potential to reduce some barriers to adoption by giving growers an opportunity to try the technology prior to purchasing and giving extension agents and technicians hands-on experience to share with growers. The future of this program will be to expand it statewide.

*Corresponding author; email: cebarrett@ufl.edu

Proc. Fla. State Hort. Soc. 132:258. 2019.

—Scientific Note—

Utilization of Cover Crops for Agroecosystem Evaluation

Stacy Swartz[1] and Zachary Brym*[2]

[1]*ECHO Global Farm, 17391 Durrance Rd., North Fort Myers, FL 33917*

[2]*University of Florida, Institute of Food and Agricultural Sciences, Tropical Research and Education Center, 18905 SW 280th St., Homestead, FL 33031*

Additional index words. agroecology, biomass production, cover crop mixture, green manure, land equivalent ratio

Cover crops are grown around the world to promote weed and erosion control, soil improvement, and nutrient cycling. Evaluation of cover crops has predominantly focused on impacts of cover crops on subsequent or nearby production systems in different environmental and ecological conditions. Aside from their application, little is known about the physiology and ecology of cover crop systems including environmental variation and potential competition between species when grown in mixture. Furthermore, cover crops may provide a model for understanding crop growth and competition in other cropping systems. We evaluated cover crop growth and production in diverse mixtures and habitats in southern Florida within an agroecological framework.

A cover crop trial was established in North Fort Myers, FL to evaluate the growth and competition of three sub-tropical cover crop species in two unique habitats: natural and agricultural. The natural habitat was a flatwood dominated by slash pines (*Pinus elliottii*) and saw palmetto (*Serenoa repens*) with no previous history of crop production. For the experiment, a small area was cleared and leveled. The second habitat was under active agricultural management, primarily for seed production. Management of the agricultural area includes application of fertilizers, pesticides, and plastic row-covers. The farm apiary is also located adjacent to the seed production area. Sunn hemp (*Crotalaria juncea*), sorghum sudangrass (*Sorghum bicolor* × *S. bicolor* var. *Sudanese*) and velvet bean (*Mucuna pruriens*) were planted in a 400 m^2 block in each habitat. Blocks were divided into a 6 × 6 Latin square containing each of the three cover crops and each pair-wise mixture of the same cover crops. Seeding rates used were 30 lb/acre for sorghum sudangrass and sunn hemp and 50 lb/acre for velvet bean. Seeding rates were halved for each cover crop in pair-wise mixture. Cover crops were sown in May 2018 (rainy season) and Nov. 2018 (dry season) and monitored for plant growth during a 90-day and 60-day growth period, respectively. Plant biomass was evaluated at the end of each growing period.

Differences in cover crop biomass were detected between agroecosystems. Total cover crop biomass was significantly greater in the block in the agricultural habitat than in the block in the natural habitat in both rainy (10.3 vs. 0.45 t/ha) and dry (0.85 vs. 0.13 t/ha) seasons (t-test, $P < 0.001$). Lower biomass production in the natural habitat may be connected to the acidic nature of natural southwestern Florida flatwood soils. Nutrient content, plant access to nutrients, and soil structure may also drive the difference between habitats due to prior land management practices.

Land equivalent ratios (LER) were calculated to evaluate yield potential of cover crop mixtures compared to sole cropping the three species. LER relates the area under sole cropping required to provide equal amounts of production as an intercropped area under the same management. In the plot located in the agriculturally managed habitat, all three pairwise species mixtures had LER values greater than one over both growing seasons. Therefore, growing these three cover crop species in any of the pairwise mixtures produced more biomass per land area than cropping with only one of the three species. During the rainy season, mixtures with velvet bean had numerically greater land equivalent ratios (2.1 with sunn hemp and 1.97 with sorghum sudangrass) than the mixture of sunn hemp and sorghum sudangrass (1.23). Little difference in LER values between mixtures was observed during the dry season (1.37–1.41). Increased production of biomass per unit land area observed by mixing cover crop species provides farmers with an insight to the maximization of organic matter returns to the field through rotations of cover crops.

Future directions include continued monitoring of crop physiology and competition over time in both habitats. Additional agroecosystem evaluations of each habitat include plant diversity sampling, soil testing, and weather data.

*Corresponding author. Email: brymz@ufl.edu

Proc. Fla. State Hort. Soc. 132:259. 2019.

—Scientific Note—

A Summary of Plant Diversity at the Tropical Research and Education Center, University of Florida, Homestead, and at Selected Areas of the ECHO Global Farm, North Fort Myers

Cliff G. Martin[1], Stacy Swartz[2], Thioro Fall[3], and Zachary T. Brym*[1]

[1]*Tropical Research and Education Center, University of Florida, IFAS, 18905 SW 280th St., Homestead, FL 33031*

[2]*ECHO Global Farm, 17391 Durrance Rd., North Fort Myers, FL 33917*

[3]*Horticultural Sciences Department, University of Florida/IFAS, P.O. Box 110690 Gainesville, FL 32611*

Additional index words. botany, species assemblage, community composition, habitat

Southern Florida has a wide diversity of land uses and plant species. The environment and management by humans are factors that contribute to the existence and assemblage of plant species. Different land uses occur in close proximity, which allows plant species to disperse and survive in multiple habitats. We investigated the effects of habitat and location on plant diversity at two agricultural research centers in southern Florida. The objective was to understand how plant species assemble in closely arranged yet different habitats under various regimes of cultivation and management.

The University of Florida Institute of Food and Agricultural Sciences Tropical Research and Education Center (TREC) occupies 160 acres near Homestead, FL, while the ECHO Global Farm occupies 55 acres near North Fort Myers, FL. Both sites have a wet season from May to October, mean annual temperatures of 23.3 °C to 24.0 °C, and nearly identical precipitation (1400 mm). Soil at TREC is high-pH, well-drained, shallow, and rocky while ECHO soil is variable in pH, poorly drained, deep, and sandy. At TREC, the soil is uniformly drained, while at the ECHO, the water table is more variable with surface flooding in the wettest areas. The habitats at TREC and ECHO include both agricultural and natural areas. Habitat classifications at each location were defined as agricultural field, lawn, tree fruit orchard, and natural area. We identified plant species in areas representing multiple habitats at TREC and ECHO using a 20 × 20 m sample grid from the National Ecological Observatory Network (NEON; Barnett, 2017). Pairs of plots were considered blocks; four blocks with eight plots were studied at TREC and two blocks with four plots at ECHO. Subplots were arranged and nested within each plot according to NEON protocol to include eight each at 1 m^2 and 10 m^2, and four at 100 m^2. Plots were surveyed during Dec. 2017–Apr. 2018 at TREC and during June 2018 at ECHO. Identification of plant species involved the use of botanical keys (Wunderlin and Hansen, 2011), followed by visual comparison with online specimens (Wunderlin et al., 2005–2018).

The study area at TREC (eight plots) was twice as large as at ECHO (four plots), yet the number of species and families at TREC was less than double that of ECHO. The most species-rich group within TREC plots was established plants not native to the State of Florida, whereas native plants were most species rich at ECHO. TREC plots yielded 159 species including 67 native, 88 established non-native, 2 non-established cultivated, and 2 unknown, while ECHO plots had 128 species with 89 native, 25 established non-native, 0 non-established cultivated, and 14 unknowns. At each location, four of the six most species-rich families were the same: Asteraceae, Poaceae, Fabaceae, and Euphorbiaceae, respectively. The block at TREC with the most species per block represented lawn and natural habitats. Plant species richness increased with increasing size of nested sampling unit. Plant species assemblages per 100 m^2 subplot were analyzed for similarity using PERMANOVA. At TREC, the differences among subplots were significant at multiple scales ($P < 0.01$). Subplots were more similar within plots than among blocks, which were more similar than among habitats. These results were consistent when TREC and ECHO species lists were analyzed together ($P < 0.001$).

Future investigation of plant diversity, habitat, and land management will include the proximity and mixture of plants in agricultural and natural areas. Some will be considered weeds, while others may prove beneficial.

Literature Cited

Barnett, D.T. 2017. Terrestrial Observation System (TOS) protocol and procedure: Plant diversity sampling, Revision G. National Ecological Observatory Network (NEON) Doc. # NEON.DOC.014042. 31 Jan. 2017. <https://data.neonscience.org/documents/10179/1883155/NEON.DOC.014042vH/bb428857-219e-4b70-be40-710b2609ba48>

Wunderlin, R.P., and B.F. Hansen, 2011. Guide to the vascular plants of Florida. 3rd ed. University Press of Florida, Gainesville, FL.

Wunderlin, R.P., B.F. Hansen, A.R. Franck, and F.B. Essig. 2005–2019. Atlas of Florida plants. (S.M. Landry and K.N. Campbell, application development, USF Water Institute.) Institute for Systematic Botany, University of South Florida, Tampa. 1 Aug. 2018. <http://florida.plantatlas.usf.edu>.

*Corresponding author. Email: brymz@ufl.edu

Proc. Fla. State Hort. Soc. 132:260. 2019.

—Scientific Note—

Linkages and Interactions: A Systems Concept for Agriculture and Natural Resource Management

Zachary T. Brym*

Tropical Research and Education Center, University of Florida, IFAS, Agronomy Department, 18905 SW 280th Street, Homestead, FL 33031

Additional index words. environment, agricultural system, natural system, food system

The agroecosystems concept facilitates the integration of agricultural and natural resource management by describing the linkages and interactions of key characteristics, and these environmental and ecological characteristics are well represented by the 2019 Florida State Horticultural Society's (FSHS) Agroecology and Natural Resources Section program.

Agroecosystems are defined by interacting environmental and ecological characteristics of farming systems and these characteristics act as dynamically linked parts of the system. The agroecosystem concept structures goal-oriented management plans within the agroecology framework that considers the characteristics of a farm and the surrounding area. Agroecosystems are complex and challenging to manage and require a multidisciplinary understanding from specialties such as horticultural sciences, agronomy, economics, ecology, engineering, entomology, natural resource management, soil and water sciences and sociology. Investigations of agroecosystems can be defined by discipline and also ecologically meaningful boundaries and scales.

The agroecosystem concept was introduced to the FSHS Agroecology and Natural Resources Section where participants were encouraged to produce a diagram of a familiar agroecosystem. In 2019, the FSHS Agroecology and Natural Resources Section program was organized using agroecosystem boundaries and scales to provide a meaningful thread of agroecology throughout the program.

The introduction of the agroecosystem concept to the section motivated the overall organization of the section's program and a visual depiction of presenters' agroecosystems. The organization of the program by agroecosystem scale also encouraged identification of linkages between presentations and discussion of the agroecological approach through the program. The presentations of the 2019 FSHS Agroecology and Natural Resources Section were organized in the following manner:

Environment. The environmental characteristics of an agroecosystem set the context for the biological activity in the system. Water availability is a key environmental characteristic that should be monitored and managed for conservation. Soil is another environmental characteristic that can be manipulated to impact plant growth.

Agricultural and Natural Systems. A focus on one specific crop plant is the organism scale, while the cultivation of crop plants in a group is the population scale. The interaction of the crop and other plant species is the community scale. Some plants in the community benefit the growth of the crop plant, such as cover crops, while other plants invade to negatively impact the environment and ecosystem health. Naturally occurring and cultivated plant diversity can be a valuable resource genetically, ecologically, and aesthetically.

System-wide characteristics and interactions that include the environment and the diversity of its populations and communities is the ecosystem scale. Invertebrate communities act at the ecosystem-scale of an agroecosystem. Some naturally occurring invertebrates can be impacted by agriculture and urban development, such as butterflies. Many invertebrates are detrimental to crop health and must be managed for crop production and quality. The ecosystem-scale may also be considered spatially across fields, landscapes and regions. Regional management plans can link the interacting characters of farms and appreciate the characteristics that operate beyond farm boundaries, such as water.

Food Systems. The management, production, and distribution of farm products and economies is the food system scale. Food systems include community gardens and gathering places, broader urban initiatives, and meaningful access of farmers' products to the communities they support.

The FSHS Agroecology and Natural Resources Section will continue to solicit presentations from a diversity of topics that represent a diversity of agroecosystems and agroecosystem scales. The section may continue to facilitate linkages and interactions among presentations in future meetings through the agroecology framework and the agroecosystem concept.

Corresponding author. Email:brymz@ufl.edu

Proc. Fla. State Hort. Soc. 132:261. 2019.

—Scientific Note—

Management of Silverleaf Whitefly and Tomato Yellow Leaf Curl Virus with Insecticides in Field Grown Tomatoes

Jawwad Qureshi*, Barry Kostyk, and Monica Triana

University of Florida/FAS, Southwest Florida Research and Education Center, 2685 State Rd. 29 North, Immokalee, FL 34142

Additional index words. silverleaf whitefly, tomato yellow leaf curl virus, chemical control

Bemisia tabaci biotype B commonly known as silverleaf whitefly is the key pest of Florida grown tomatoes because of its ability to vector tomato yellow leaf curl virus (TYLCV). TYLCV threatens both commercial tomato production fields and home gardens and was identified in 1997 in south Florida (Polston et al., 1999). Disease management strategies include effective vector control, sanitation and use of TYLCV-resistant cultivars. Tomatoes should be planted with intervals of time and space from plantings of hosts (cabbage, collards, cucurbits, tobacco, soybean, cotton, and weeds), which are good sources of whiteflies.

TYLCV resistant cultivars and vector control are important tools in reducing the incidence of the *B. tabaci* B and TYLCV. Two experiments were conducted; one each in 2017 and 2018. The objective was to evaluate several insecticide regimens and with and without a TYLCV tolerant variety. In both years there were 4 replicates in a randomized complete-block design and main plots were divided into sub plots containing tolerant and susceptible varieties. In 2017, seedlings of 'Brickyard' (Susceptible) and 'Skyway' (Tolerant) varieties were transplanted on 10 Mar. at 18-inch plant spacing using 20 plants (10 'Brickyard' + 10 'Skyway') per plot. The split plots were separated by a single susceptible plant planted on 23 Mar. and previously infected with TYLCV by exposure to a caged colony of infected whiteflies. An empty 3-foot area was left between plots as a buffer. There were 4 treatment programs in which plants received a drench application of Venom made 13 Mar. by delivering a 90-mL suspension to the base of each plant using an EZ-Dose® sprayer operating at 45 psi. Following the drench applications, programs received spray applications of 1) Closer® and Movento® rotation, 2) Closer® and Knack® rotation, 3) Sefina® only rotation, and 4) Sivanto® and Movento® rotation. Foliar sprays were applied with a single-row high clearance sprayer operating at 180 psi and 2.3 mph. The sprayer was fitted a vertical boom on each side of the plant row equipped with yellow Albuz® hollow cone nozzles, each delivering 10 gal/acre. Total spray volume increased from 40 to 60 gpa as nozzles were added to accommodate plant growth.

In 2018, seedlings of either TYLCV tolerant 'Charger' or susceptible 'HM1823' cultivars were planted on 6 Mar.using same spacing as above. Some other procedures were also similar to 2017. Four plants were left untreated between plots acting as a spray buffer. All five treatment programs received a 10.5 oz/acre drench of Admire Pro on 12 Mar.. The main insecticides tested in these programs were 1) Exeril®, 2) Sefina®, 3) Movento®, 4) Sivanto®, and 5) Beleaf®. All five programs also received applications of Knack. Foliar sprays were applied with a single-row high clearance sprayer operating at 180 psi and 2.3 mph. The sprayer was fitted a vertical boom on each side of the plant row equipped with yellow Albuz® hollow cone nozzles, each delivering 10 gpa. Total spray volume increased from 40–80 gpa as nozzles were added to accommodate plant growth.

Whiteflies were monitored on the tolerant plants using the number of adults/leaflet, and nymphs/4 square inches, followed by the determination of the incidence of TYLCV using percentage of infected plants. Programs including the use of Sefina®, Exeril®, Closer®, Sivanto®, and Movento® provided significant reduction in populations of whitefly. Fewest TYLCV symptomatic plants were observed in treatments including Venom drench followed up by sprays of Sefina® or in the treatments using Admire drench with sprays of Sefina®, Exeril®, Movento®, and Sivanto®. Most reduction in TYLCV was seen in the program with Sefina®. Findings suggest that integrating and rotating these new insecticides in the management programs for this vector-disease complex will be useful in reducing vector populations, potential for resistance and delaying and/or reducing the incidence of TYLCV.

Literature Cited

Polston, J.E., R.J. McGovern, and L.G. Brown. 1999. Introduction of tomato yellow leaf curl virus in Florida and implications for the spread of this and other geminiviruses of tomato. Plant Disease 83:984–988.

*Corresponding author. Email: jawwadq@ufl.edu

Proc. Fla. State Hort. Soc. 132:262. 2019.

—Scientific Note—

A Regional Strategy for Confronting the Challenges of a Basin Management Action Plan (BMAP) in North Florida

De Broughton*, Charles Barrett, Robert Hochmuth, and Kevin Athearn

University of Florida, IFAS North Florida Research and Education Center, Suwannee Valley, 7580 County Rd. 136, Live Oak, FL 32060

Additional index words. basin management action plan (BMAP), nitrogen loading, crop sustainability

The Regional Specialized Agents (RSAs) within the Suwannee River Basin are charged with helping agricultural producers overcome challenges associated with an emerging Basin Management Action Plan (BMAP), while ensuring their operational sustainability.

In 2016, the Florida Legislature found that Florida's natural springs were threatened due to excessive withdrawal and increasing nutrient (nitrogen) levels. This information led to the passage of the Florida Springs and Aquifer Protection Act. This formal legislative action generated the development of a BMAP for portions of the Suwannee River Basin, as well as other areas in the state. The plan targets impaired waterbodies and surrounding ecosystems affected by the amount of nitrogen loading in certain springs. Due to the amount of cultivated, irrigated farmland acreage in the north Florida region, the Suwannee River BMAP will focus heavily on agriculture and will enable regulatory efforts of production practices that are not in compliance with the Best Management Practices (BMPs) known to achieve nutrient reductions. The RSAs determined that fewer agricultural water permits would be issued to farmers who were not BMP compliant, and no new permits will be issued to producers in areas identified with the most sensitive waterbodies nearby. Producers will be required to implement BMPs (that will be verified for compliance), or more strenuous regulatory action will occur by the governmental monitoring agency.

In order to help farmers develop sustainable goals and to manage this impending challenge, the RSAs initiated projects at the North Florida Research and Education Center, Suwannee Valley and farm locations throughout the region. These projects demonstrate optimal BMPs that will help growers meet their goals and comply with the standards set for reduced nitrogen usage in agricultural crop settings. These extension efforts showcase the 4Rs concept of Nutrient Stewardship. Using the 4Rs approach as a consistent theme, producers are learning how to apply nutrients using the right source of fertilizer, at the right rate, at the right timing of development and in the right place for the crop. An example of grower adoption took place during a 2018 demonstration, when a large-scale corn producer utilized a fertilizer side-dressing implement to incorporate nitrogen directly by the plant row, instead of broadcasting the nutrient overhead through pivot irrigation. As a result of this new fertilizer application method implemented, on 4000 acres of grain corn, the producer reduced the usual amount of nitrogen applied by 50 lb/acre. The total reduction of nitrogen fertilizer being applied during the 2018 corn growing season equaled 200,000 pounds for the grower. Additionally, commercial producers have tried using a new source of fertilizer by testing controlled release fertilizer (CRF) sources instead of conventional liquid and dry bulk blends that were traditionally broadcast routinely. An added advantage of the CRF source is that farmers chose to side-dress the product due to its high cost, as this method is known to waste less fertilizer.

Efforts are underway to quantify the efficacy of controlled-release fertilizers in commercial production settings. Additionally, new ways to implement the 4Rs of Nutrient Stewardship are being identified and studied in a research setting, so that the practices can be demonstrated on farms within the Suwannee Valley area.

*Corresponding author. Email: deonne@ufl.edu

Proc. Fla. State Hort. Soc. 132:263. 2019.

—Scientific Note—

Applying Principles of Sustainability and Agroecology to the Community Garden—A Systems Approach at Derbyshire Place, a Daytona Beach Food Desert

Joseph J. Sewards*

University of Florida/IFAS, Volusia County Extension, 3100 E. New York Ave., DeLand, FL 32724

Additional index words. agroecology, community gardens, sustainability

Interest in creating community gardens in Volusia County is growing. Municipalities, community centers, churches and civic organizations are interested in creating them, particularly in recognized food deserts in urban areas. These areas are usually surrounded by buildings and pavement and lack ecological diversity that is present in natural systems. By applying a systems approach to community gardens and including the principles of agroecology, garden sustainability and productivity can be enhanced.

This community garden includes agronomic and Florida-Friendly Landscape principles, alternative production methods, native pollinator habitat, sociology and ongoing education. Such a garden will appeal to the surrounding community and provide a source of healthy foods and exercise in a recognized food desert. It will also serve as an example to other communities fostering the creation of additional community gardens throughout Volusia County

As a result of the efforts of Derbyshire Place, a vibrant community garden with thirty-six, 4 ft × 12 ft raised beds, hydroponic towers, ADA-compliant beds, compost bins, native pollinator habitat, and banker plants is now operational. To date, all the raised beds are fully occupied and the ten hydroponic towers provide lettuce and other greens to a local food bank.

In addition to this fully functional community garden, we have suggested adding an urban hydroponic farm in an adjacent empty lot. The intent is to provide a source of affordable, healthy and pesticide-free produce to community members who don't participate in the community garden. The urban farm will also produce an additional revenue stream for Derbyshire Place Community Center and will inspire others in other recognized food deserts in Volusia County. We hope to partner with Bethune-Cookman University and Stetson University to incorporate such projects as entrepreneurial enterprises in their curricula.

*Corresponding author. Email: sewards@ufl.edu

Proc. Fla. State Hort. Soc. 132:264. 2019.

Alternative Crops Extension Program Aims to Enhance Sustainability of Hastings Area Farms

Bonnie C. Wells*

Brevard County Extension, University of Florida/IFAS
3695 Lake Dr., Cocoa, FL 32926

ADDITIONAL INDEX WORDS. alternative crops, sustainability, vegetables

Alternative crops are defined as agronomic crops unusual for a specific region yet selected for production due to their high marketing potential or specialized benefit to the farming system.

In St. Johns County, the "Potato Capital of Florida," what once was an economic powerhouse for potato production providing a significant portion of the nation's annual spring crop, has in recent years seen acreage significantly shrinking due to low profit margins. Within the past few years, the area has lost several potato farms altogether, while the ones that remain are seeking alternative crops to improve farm profitability and sustainability.

Adopting alternative crops increases opportunities but, due to unfamiliarity with how the new crop should be grown, risk increases as well. Local growers are turning to University of Florida's Institute of Food and Agricultural Sciences (UF/IFAS) Extension for research and education on alternative crop production to help mitigate the risks and ease transition to unfamiliar cropping systems.

In 2015, responding to this need, we implemented an Extension program in alternative crops. The overall objective of the program in St. Johns County is to provide research-based production information on alternative crops for area potato growers to improve farm profitability and sustainability.

Field research and demonstration trials are on-going at the UF/IFAS Hastings Agricultural Extension Center (HAEC) and on-farm in producer fields. These explore the production potential of various alternative crops including Asian vegetables, sweetpotatoes, brussels sprouts, artichokes, and cauliflower. Field investigations have been diverse and include cultivar selection, nutrient management, irrigation needs, and pest management.

Since 2015, field trials have yielded a variety of outcomes, most notably the establishment of nutrient standards for select Asian vegetables, commercial production of purple sweetpotato, and increased knowledge and interest in other alternative crops such as brussels sprouts, cauliflower, artichokes, and sweet corn. Other results include:

- Eighty-nine percent of commercial vegetable growers in St. Johns County have reported an increase in knowledge about alternative crops.
- A 90-lb nitrogen per acre rate recommendation for sweetpotato production in the Hastings area has been established through field trials at HAEC.
- One grower learned how to determine optimal fertilization of Asian vegetable crops using petiole sap testing after demonstrations in on-farm trials.
- Approximately 25 acres of sweetpotato are now in commercial production in St. Johns County after successful demonstration at the HAEC and on-farm in producer's fields.
- Potential artichoke cultivars have been identified for Hastings area production

Crop diversity is vital for farm sustainability. Adopting alternative cropping systems will be essential for revenue enhancement and farm productivity. Approximately forty acres in St. Johns County now produce alternative crops. Growers are increasing the sustainability and productivity of their farming operations. By utilizing UF/IFAS Extension research and demonstration centers such as the HAEC, agents can help growers transition to alternative crops through demonstrated field success that yields a well-thought out production plan derived from un-biased research.

*Corresponding author: bcwells@ufl.edu

Proc. Fla. State Hort. Soc. 132:265. 2019.

How Much Compost Do Homeowners Produce? A Case Study

Adrian G.B. Hunsberger*

University of Florida/IFAS, Miami-Dade County Extension, 18710 SW 288th St., Homestead, FL 33030

Additional index words. residential home composting, compost, recycling, landscape waste, food waste

According to the U.S. Environmental Protection Agency, food scraps and yard waste currently make up 20 to 30% of what is thrown away and should be composted instead. Making compost keeps these materials out of landfills where they take up space and release methane, a potent greenhouse gas. A proven method of getting residents started in home composting is to provide the equipment they need. Miami-Dade County Extension partnered with the Miami-Dade Solid Waste department to start a pilot program teaching homeowners how to compost. Each participant receives a free Earth Machine™ compost bin after training (Fig. 1).

To gather data of how much waste is composted by typical residents, 25 Master Gardener volunteers tabulated how much material they composted for three months (Table 1). On a per person, per month basis: Indoor waste (kitchen scraps, etc.)—an average of 8.52 gal was composted. Outdoor waste (plant clippings, etc.)—an average of 19.61 gal was composted. The overall average was 14.1 gal of indoor/outdoor waste composted. While density figures for food scraps and yard trimmings can vary widely, it was estimated this volume equates to approximately 40–60 lb per month. It was extrapolated that per annum, over 4230 gal (21 cubic yards) of waste was composted and therefore diverted from the landfill by these 25 participants. This compost was then used as a source of plant nutrients for landscape and edible plants, which had the added benefit of reducing the amount of added fertilizer that would normally have been used.

Table 1. Instructions on how to collect and report compost collection given to the compost study participants.

We want to quantify the amount of waste that you will be adding to your composter. We need to know how much you are adding as well as how often. The data collection form is to help you track your progress.
Please place your data sheet on your refrigerator to remind you about keeping track of the data.
For indoor waste, use a container that will be dedicated for scrap collection. Measure the diameter and height in inches and record the dimensions on the volume line on the form. Of if you already know the volume of the container (Example: 1 gallon), record its size.
For outdoor waste, use the same method of measurement as above.
Choose months that you can track for the whole month and label the month above the box on the form.
For consistency sake, fill your collector to the top but not overflowing. Do not compress the contents.
Each time you fill your collector and empty it into your composter, make a check mark or tick mark in the appropriate box.
For outdoor collections, if you need to use different size collectors, just make a duplicate of the form and record each size of collector.
At the end of three completed months of data collecting, please return the form.

Fig. 1. Filling the Earth Machine™ compost bin with landscape waste.

Studies indicate that 23–83 lb/household/month could be diverted through home composting (Leboe, 2011). Personalized training and support increase the potential. For every 10,000 households composting at home, between 1400 and 5000 t/year could be diverted from curbside collection, with potential savings in avoided disposal costs alone ranging from $72,000 to $250,000.

When added to soil, compost sequesters carbon, improves plant growth, conserves water, reduces reliance on fertilizers, and helps prevent nutrient runoff. At-home or backyard composting has additional benefits. It saves local governments money by avoiding the need to collect and process material. By directly engaging citizens in the act of converting waste into a resource, home composting also builds a critical culture of composting know-how.

Literature Cited

Leboe, E. 2011. Value and benefits of backyard composting. Biocycle, Vol. 52, No. 10, p.35–36. Accessed Feb. 2019. <https://www.biocycle.net/2011/10/19/value-and-benefits-of-backyard-composting/>

*Corresponding author email: aghu@ufl.edu

Proc. Fla. State Hort. Soc. 132:266. 2019.

Contacts and Work Accounting for Efficient Reporting

Tatiana Sanchez*

Alachua County Extension, University of Florida/IFAS
2800 NE 39 Ave., Gainesville, FL 32609

Additional index words. data collection, recordkeeping

Data collection in Extension is a never-ending process. To avoid drowning in a sea of data with no order, the agent has adopted a couple of tools to help track daily activities and ultimately, facilitate county quarterly reports and annual report of achievement for extension agents.

Methods

The majority of data collection is conducted with MS Access and Qualtrics. Daily activities are recorded on a form linked to a table on MS Access where the information is stored. Some of the fields include demographic and contact information, date, agricultural type (conventional, organic), inquiry type (email, phone, office visit), recommendation, inquiry status (complete or follow up) and programmatic area (connected to Report of Achievement). Data can be analyzed by either generating reports on MS Access or by exporting it to MS Excel. The "Offline Surveys" mobile app by Qualtrics is used to collect data on the go when access to an internet connection is unavailable. Questions include adoption of recommended practices, number of acres that apply, benefit received from using Extension (e.g. reducing fertilizer or pesticide use, water savings, etc.) and, economic impact of practices adopted. These data can be analyzed directly on Qualtrics or after exporting it to MS Excel.

Results

The template of the database used for collecting daily activities has been shared with more than six extension agents, one specialist and the UF/IFAS Program Development & Evaluation Center. One of the agents that adopted the database modified the form to improve the recordkeeping by Master Gardeners. Recently, the author started to use the offline survey app for field data collection. To date, three farms have completed the survey allowing real-time assessment of indicators increasing the accuracy of the data that is reported.

Conclusion

Integration of technology tools can help organize and reduce the time dedicated to reporting. By aligning data collection structure with workload indicators and programmatic areas, it becomes easier to filter the data and generate reports.

*Corresponding author. Email: tataiana.sanchez@ufl.edu

Posters Section

Proc. Fla. State Hort. Soc. 132:267. 2019.

Development of *Antirrhinum* Mutants with Enhanced Axillary Branching Using Transposon Transposition

Zhaoyuan Lian*[1,2], Sandra Wilson[1], and Heqiang Huo[1,2]

[1]*Department of Environmental Horticulture, University of Florida /IFAS*
P.O. Box 110670, Gainesville, FL 32611

[2]*Mid-Florida Research and Education Center, University of Florida/IFAS*
2725 S. Binion Rd., Apopka, FL 3270

Additional index words. *Antirrhinum,* transposon, mutants

Snapdragon (*Antirrhinum majus*) is one of top ten fresh cut flowers in the United States. Current commercial snapdragon varieties have one primary stem with no or rare axillary branching, an attractive characteristic for nursery growers since it can multiply cut-flower production from each plant. Although horticultural practices such as removing the shoot tips can be adopted to overcome the apical dominance effect to promote axillary branching, this practice is cost-inefficient, time-consuming and may cause severe pathogenic infection through stem wounds. While breeding programs in the United Stated intensively have focused on improving snapdragon aesthetic characteristics such as color and size of flowers, little attention has been given to breeding enhanced axillary branching cultivars. In this study, we have used transposon transposition to generate several mutants with enhanced axillary branching. Transposition of the active transposon *Tam3* is strictly regulated by temperatures, and can be activated in the inducer snapdragon "J2" grown at 15 °C, resulting in random mutations in its genome. To pin out the mutated gene(s) for axillary branching, recessive mutants will be crossed with a highly inbred *Antirrhinum* line "JI7" to develop an F_2 mapping population. Two F_2 pools of mutants, with or without the axially branching trait, will be generated for bulked segregant analysis and whole genome sequencing to identify the mutated gene(s). Molecular complementation or knocking out candidate genes through CRISPR/Cas9 genome editing will be conducted to validate functions of the candidate mutated gene(s). Molecular markers will be developed for snapdragon breeding toward improving axillary branching using our mutants.

*Corresponding author email: lianzhaoyuan@ufl.edu

Proc. Fla. State Hort. Soc. 132:268. 2019.

Fruit Firmness and Susceptibility to Splitting and Spotted-wing Drosophila on Different Grape Cultivars

Amir Rezazadeh*[1], Eric T. Stafne[2], and Blair J. Sampson[3]

[1]*St. Lucie County Extension, University of Florida/IFAS*
8400 Picos Rd., Ste. 101, Ft. Pierce, FL 34945

[2]*Coastal Research and Extension Center, Mississippi State University, Poplarville, MS*

[3]*USDA-ARS Thad Cochran Southern Horticultural Laboratory, Poplarville, MS*

Additional index words: fruit splitting

Fruit splitting is a physiological disorder that produces surface cracks that promote disease and insect damage. The spotted-wing drosophila (SWD), *Drosophila suzukii* (Matsumura), is a species of vinegar fly that attacks berry crops worldwide, including grapes, and reduces fruit quality and yield. In this study, we tested the susceptibility of various hybrid grapes and muscadines to berry splitting and SWD attack.

Ten grape cultivars were harvested in 2016 and 2017, and 'Thompson Seedless' (*Vitis vinifera*) was purchased locally. Fruit quality traits examined included Brix (soluble solids), total acidity (TA), pH, fruit firmness, and skin break force. 'Villard Blanc' and 'OK392' were the least susceptible to berry splitting (0%). Although muscadines showed the highest fruit firmness, they were also susceptible to splitting, especially 'Fry Seedless' (88% splitting incidence). There were no significant correlations among soluble solids, firmness, and splitting; however, there was a negative correlation between splitting and TA. Pierce's disease (PD)-resistant and PD-tolerant grapes also appear to be mostly resistant to or highly tolerant of SWD infestation. SWD females do not appear to be able to oviposit in fruit hosts that have a fruit firmness exceeding 300 g/mm. Wounds or splits in the grape epidermis increased reproductive success of SWD by 400%, suggesting that a well-managed vineyard will not host large SWD populations. Vineyards suffering from severe berry damage might provide SWD with a summer fruit host.

Overall, PD-resistant hybrids and muscadines are highly adaptive to subtropical climates and are tolerant of this new invasive vinegar fly.

*Corresponding author. Email: amir2558@ufl.edu

Posters Section

Proc. Fla. State Hort. Soc. 132:269. 2019.

Phenotyping Dual-purpose Cowpea for Agronomic Traits

Rocheteau Dareus*[1], Esteban F. Rios[1], Yolanda Lopez[1], Carlene Chase[2], and Beatriz Tome Gouveia[3]

[1]*Agronomy Department, University of Florida/IFAS, P.O. Box 110500, Gainesville, FL 32611*

[2]*Horticultural Science Department, University of Florida/IFAS, P.O. Box 110690 Gainesville, FL 32611*

[3]*Universidade Federal de Lavras, Brazil*

Additional index words. *Vigna unguiculata* L. Walp

Cowpea (*Vigna unguiculata* L. Walp) is a legume cultivated for food and fodder in warm regions around the world. Annual cowpea production is estimated at 3 million tons of dry grain harvested on about 12.5 million hectares worldwide. Cowpea grain and fodder have a high nutritive value. On a dry weight basis, crude protein in grain and leaves ranges from 22 to 30%, making it a good substitute for milk/meat products; while its haulms represent valuable fodder source. In the United States, cowpea is cultivated on over 80,000 ha for grain production and as cover crop to improve soil fertility. Therefore, the development of dual-purpose cultivars (forage + grain) is crucial to make cowpea more marketable. The objective of this research was to screen a worldwide cowpea germplasm collection and commercial cultivars for morphological and agronomic traits.

A field experiment was established in the Fall 2018 at the Plant Science Research and Education Unit (PSREU), Citra, FL with 302 genotypes using a row and column experimental design with augmented representation of controls in two blocks. The highest yielding genotype was the cultivar USDA 1137, followed by 79 genotypes from the germplasm collection. Most lines exhibited an erect, semi-erect or vine-like shoot architecture, indeterminate flowering, early maturing (39–45 days to flowering), and plant height varying from 31–60 cm. These results revealed genetic potential to develop dual-purpose cultivars. Significant differences were observed for all the agronomic traits under investigation. Biomass production was highly correlated (R^2 = 0.77) to the number of days to first flower.

*Corresponding author. Email: rocheteau.dareus@ufl.edu

Posters Section

Proc. Fla. State Hort. Soc. 132:270. 2019.

Evaluation of Egg-mass-based Bioassay and Molecular Markers for Identifying Root-knot Nematode Resistance in Pepper

Dominick Padilla*[1], Mary Ann Maquilan[1,2], Donald Dickson[2], and Bala Rathinasabapathi[1]

[1]*Horticultural Sciences Department, University of Florida/IFAS*
P.O. Box 110690, Gainesville, FL 32611

[2]*Entomology and Nematology Department, University of Florida/IFAS*
P.O. Box 110620, Gainesville, FL 32611

Additional index words. *Capsicum annuum*, *Meloidogyne incognita*, nematode–plant interaction

Bell pepper is an important commodity in Florida with a farm value of $183 million. This crop is susceptible to damage by root-knot nematodes (RKNs, *Meloidogyne* spp.). RKNs are pathogens that severely damage susceptible crops in the Solanaceae family. Ultimately, the goal of this project is to provide a sustainable alternative to chemical inputs for managing RKNs by developing nematode-resistant pepper varieties adapted to Florida field conditions. Greenhouse and lab experiments were conducted to assess RKN resistance among progeny from crosses between resistant sweet pepper (*Capsicum annuum* L.) cultivar 'Charleston Belle' with the Florida-adapted cultivar 'Jimmy Nardello Italian' when artificially inoculated with *Meloidogyne incognita* (Southern RKN). We report the results of our phenotypic evaluation of the two parental cultivars and of the F_1, F_2, and backcross progenies based on the number of egg masses. We also conducted a survey of DNA polymorphisms in "root-knot nematode resistance protein coding gene" (CaMi) that could potentially be used to develop PCR-based genetic markers for RKN resistance in pepper.

*Corresponding author. Email: dpadilla@ufl.edu

Proc. Fla. State Hort. Soc. 132:271. 2019.

The Effect of Chlorine Dioxide (ClO_2) Gas on Reducing Postharvest Decay Caused by *Rhizopus stolonifer* in Strawberries

Tian Zhong[1, 2], Jiuxu Zhang[2], Jingjing Kou[3], Xiuxiu Sun[4], Jinhe Bai[4], Jeffrey K. Brecht[5], Steven A. Sargent[5], Anne Plotto[4], and Mark A. Ritenour[2]*

[1]*School of Pharmacy and Food Science, Zhuhai College of Jilin University, P.R. China*

[2]*Indian River Research and Education Center, University of Florida/IFAS 2199 Rock Rd., Ft. Pierce, FL 34945*

[3]*College of Horticulture, Agricultural University of Hebei, P.R. China*

[4]*USDA-ARS US Horticultural Research Laboratory 2001 South Rock Rd., Ft. Pierce, 34945*

[5]*Horticultural Sciences Department, University of Florida/IFAS P.O. Box 11690, Gainesville, FL 32611*

Additional index words. fresh fruits, postharvest disease control, Rhizopus rot

The effect of gaseous chlorine dioxide (ClO_2) on reducing Rhizopus decay and maintaining quality of fresh strawberries was studied. In the in vitro tests, *Rhizopus stolonifer* growth on potato dextrose agar (PDA) plugs in petri-dishes was totally inhibited after a 24-h treatment with 0.2 g of ClO_2 generating media, which produced an approximate ClO_2 gas concentration between 3.27–16.77 mg/L at 20 °C in 7.7-L sealed containers. During in vivo experiments of inoculated strawberries, four dosages of ClO_2 generating media including control (0 g), single dose (1.0 g), double dose (2.0 g) and triple dose (3.0 g) were employed to treat fruit at 20 °C for 24 h in 7.7-L sealed containers. Fruit decay was recorded 42, 48, 54, and 66 h after inoculation. The results showed that the triple-dose treated resulted in the least disease incidence and severity over time. After 48 h incubation, Rhizopus decay incidence and severity, and fungal mycelial growth incidence and severity of the triple-dose treated group fruit were 55.56, 16.67, 2.22, and 0.56%, respectively, while the values of control group fruit were 100, 40, 73.33, and 18.89%. It indicates that the application of gaseous ClO_2 efficiently reduce postharvest decay incidence and severity of fresh strawberries inoculated with *R. stolonifer*. No significant differences in fruit weight loss, firmness, total soluble solid and titratable acidity were found between the ClO_2-treated and control fruit. However, exposure to the higher ClO_2 concentrations resulted in some phytotoxicity to the fruit and calyx (such as browning).

*Corresponding author. Email: ritenour@ufl.edu

Proc. Fla. State Hort. Soc. 132:272. 2019.

The Effect of Thinning on Subtropical Peach 'UFSun' on Fruit Weight, Size, and Dry Matter Content

Yuru Chang* and Ali Sarkhosh

Horticultural Sciences Department, University of Florida/IFAS
P.O. Box 110690 Gainesville, FL 32611

Additional index words. peach fruit thinning, fruit size, fruit weight, dry matter content

Larger fruit size of subtropical peach (*Prunus persica*. L) has been a desirable trait since production began. The fruit development period (FDP) of UF early ripening cultivars is as short as 60–90 days. Growers can take advantages of an early market window, but also face the problem of small fruit size because of the short FDP. Fruit thinning has been suggested as an effective method to increase fruit size. Twenty grafted peach trees were grown with 'UFSun' as scion and 'Flordaguard' as rootstock, half were thinned during the second week of Mar. 2018 with 6 inches interval and the remaining ten trees were left unthinned as control. The fruits from unthinned trees, weight, average diameter, and length were significantly increased compared to unthinned trees while the fruit shape index and dry matter content did not show significant differences.

According to our results, fruit thinning for tropical peach cultivation is strongly recommended to increase fruit size and therefore marketability. Based on our tests, flower thinning appears to be a promising technique for producing fresh fruit, however it is not important for juicing due to the similar dry matter content.

Further data are being collected so that yield and fruit quality can be compared between thinned and unthinned peach trees, thus giving more particular and useful suggestions to growers under different circumstances.

*Corresponding author. Email: changyuru@ufl.edu

BY-LAWS OF
FLORIDA STATE HORTICULTURAL SOCIETY, INC.
A Florida Non-Profit Corporation

ARTICLE I.

NAME

The name of the Corporation shall be Florida State Horticultural Society, Inc.

ARTICLE II.

PRINCIPAL OFFICE

The principal office of this Corporation shall be designated as required by the Board of Directors.

ARTICLE III.

PURPOSES

A. To serve as an information center to collate, enhance and disseminate information in the broad field of Florida horticulture.

B. To create and publish the proceedings of the Florida State Horticultural Society to advance Florida horticulture.

C. To advance charity education or any other related or corresponding charitable purpose by the distribution of its funds for such purposes.

D. To advance and develop horticulture and agroecology in the State of Florida.

E. To operate exclusively for charitable and educational purposes as will qualify it as a tax-exempt Corporation under Section 501(c)(5) of the Internal Revenue Code including private foundations and private operating foundations.

ARTICLE IV

PROHIBITED ACTIVITIES

Notwithstanding any other provision of these by-laws or any other provision of these by-laws or the Articles of Incorporation this Corporation shall not conduct or carry on any activities not permitted to be conducted or carried on by any organization that shall be exempt under Section 501(c)(3) or Section 501(c)(5) of the Internal Revenue Code and its regulations, now existing or hereafter amended.

ARTICLE V.

MEMBERSHIP

Section 1. There shall be five classifications of membership, all of which carry voting privileges; a. Annual, b. Patron, c. Student, d. Honorary and e. Life.

Section 2. Any individual, corporate entity or partnership interested in the development and the advancement of horticulture in society shall be eligible for membership provided they agree to be bound by the Articles of Incorporation and By-Laws thereof and by such rules and regulations as may from time to time be adopted by the Board of Directors of this organization and upon the payment of the proper dues as hereinafter provided.

Section 3. Classification of members.

A. Annual Members. Annual members shall be full members of the Corporation.

B. Patron Members. Patron members qualify as such based on dues paid to the society at a level set by the Board of Directors. Patron members shall be full members of the Corporation.

C. Student Members. Student members shall be full members of the Corporation.

D. Honorary Members. Any individual who has rendered a special meritorious service to the society and to the advancement of horticulture in Florida may be designated as an Honorary Member. Honorary members are nominated by the members of the society and approved by two-thirds (2/3) vote of the Board of Directors. Honorary members shall not be required to pay annual membership fees. Honorary members shall be permanent members of the Corporation.

E. Life Members. Current members who are age 65 or older may choose to pay a one-time membership fee to become life members and shall not be required to pay annual membership fees thereafter. Life members shall be full members of the Corporation.

F. Membership fees for Annual, Patron, Student, and Life Members shall be established by the Board of Directors.

Section 4. Duration. All classes of membership save and except honorary and life shall be one (1) year in duration and shall be required to be renewed annually by the payment of the required dues. Dues shall be payable in advance on the last day of December in each fiscal year, and will be considered delinquent after that date.

Section 5. The right of a member to vote and all of his or her other rights and interests in the organization shall cease on the termination of his or her membership. No member shall be entitled to share in distribution of the Corporate assets upon the dissolution of the organization's corporate structure.

Section 6. Application of membership. A prospective member shall be eligible for membership upon the approval of his or her application and payment of the applicable membership fees.

Section 7. Resignation. Any member may resign from the organization by delivering a written resignation to the President or Secretary.

Section 8. Reinstatement. A member who has resigned in good standing may apply to the Membership Coordinator for reinstatement.

ARTICLE VI.

MEMBERSHIP MEETING

Section 1. Annual meeting. The membership shall hold annual meetings each year at such place or places as may be determined by the Board of Directors.

Section 2. Special meetings. Special meeting of the members may be called at any time by the President or Secretary or by any two (2) members of the Board of Directors. Such meeting must be called by the President or Secretary upon receipt of the written request of 1/3 (one-third) of the members. Written notice of such meeting, stating the time, place and purposes shall be served by mail upon each member of the organization not less than ten (10) days nor more than fifteen (15) days before such meeting.

Section 3. Quorum. At any meeting twenty-five (25) members or more present shall constitute a quorum for all purposes, and the act of the majority of these members present at which there is a quorum shall be the act of the entire membership, except as may be otherwise provided for by statute or by the charter of the organization. In the absence of a quorum, or when a quorum is present, a meeting may be adjourned by the vote of a majority of the members present in person or by proxy without the notice other than by announcement at the meeting and without further notice to the absent members. At any adjourned meeting at which the quorum shall be present any business may be transacted which has been transacted at the meeting as originally notified.

Section 4. Voting Rights. At every meeting of members, each member entitled to vote, shall be entitled to vote in person. The vote for the election of directors and on any question before the meeting when deemed necessary by either the President or the Secretary shall be by ballot. All elections and all questions to be decided at such meeting shall be by majority vote of the members present and entitled to vote.

Section 5. Order of Business. The order of business at membership meetings shall be as follows:

A. Calling of the roll of members.
B. Proof of notice of meeting or waiver of notice submitted.
C. Reading of the minutes of the previous meeting.
D. Reports of officers.
E. Reports of committees.
F. Election of Board of Directors.
G. Unfinished business.
H. New business.

Any question concerning the priority of the business to be conducted before the meeting shall be decided by the chair of the meeting. The order of business may be amended or changed at any meeting by majority of vote of the members present at such meeting.

Section 6. Informal actions by members. The members of the Society may take any action allowed at the annual meeting if the majority of a quorum as described in Section 3 of this Article sets forth the action and files it by any traceable means with the Secretary of the Corporation. Within ten (10) days after obtaining such a filing, notice must be given to those members who have not consented to such action taken.

ARTICLE VII.

BOARD OF DIRECTORS

Section 1. General management of the affairs of the organization shall be vested in the Board of Directors.

Section 2. The number of directors shall not be less than three (3) nor more than twenty-one (21) which may be changed from time to time by amendment of these By-laws in the manner provided herein.

Section 3. The Election of Directors. The Board of Directors shall be elected by the members of the organization at the annual meeting of the members by a majority vote of the members present at such meeting as provided in Section 9 of Article VIII herein.

Section 4. Duties and Powers of the Directors. The Board of Directors shall have the authority to:

A. Hold meetings at times and places that may deemed proper and necessary.
B. Admits, suspend or expel members.
C. Appoint committees on particular subjects for members of the board or from the membership of the organization.
D. Audit bills and disburse the funds of the organization.
E. Print and circulate documents and publish articles, pamphlets and papers.
F. Carry on correspondence, communicate with other associations with the same interests.
G. Employ agents.
H. Devise and carry into execution such other measures as deemed proper and expedient to promote the objects of the organization and protect the interests and welfare of the members.
I. Remove any or all of the officers of the organization with due cause prior to the termination date of such office.
J. Elect substitute directors in the event any director resigns or is removed from office prior to the termination date from such office.
K. Terminate the contract of any firm, individual or any other entity employed by the organization to perform any and all nature of services to the organization.
L. Employ, retain or terminate any employee of the corporation who it deems appropriate.

ARTICLE VIII.

MEETINGS OF THE BOARD OF DIRECTORS

Section 1. Annual Meetings. The annual meeting with the Board of Directors shall be held at a place or places determined by the Chairman the Board.

Section 2. Special Meetings. Special meetings of the Board of Directors may be called by the President or by any two (2) members of the Board of Directors. Written notice shall be given stating the purpose of such meeting and shall be sent by any traceable means to each member of the Board of Directors at least five (5) days prior to such meeting date.

Section 3. Regular Meetings. The Board of Directors shall hold regular meetings at such time and place as may be approved by a majority of the board. A majority of the board shall constitute a quorum. The board may be canvassed by any traceable means and vote by ballot in like manner.

Section 4. Absences. Should any member of the Board of Directors be absent without just cause from three (3) consecutive meetings of the board without notifying the President or Secretary for his/her reason for doing so and if his/her reason should not be accepted by the members of the board, his/her seat on the board may be declared vacant and the board may vote to select a substitute director from the membership of the organization to serve the remainder of his/her term.

Section 5. Resignation. Any director may resign at any time by giving written notice of such resignation to the Board of Directors.

Section 6. Removal, Termination of Office. Anyone or more of the directors may be removed with cause at any time by the Board of Directors in the same manner set forth for voting on any issue herein above.

Section 7. Delegation of Authority. Board of Directors shall delegate authority to any executive committee created by it to conduct the business of the organization in accordance with the policies prescribed by the Board of Directors from time to time.

Section 8. Eligibility for Membership. Only members in good standing shall be qualified to become members of the Board of Directors.

Section 9. Election of Directors. The President shall appoint a nominating committee consisting of not less than two (2) persons from each section, one of whom shall be the most recent past sectional vice-president able to serve. This committee shall at each annual meeting make nominations for members of the Board of Directors for the ensuing year provided the members representing various sections shall seek advice of each section in open meetings concerning the nomination of Vice-President elect for that section. Such nominations by the nominating committee, however, shall not preclude nominations from the floor.

1. At the annual meeting held after the adoption of the By-laws, an election shall be held and determined by the majority of the members present.
2. At all elections for officers of the society, only active voting members in good standing may be qualified to cast their votes for such officers.

Section 10. Order of Business. The order of business of the Board of Directors meeting shall be the same as provided in Section 5 of Article VI except F. Election of Board of Directors.

Section 11. Directors shall receive no compensation for their services.

Section 12. The directors of the corporation shall not be personally liable for its debts, liabilities or other obligations.

ARTICLE IX

OFFICERS

Section 1. The officers of the corporation: Chairman of the Board who is the immediate past President, President who is the immediate past President-elect, President-elect, six (6) sectional Vice-Presidents and Vice-Presidents-elect, as more particularly hereinafter described, Secretary, Treasurer, Editor, Program Coordinator, Marketing Coordinator, Membership Coordinator, Student Affairs Coordinator, In-Service Training Coordinator, Newsletter Editor, two Members-at-Large and such other officers with such powers and duties not inconsistent with these By-laws as may be appointed and determined by the Board of Directors from time to time.

Section 2. Term of Office. The terms of office for the Editor, Program Coordinator, Secretary, Treasurer, and Marketing Coordinator shall be five (5) years on a staggered basis with no member serving more than two (2) terms in such offices. The term of the Membership Coordinator, Student Affairs Coordinator, In-Service Training Coordinator, and Newsletter Editor shall be three (3) years. The terms of the Members at-Large shall be two (2) years, beginning in alternate years. All other offices shall be for a term of one (1) year.

Section 3. Installation and Commencement of Duties. The officers newly elected at the annual meeting shall be installed and take office immediately following the annual meeting as established by the Board of Directors. All officers who are elected by the members of the society except the vice-presidents-elect shall be voting members of the Board of Directors.

Section 4. Election of Officers. The President shall appoint a Nominating Committee consisting of not less than two (2) persons from each section, one of whom shall have been the most recent past sectional vice-president able to serve. This committee at each annual meeting shall make nominations for officers for the ensuing year provided the members representing various sections shall seek advice of each section in open meetings concerning the nomination of Vice-President-elect for that section. Such nomination shall not preclude nominations from the floor. The names of the proposed officers shall be submitted to the board prior to the annual meeting.

Section 5. Consent to Election. Only those persons who have signified their consent to serve if elected shall be nominated for or elected to such office.

Section 6. Multiple Offices. No person shall be on the ballot for more than one (1) office.

Section 7. Vacancies in Office. If the office of Chairman of the Board or President becomes vacant by reason of termination or resignation during the term of office, the Board of Directors shall select a successor to succeed to the office for the unexpired term. Vacancies in all other elected offices shall be filled for the unexpired term by the Board of Directors. During the absence or the inability of the President to serve, the Chairman of the Board of Directors shall temporarily fulfill the role of President.

Section 8. Duties of Officers.

A. Chairman of the Board. It shall be the duty of the Chairman of the Board to chair the board meetings; appoint all standing committees as prescribed by the board, and he or she shall perform such other duties as may be delegated to him or her by the Board of Directors.

B. President. It shall be the duty of the President as a Chief Executive Officer to be the official head of the society and preside at the general session of the annual meeting. The President shall be directly responsible to the Board of Directors and may be removed from office for cause by an affirmative vote of a majority of the full Board of Directors. The President shall the power to sign all contracts and any other obligations on behalf of the corporation approved by the Board of Directors. The President shall be ex-officio member of all sections and committees except the nominating committee. He/she shall be authorized to sign checks on the corporation's bank account. In addition, the President shall have and perform such other duties as may be delegated to him/her by the Board of Directors.

C. President-elect. The President-elect shall be a member of the Board of Directors and shall have and perform such duties as may be delegated to him/her by the Board of Directors. Upon the expiration of his or her term of office, the President-elect shall automatically assume the office of President of the Society.

D. Sectional Vice-Presidents and Vice-Presidents-Elect. The sectional vice-presidents shall be members of the Board of Directors. The sections comprising the corporate body shall be: Citrus Section, Vegetable Section, Krome Memorial Institute (tropical and subtropical fruits), Ornamental, Garden & Landscape Section, Handling and Processing Section, and Agroecology and Natural Resources Section. The vice-presidents of the various sections shall develop the programs for the particular sections of which they are representatives at the annual meeting, preside over the sectional programs, and support the Editor in collecting papers at the annual meeting. The vice-presidents-elect assist the vice-president of their section as requested and work with the Editor in carrying out his or her duties as requested. The vice-presidents-elect become vice-presidents after serving their initial one (1) year term of office.

E. Secretary. The Secretary shall record all records of the meetings of the Board of Directors and the membership. The Secretary shall also perform such other duties as are designated by the Board of Directors.

F. Treasurer. The Treasurer shall be responsible for all funds paid into the corporation and shall issue and countersign all vouchers paying bills or accounts against the corporation. The Treasurer reviews reports prepared by administrative staff on the financial status of the corporation and reports on such at all meetings of the Board of Directors and membership. The Treasurer shall also perform such other duties as are designated by the Board of Directors.

G. Editor. The Editor shall oversee production of the Proceedings of the annual meeting. The Editor shall coordinate collection of manuscripts by the Sectional Vice-Presidents, supervise the review process with the Associate Editors and work with administrative staff to ensure that that all papers in the Proceedings have the proper style and format. The Editor shall also perform such other duties as are designated by the Board of Directors.

H. Program Coordinator. The Program Coordinator shall develop the program for the annual meeting. The Program Coordinator shall work with the Sectional Vice-Presidents to develop the sectional programs workshops, and special events, and shall work with the President to arrange for invited speakers at the annual meeting. The Program Coordinator shall work with the administrative staff meeting planner to arrange for appropriate facilities and equipment for the annual meeting. He/She shall work closely with the In-Service Training Coordinator to plan the Extension Luncheon, invite a Luncheon speaker, and coordinate IST programs associated with the annual meeting. The Program Coordinator shall also perform such other duties as are designated by the Board of Directors.

I. Marketing Coordinator. The Marketing Coordinator shall serve as the chair of the Publicity Committee and is responsible for leading the development and implementation of the Society's marketing plan. The Marketing Coordinator shall also perform such other duties as are designated by the Board of Directors.

J. Membership Coordinator. The Membership Coordinator shall serve as the chair of the Membership Committee and lead efforts to evaluate member satisfaction and improve member services. The Membership Coordinator shall also serve as a member of the Publicity Committee and perform such other duties as are designated by the Board of Directors.

K. Student Affairs Coordinator. The Student Affairs Coordinator shall serve as the chair of the Student Affairs Committee and is responsible for organizing and conducting the student awards activities at the annual meeting. The Student Affairs Coordinator shall direct all affairs of the society related to student activities.

L. In-Service Training Coordinator. The IST Coordinator shall serve as liaison between the society and the University of Florida/IFAS Extension to solicit relevant, in-service training programs for county extension faculty who work in plant sciences (horticultural and agronomic crops), to be held in conjunction with the annual meeting. The IST Coordinator works closely with the Program Coordinator regarding arrangements for in-service trainings.

M. Member-at-Large. The two members-at-large shall lead efforts to improve the Society's impact on the diverse industry groups within Florida. Both Members-at-Large will serve as members of the Publicity Committee.

N. Reports of Officers. All officers shall perform the duties prescribed and assigned to them by the President from time to time and as outlined herein and deliver to their successor all official material not later than thirty (30) days following the election and installation of their successors.

O. Compensation. Officers of the corporation shall receive no compensation for their services.

ARTICLE X
COMMITTEES

Corporation shall have such committees as shall be necessary for the conduct of the organization's business and to carry out its objects and purposes. All committees shall serve for one (1) year. The committees shall be as established by the Board of Directors and initially are as follows:

1. Nominating Committee. There shall be a nominating committee established as provided above and appointed by the President who shall designate a member of the committee as chair and shall have such duties as above provided.
2. Membership Committee. The Chairman of the Board of Directors shall appoint a membership committee consisting of the Membership Coordinator, who shall serve as chair of the committee, and one other member. Within each section, the sectional Vice-President shall appoint a section member to this committee. The Membership Committee shall ensure that the needs of the membership are being meet by the services and activities of the society.
3. Publicity Committee. The Publicity Committee shall consist of the Marketing Coordinator, who shall serve as the chair, the In-Service Training Coordinator, the Membership Coordinator, the Newsletter Editor, the webmaster of the society's website, and the two Members-at-Large. The Publicity Committee will develop outreach and recruiting programs and materials for the Society.
4. Student Affairs Committee. The Chairman of the Board of Directors shall appoint a student affairs committee. The Student Affairs Coordinator shall serve as chair of the student affairs committee. This committee shall coordinate all affairs of the society related to student activities, including distribution of patron funds for support of student attendance at meetings, recognition of students at meetings, reports of students, and other student related activities.
5. Editorial Committee. The Chair of the Board of Directors shall appoint an editorial committee consisting of one (1) associate editor from each of the six (6) sections and additional members who conduct the reviews for the peer reviewed papers. The Editor shall serve as the chair of the Editorial Committee. This committee shall meet with the Editor as called and assist him or her in the editing and preparation of manuscripts for publication in the proceedings as needed. Names of nominees for associate editors shall be presented to the Board of Directors along with brief resumes of their background and qualifications. Associate editors shall then be appointed by the Chairman of the Board of Directors with the approval of the Editor. Such appointment shall be for a three (3) year term with an option for renewal.
6. Auditing Committee. The President with the approval of the Board of Directors shall appoint an auditing committee which committee shall confer with the Treasurer in preparing an audit to be presented by the Treasurer at the annual meeting.
7. Awards Committees. There shall be separate committees on awards as follows:

A. Presidential Gold Medal Award Committee - the presidential gold medal award committee shall consist of the Chairman of the Board of Directors who shall act as chair and three or more other members of the society of the corporation appointed by the President. The presidential gold medal shall be awarded to one (1) individual who has contributed most to Florida horticulture through work published in the proceedings over the preceding time periods since the given section was last eligible. The award will rotate each year from section to section with the Citrus Section eligible in 1975 followed in order by the Krome Memorial Institute, Vegetable Section, Ornamental, Garden & Landscape Section, Handling & Processing, and Agroecology and Natural Resources Sections. The awards shall consist of a gold medal suitably inscribed, a printed certificate, and an honorarium which amount will be established by the Board of Directors.

B. Best Paper Award Committee - the best paper award committees shall be appointed by the President annually from each of the society's sections. Each committee will consist of three members who:

a. Most recently have served as Vice-Presidents of respective sections but;

b. Who were not authors of papers in that volume of the proceedings from which the selection is being made,

c. Who remain members of the corporation.

If less than three (3) members qualify for appointment in any section, the President may appoint members lacking qualification (a) above. Membership of these committees will not be announced. Each sectional best paper will select the best and most meritorious paper as printed in the previous year's proceedings for its respective section. This award shall consist of a medal and printed certificate suitably inscribed and an honorarium which amount will be established by the Board of Directors.

C. Presidents Industry Award Committee. The selection of the paper qualifying for the president's industry award will be made by a committee consisting of at least one (1) member from each section of the society, appointed by the President. The award will be presented to the senior author of a single best paper given at the Florida State Horticultural Society annual meeting by an industry author. An industry author is defined as anyone other than a staff member of an academic institution or a government agency. In those instances where there are both senior and junior authors, all must meet the qualifications of industry authors. The senior author of the paper must be a member of the society. Any paper which receives recognition as the best paper in a section will also be eligible for the President's Industry Award if other qualifications are met. The award shall consist of a plaque suitably inscribed and presented at the Presidents discretion.

D. Outstanding Commercial Horticulturist Award. This award shall be presented annually to an individual who has made significant contribution to the commercial Florida horticultural industry and to the FSHS. Criteria for the selection will be based on the following: Accomplishments: 1. Membership and participation in the Florida State Horticultural Society. 2. Leadership in the Florida horticultural industry. 3. Excellence in the production and/or marketing of horticultural crops. Procedures: 1. The award will be rotated annually among the six Sections of the FSHS in order as follows: Vegetable Section, Citrus Section, Krome Memorial Institute, Ornamental, Garden & Landscape Sections, Handling & Processing, and Agroecology and Natural Resources

Section, starting in 2002 with the Handling & Processing Section. 2. The president shall appoint a selection committee of five (5) members, three from the section to receive the award. Members are encouraged to submit nominations in written format to the Secretary or the President. 3. Recipients are not required to present a paper at the annual meeting. 4. The award will consist of a suitably inscribed plaque and printed certificate.

E. Student Best Presentation and Best Paper Awards. Students are encouraged to attend the annual meeting and to compete for these Awards. The student and the student advisor must be members to participate, and the student must be the senior author of the paper and deliver the oral presentation. Papers and presentations will be judged by a committee chaired by the Student Affairs Coordinator and at least two individuals from different Sections appointed by the President. Entry into the competitions must be declared at the time of title submission.

Student oral presentations at the annual meeting will be judged on importance to Florida horticulture, scientific merit, organization, awareness of current literature, fluency, clarity of presentation, effective use of figures and tables, report of conclusions, completeness, timing, and response to questions. Student papers published in the proceedings of the meeting will be judged on relevance to Florida horticulture, scientific merit, experimental design rigor, literature review, completeness and clarity. Students may participate in either or both competitions.

The committee members shall score each student oral presentation, determine a first, second and third place winner, and announce the Student Best Presentation winners at the Annual Business meeting. The committee members shall score each student paper, determine a first, second and third place winner, and announce the Student Best Paper awardees at the Annual Business meeting the following year. Student winners will receive prize money with monetary values set by the Board of Directors and be recognized in the Proceedings of the Florida State Horticultural Society.

F. Tomato Research Award. This award shall be presented based on an annual donation to the corporation by the Florida Tomato Committee. The award committee shall consist of the Chairman of the Board of Directors and four (4) other members of the corporation appointed at large by the President whose names will not be announced. This award shall be given to the individual or group who have done work with the most potential to further the fresh market tomato industry in Florida through advances reported in any single publication during the two (2) previous calendar years. The senior author of the publication upon which the award is based must be an active member of this corporation. At the discretion of the Chairman of the Board, the award may not be presented in any given year when there does not appear to be suitable nominees. The award shall consist of a certificate suitably inscribed and an honorarium which amount will be established by the Board of Directors.

8. The President shall appoint such other committees as may be deemed advisable and approved by the Board of Directors.

ARTICLE XI

BY-LAW AMENDMENT

The By-laws or the Articles of Incorporation of this corporation may be amended, repealed or altered in whole or in part by a majority vote at any duly organized meeting of the members at which a quorum shall be present. Notice of the proposed change shall be published in the newsletter and sent by any traceable means to each member at least ten (10) days prior to the time and date of the meeting which is to consider and vote upon such change or amendment. The proposed change or amendment to the By-laws or the Articles of Incorporation shall, prior to notice being given of such meeting, be ratified and approved by the Board of Directors by a majority vote of the officers present at such Board of Director's meeting. Only those officers present may cast their vote on the action before the meeting.

Copies of such revised or amended By-laws or Articles shall be given to any member upon request.

ARTICLE XII

PARLIAMENTARY AUTHORITY

The Rules contained in Roberts Rules of Order as Revised shall govern the Board of Directors, Officers, Chairmen of various Committees, and the Members in all cases in which they are applicable, provided, however, that they do not conflict with the By-laws the Articles of Incorporation of this Corporation, or with the laws of the State of Florida.

ARTICLE XIII

FISCAL YEAR

The fiscal year of the Corporation shall commence on the 1st day of January of each year and terminate on the 31st day of December of each calendar year.

ARTICLE XIV

SEAL

The Corporation shall have a seal of such design as may be approved by the Board of Directors.

ARTICLE XV

DISTRIBUTION OF ASSETS

Upon dissolution, liquidation and winding up of the Corporation, the Board of Directors shall, after paying or making provision for the payment of all of the liabilities of the Corporation, dispose of all assets of the Corporation exclusively for the purposes of the Corporation in such manner, and to such organization or organizations organized and operated exclusively for horticultural or scientific purposes as shall at the time qualify as ~~an~~ tax-exempt organization under Section 501(c)(3) or 501(c)(5), as the Board of Directors may determine. Any assets not so disposed of shall be disposed of by a court of competent jurisdiction in the county in which the principal office of the Corporation is then located, exclusively for such purposes, or to such organization or organizations as such Court shall determine which will not impair the tax-exempt status of this Corporation.

ARTICLE XVI

INDEMNIFICATION

The Corporation may be empowered to indemnify any officer or director, or any former officer or director, by a majority vote of a quorum of directors or by a majority vote of a quorum of members, who were not parties to such action, suit or proceeding, in the manner provided in Section 607.014 of the Florida Statutes, as amended. If such indemnification is authorized by the directors or members, expenses incurred in defending such civil or criminal action, suit or proceeding may be paid by the Corporation in advance of the final disposition of such action, suit or proceeding in the manner described in Subsection 5 of Section 607.014 of the Florida Statutes, as amended, upon receipt of an undertaking by or on behalf of the director, officer, employee or agent to repay such amount unless he/she is found to be entitled to such indemnification.

ARTICLE XVII

CONTRACTS, CHECKS, DEPOSITS

Section 1. Contracts. The Board of Directors may authorize any officer or agent of the Corporation to enter into any contract or to execute and deliver any instrument or document on behalf of the Corporation, which authority may be general or specific.

Section 2. Deposit. All funds received by the Corporation shall be deposited in such depositories as selected by the Board of Directors or in such Trusteeship for said funds as the Corporation through its

Board of Directors may deem in the best interests of the Corporation. All uncommitted funds shall be invested by the Treasurer in Federal Insured Investments approved by the Board of Directors. For prudent operation and financial security of the Corporation, funds in an amount at least one and one-half (1.5) times the annual expenses shall be maintained, whenever possible, in such reserve funds and shall not be allowed to exceed two and one-half (2.5) times annual expenses. Deviations from these practices may be authorized by a majority vote of the Board of Directors.

Section 3. Checks. All checks, drafts, or any authorization for the payment of any notes, sums of money, or other evidence of debt issued in the name of the Corporation shall be signed by such officers or agents as shall from time to time be designated and determined by the Board of Directors. Unless otherwise authorized, such instruments shall be signed by the Treasurer and the President or the Chairman of the Board.

Section 4. Approval of Bills. All bills before being paid shall be approved by the President or Treasurer and vouchers drawn to pay such bills shall be signed as herein above provided.

ARTICLE XVIII

RESOLUTIONS

Resolutions must originate either regular scheduled sectional business meetings. The resolutions must be supported by a majority vote of those members present voting. The Chair of the Board of Directors will place them before the full Board of Directors by either:

A. Calling a special meeting of the Board of Directors or;

B. Instructing the Secretary to poll the members of the Board of Directors.

Those resolutions approved by a majority of the Board of Directors will be presented to the membership at the annual meeting for action by the Corporation as a whole.

ARTICLE XIX

STANDARD OPERATING PROCEDURES

The Board of Directors shall create, update, and follow a Standard Operating Procedures Manual in a manner consistent with the provisions of the By-laws.

ARTICLE XX

RECORDS

The Corporation shall maintain correct and proper books and records and shall keep minutes of all meetings of the members and Board of Directors, at the principal office of the Corporation. All such records may be inspected by any director, member, or agent or attorney of either, or any proper person at any reasonable time.

DATED & ADOPTED: June 12, 2019

By: Gene McAvoy
President

Attest: Jamie Burrow
Secretary-Treasurer

Florida State Horticultural Society
2019 Membership List

2019 Patron

Plotto, Anne
Simonne, Eric

Honorary Members

Albrigo, Leo
Brecht, Jeffrey
Calvert, David
Campbell, Becky
Cantliffe, Daniel
Castle, William
Everett, Paul
Guzman, Victor
Ismail, Mohamed
Jackson, Larry
Jones, John
Kender, Walter
Locascio, Salvadore
Matthews, Richard
Miller, Leon
Overman, Amegda
Sargent, Steven A.
Saunders, Fred
Sherman, Wayne B.
Spalding, Donald
Stamps, Robert
Syvertsen, James
Todd, Norman
Tucker, David
Wilfret, Gary
Winsberg, Theodore W.

Lifetime Members

Brown, Reginald
Crovetti, Aldo
Fischer, Everette
Fitzpatrick, George
Hurner, Tim
Lamberts, Mary
Price, James
Purcifull, Dan
Read, Paul
Roe, Nancy
Timmer, L.
White, James

Professional

Acosta Rangel, Aleyda
Agehara, Shinsuke
Albrecht, Laurie
Albrecht, Ute
Alferez, Fernando
Alvarado, Veria
Ampatzidis, Yiannis
Athearn, Kevin
Austin, David
Bai, Jinhe
Barcelos Bisi, Rayane
Barrett, Charles
Batuman, Ozgur
Boatwright, Logan
Bolques, Alejandro
Broughton, De
Brym, Zachary
Burrow, Jamie
Campbell, Craig
Chambers, Alan
Chapin, Travis
Chase, Carlene
Chase, Christine
Clem, Taylor
Crane, Jonathan
Creech, Matthew
da Silva, Andre
Danyluk, Michelle
Demesyeux, Lynhe
Deng, Zhanao
Diepenbrock, Lauren
Dittmar, Peter
Donnsn, Van
Dorado, Christina
England, Gary
Etxeberria, Ed
Ferrarezi, Rhuanito
Figart, Lawrence
Franck, Alan
Futch, Stephen
Gettys, Lyn
Gioeli, Kenneth
Gonzalez-Blanco, Pedro
Goodiel, Yvette
Griffis, John
Guzman, Sandra
Hickey, Lisa
Hobbs, Wayne
Hu, Cuifeng
Hunsberger, Adrian
Huo, Heqiang
Hurner, Laurie
Kadyampakeni, Davie
Kelly-Begazo, Christine
Khachatryan, Hayk
Killiny, Nabil
Kopsell, Dean
Krug, Matthew
Lamborn, Alicia
Larsen, Nicholas
Ledesma, Noris
Leonard-Mularz, Michelle
Litzinger, Marion
Liu, Tie
Liu, Guodong
Mankin, Richard
Martin, Cliff
Mayer, Henry
McAvoy, Camille
McAvoy, Eugene
McCormick, Kaydie
McIntyre , Tina

Medina, Karla
Meru, Geoffrey
Meyering, Bryce
Miller, Christian
Munroe , Leslie
Murray, Jesse
Mussoline, Wendy
Orellana, Luis
Oswalt, Chris
Page, Brandon
Pinson, Nicole
Popenoe, Juanita
Qureshi, Jawwad
Ren, Zhongbo
Rezazadeh, St. Lucie County
Rice, Ronald
Ricketts, Grantly
Rihn, Alicia
Ritenour, Mark
Rivera Melendez , Francisco
Rogers, Steve
Rossi, Lorenzo
Rotindo, Kate
Sanchez, Mayerling
Sanderson, Lisa
Sandoya, German
Sargent, Steven
Sarkhosh, Ali
Schumann, Arnold
Seal, Daskshina
Sewards, Joseph
Silvasy, Tiare
Skvarch, Ed
Smith, Cody
Sprague, Danielle
Stauderman, Karen
Steed, Shawn
Sun, Xiuxiu
Swanson, Stewart
Tang, Lisa
Tyson, Richard
Vashisth, Tripti
Vendrame, Wagner
Vincent, Christopher
Wade, Tara
Waldo, Laura
Waldo, Eric
Wang, Weining
Wang, Qingren
Wang, Yu
Wei, Xuan
Wells, Bonnie
Williamson, Jeffrey
Wilson, Sandra
Yeager, Thomas
Zekri, Mongi
Zhang, Jiuxu
Zhang, Zhike
Zhong, Tian
Zotarelli, Lincoln

International
Hernandez, Gonzalo
Pinochet, Dante
Sanada, Atsushi

Student
Adhikari, Bikash
Belisle, Catherine
Boakye, Daniel
Bodaghi, Shahrzad
Brewer, Sarah
Castro, Gustavo
Chang, Yuru
Chang, Lanyen
Dorval, Marie
Edmond, Vovener de Verlands
Feng, Shi
Feuille, Cassandre
Freidenreich, Ariel
Gaire, Susmita
Gairhe, Biwek
Ghimire, Lushan
Glatting, Caitlyn
Gomez Pesantes, Luis
Jiao, Yanli
Kalaman, Heather
Kou, Jingjing
Kreutz, Gustavo
Kunwar, Sudip
Li, Sheng-yang
Lian, Zhaoyuan
Lin, Syuan-You
Louizias, Jean Maude
McGee, Trequan
Mungofa, Perseveranca
Murray, Jesse
Mwatuwa, Rodrick
Njung'e, Michael
Orrock, James
Padilla , Dominick
Phuyal, Dinesh
Pokhrel, Sameer
Reuss, Laura
Rycyna, Julia
Sandhu, Ravneet
Shahkoomahally, Shirin
Shahzad, Faisal
Singh, Garima
Singh, Sukhdeep
Soh, Moonwon
Steppe, Carlee
Sung, Jeehye
Swartz, Stacy
Sweeney, Kenneth
Theodore, Carina
Uthman, Qudus
Yin, Melinda
Zhang, Xumin
Zou, Zhou

Author Index