SURVEY AND CONTROL OF BRAZIL PUSLEY (*RICHARDIA BRASILIENSIS*) IN FLORIDA CITRUS

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Abstract. **Surveys for Brazil pusley (*Richardia brasiliensis*) and Florida pusley (*Richardia scabra*) in leading citrus producing counties of Florida indicated the presence of only Brazil pusley, where Florida pusley was considered to predominate. Field and greenhouse studies were conducted to evaluate 2,4-D and glyphosate, applied separately, or as a tank mixture, or as a commercial prepackage mixture for Brazil pusley control. In separate field studies, tank mixing 2,4-D and glyphosate at 1.2 and 0.68 kg ae/ha provided 95% and 94% control at 5 weeks after treatment (WAT), compared to 85% and 53% control when glyphosate was applied alone at 0.68 kg·ha^{-1}. The prepackage mixture containing the same concentrations of 2,4-D and glyphosate provided 92% and 79% control of Brazil pusley, in the two field studies, respectively. In greenhouse studies, Brazil pusley control with 2,4-D + glyphosate tank mixed at 1.2 and 0.68 kg ha^{-1} was 91% and control with the prepackage mixture was 44%. Viability and regrowth of Brazil pusley rootstocks were not affected by the different herbicide treatments.**

Florida pusley (*Richardia scabra*) is considered to be an economically important weed species in Florida citrus groves (Mersie and Singh, 1989). Brazil pusley (*Richardia brasiliensis*), a member of the same genus, is seldom mentioned in the literature. Both species belong to the family *Rubiaceae* and are characterized by the presence of hairy stems and leaves and resemble each other during the seedling and vegetative phases. Florida pusley is an annual found in the Southeastern U.S., Central and South America, and can be distinguished from Brazil pusley by the presence of tubercles on fruits (Wunderlin, 1998). Brazil pusley is a perennial species native to South America and prevalent in the disturbed soils of the southern coastal plains of the U.S., Mexico, South Africa, Indonesia, and Hawaii. It is characterized by a hirsute fruit and a thickened rootstock capable of perennating (Murphy et al., 1996). Both species grow year-round in central and southern Florida where most of Florida's citrus groves are located.

Florida pusley is considered to be more dominant than Brazil pusley in central Florida[2] and has been studied more extensively than the latter. Effects of environmental factors and chemical scarification on germination of Florida pusley have been documented (Biswas et al., 1975; Paul et al., 1976). Singh and Tucker (1984) listed Florida pusley as one of the common broadleaf weeds in citrus groves. Similar observations were documented by other citrus weed researchers (Reddy and Singh, 1992; Singh and Mack, 1993; Singh and Tucker, 1988; Singh et al., 1990).

In a weed population dynamics study in Georgia, Florida pusley was determined to be the most prevalent weed species with densities greater than 300 plants per m^2 (Johnson and Mullinix, 1997). In 1974, Florida pusley was listed among the 10 most common weeds in nine crops in Florida, Alabama, Georgia, and South Carolina (Buchanan, 1974). During 1997-1998, Florida pusley was one of the 10 most common weeds in eight crops in Florida and Alabama (Dowler, 1997, 1998). Webster and Coble (1997) reported a mean decrease of Florida pusley by 0.9 rank units in the troublesome weed index averaged over four crops in the southeastern states between the years 1974 and 1994-1995. However, Brazil pusley is still not listed as a common or troublesome weed in any of the southern states.

Changes in weed species composition and population densities resulting from weed management and other cultural practices have been well documented. Conservation tillage systems in corn and soybean increased the perennial weed populations and reduced the densities of large seeded dicot species (Buhler, 1995). Ball (1992) determined a shift in weed seedbank dynamics in favor of weed species which were less susceptible to the herbicides applied. Herbicides are the primary means of weed control in Florida citrus, because tillage and other mechanical measures damage the shallow root system of the crop (Tucker et al., 1980). Biological and cultural methods are not determined to be cost-effective yet. Currently, residual PRE herbicides are applied twice a year during February-April and September-November. Periodically, directed sprays of non-selective POST herbicides are used to control heavy infestations of pusley species, hairy beggarticks (*Bidens pilosa* L.), guineagrass (*Panicum maximum* Jacq.), spreading dayflower (*Commelina diffusa* Burm. f.), and other troublesome weeds.

The objectives of this study were to evaluate the relative dominance of Florida and Brazil pusleys in the citrus groves of central Florida, and to determine the efficacy of a prepackage mixture containing 2,4-D and glyphosate, or a similar tank mixture, or the two chemicals applied separately, for the control of Brazil pusley.

Materials and Methods

Surveys. Two field surveys (Survey 1 & 2) were conducted in 14 leading citrus producing counties of central Florida. The counties included Hardee, Hendry, Highlands, Indian

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River, Lake, Manatee, Martin, Okeechobee, Osceola, Orange, Pasco, Polk, and St. Lucie. Surveys 1 and 2 were carried out during the last week of Oct. 1998, and during the first week of May 1999, respectively. Surveys were conducted by selecting 25 random locations within each county and by examining 25 randomly uprooted pusley plants at each location. Locations surveyed were mostly (>90%) citrus groves but also included other disturbed sites like roadsides, right of ways, and fencelines in each county. Naturally wooded areas or other undisturbed vegetation were also surveyed at one or two locations in each county. The weed was identified as Brazil pusley by the presence of a hirsute pericarp on the fruit and rootstocks or as Florida pusley if the pericarp had tubercles and the root system was devoid of rootstocks. A magnifying hand lens (10×) was used to identify the distinguishing characteristics of the fruit. Herbarium vouchers were prepared for documentation of representative samples from each location.

Field studies. Field experiments were conducted at University of Florida, Citrus Research and Education Center citrus groves at Lake Alfred and Davenport, Fla. The soil at both locations was a Candler fine sand (hyperthermic, uncoated Typic Quartzipsamments), the Lake Alfred soil had an OM content of 1.8% and a pH of 5.97, and the Davenport soil had an OM content of 1.3% and a pH of 5.57. Both locations had heavy natural populations of Brazil pusley with an average row-middle cover of >60%. Individual plots (2 m wide by 6 m long) were arranged in row middles as a randomized complete block design with four replications. Five randomly selected pusley plants from each plot were tagged using colored ribbons to monitor rootstock regeneration later on. At the time of treatment, plant height ranged from 5 to 10 cm, each plant spread over an area of 0.5 to 1.0 m^2, and most of the plants were flowering.

Herbicide treatments consisted of either a prepackaged mixture[3] to provide 2,4-D and glyphosate at 1.2 and 0.68 kg ae/ha, respectively, 2,4-D at 1.2 $kg \cdot ha^{-1}$, glyphosate at 0.68 $kg \cdot ha^{-1}$, applied separately or as a tank mixture. All herbicides were formulated as liquid (liter) and no adjuvants were added. The prepackaged mixture and glyphosate component in the tank mixture contained proprietary surfactants in the formulation. Herbicide treatments were applied on 29 Oct. 1998 at the Lake Alfred site and on 12 Nov. 1998 at the Davenport site. The herbicide formulations were applied using flat fan nozzles[4] with a tractor-mounted air-pressurized boom sprayer delivering 230 $L \cdot ha^{-1}$. Weekly weed control ratings, for a 6-week period after treatment, were recorded for Brazil pusley in the entire plot using a scale of 0 to 100% where 0 = actively growing plants and 100 = complete plant death (Frans et al., 1986).

At the end of 6 weeks, rootstocks were retrieved from each of the five tagged pusley plants and were pooled. Ten randomly selected rootstocks from each plot were planted into plastic containers (4000 mL) filled with sterile Candler fine sand and were transferred into a greenhouse to monitor regeneration. The greenhouse was maintained to provide average day/night temperatures of 30/25 °C with average light levels of 900 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ at noon and a photoperiod of approximately 12 hr, with no supplemental lighting. Rootstocks were monitored for regrowth, and shoot counts of Brazil pusley were recorded after an 8-week period. Viable rootstocks were counted after removing the soil from each container.

[3]Landmaster 1.2 liter herbicide, Monsanto Company, St. Louis, MO.
[4]Teejet 8003 flat fan spray nozzles, Spraying Systems Co., Wheaton, IL.

Greenhouse studies. Actively growing Brazil pusley plants (10 to 15-cm tall) with rootstocks were transplanted into plastic containers (500 mL) using sterile Candler fine sand. The plants were grown for 2 weeks prior to treatments. The herbicide treatments were the same as in the field studies. All herbicide formulations were applied with an air-pressurized sprayer[5] using flat fan nozzles[6] delivering 230 $L \cdot ha^{-1}$ with water as the carrier. The plants were fertilized with a liquid fertilizer containing elemental N (20%), P (8.6%), and K (16.7%) to provide 5 $g \cdot m^{-2}$ nitrogen at biweekly intervals and were irrigated daily to field capacity.

Weed control ratings were recorded on a weekly basis for a period of 6 weeks after treatment (WAT), as described in field studies. At the end of 6 weeks, shoot fresh weights were recorded after cutting at the soil surface. The containers remained in the greenhouse and were subsequently irrigated once a week to field capacity to allow rootstock regeneration. The containers were monitored for vegetative growth for a period of 6 weeks, counts of live plants were taken, and plants were harvested at soil surface to measure shoot fresh weight. Rootstocks were then recovered from each container and counts of rootstocks that appeared viable or had active buds were taken. Viability of rootstocks was determined by presence of buds. Fresh weight of all underground tissue was recorded.

All experiments were arranged in a randomized complete block design with four replications and were repeated over time. Percent weed control data were subjected to analysis of variance (ANOVA) at $P = 0.05$, after performing an arc-sine transformation, but are presented in the original form for clarity. All other data were subjected to ANOVA and the means were separated by the LSD test at $P = 0.05$. A test of homogeneity of variance indicated differences between the data collected from the two field experiments and are, therefore, presented separately. Data from greenhouse experiments were combined to present the mean values after performing a test of homogeneity of variance.

Results and Discussion

Surveys. All specimens of *Richardia* spp. collected were identified as Brazil pusley at all locations surveyed. The 14 counties surveyed for this weed represented 82% of commercial citrus acreage in Florida.[7] In >50% of the citrus groves surveyed, Brazil pusley was also among the five most common weeds in the orchard. Neither Brazil pusley nor Florida pusley was found in undisturbed areas surveyed. This may be due to their inability to survive in such areas (Murphy et al., 1996). During Survey 1 (Oct. 1998), the weed appeared to be dormant with light brown rootstocks compared to actively growing Brazil pusley having white rootstocks with active buds during Survey 2 (May 1999).

The results of this survey are contrary to earlier reports on *Richardia* spp. (Singh and Tucker, 1988; Singh et al., 1990). This indicates that the weed was either misidentified in previous studies, or there was a widespread population shift after these reports. If a population shift towards Brazil pusley occurred, it could be explained solely on the basis of selection pressure from herbicides that selectively controlled Florida pusley, an annual species. However, the complete absence of

[5]Allen track sprayer, Allen Machine Works, Midland, MI.
[6]Teejet 8002 flat fan spray nozzles, Spraying Systems Co., Wheaton, IL.
[7]Florida Agricultural Statistics Service, 1996.

Florida pusley in our surveys questions such a population shift theory. Herbicide use targeting a specific group of weeds has occasionally led to weed population shifts. Rozanski and Leiderman (1979) noted an increase in broadleaf weeds, which included Brazil pusley, in soybean fields due to frequent use of graminicides.

Field studies. The tank mixture containing 0.68 kg ae/ha of glyphosate and 1.2 kg ae/ha of 2,4-D controlled Brazil pusley >90% at 5 weeks after treatment (WAT) in both field studies (Table 1). The prepackage mixture provided 92% control in one study and 79% control in the other study at 5 WAT. Brazil pusley control from 2,4-D applied alone was lower in Study 1 compared to the tank or prepackage mixture. Similarly, glyphosate applied alone in Study 2 provided lower Brazil pusley control than the tank or prepackage mixture of 2,4-D and glyphosate. Flint and Barrett (1989b) noted increased field bindweed (*Convolvulus arevensis* L.) control as a result of tank mixing 2,4-D and glyphosate compared to the herbicides applied alone. Injury to Brazil pusley progressed until approximately 5 WAT.

The herbicide treatments did not affect the number of shoots that emerged from rootstocks of Brazil pusley (Table 1). This was perhaps due to lack of optimum conditions in the greenhouse to trigger germination of these rootstocks. Limited information is available on the biology of this weed, which indicates a need for further research.

Brazil pusley rootstock viability was reduced by all herbicide treatments in Study 1 (Table 1). The tank mixture containing 2,4-D and glyphosate reduced rootstock viability in both studies. Rootstocks of herbicide-treated plants may have developed less vigorously compared to untreated rootstocks, thereby affecting their viability. Further studies using radiolabeled compounds to monitor their uptake and translocation would provide conclusive results.

Greenhouse studies. Brazil pusley plants that received 2,4-D and glyphosate at 1.2 and 0.68 $kg \cdot ha^{-1}$, respectively, as a prepackage mixture, were controlled >90% at 5 WAT (Table 2). However, the tank mixture of the same herbicides controlled Brazil pusley only <50%. Under field conditions, the tank mixture of 2,4-D and glyphosate controlled the weed >90%, similar to that of the prepackage mixture (Table 1). Also, 2,4-D provided an average of 80% Brazil pusley control under field conditions compared to 90% control in the greenhouse at 5 WAT.

The differential response of Brazil pusley to similar treatments under field and greenhouse conditions may have resulted from differences in formulation of the herbicide mixtures and the growing conditions. The prepackaged mixture and the glyphosate component of the tank mixture contain certain proprietary surfactants. These surfactants may have caused differential uptake of the active ingredients into the plant under field and greenhouse conditions. The physiology of the plant under different environments may also have attributed to the differential response to herbicides. Fleming et al. (1988) determined that certain maize (*Zea mays* L.) inbreds were more tolerant to atrazine [6-chloro-*N*-ethyl-*N*'-(1-methylethyl)-1,3,5-triazine-2,4-diamine], and bentazon [3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide] under greenhouse conditions compared to field conditions.

Based on shoot fresh weights at 6 WAT, there were no differences between the treated and untreated Brazil pusley plants (Table 2). This may have been due to variability in shoot emergence from rootstocks following different treatments. The number of shoots that emerged from rootstocks was also not different between various treatments, which may further explain this.

Viability of rootstocks was also not affected by the herbicide treatments (Table 2). Fresh weights of rootstocks harvested at 8 weeks after transplanting were reduced by all herbicide treatments, except glyphosate at 0.68 $kg \cdot ha^{-1}$. Again, 2,4-D expressed a more pronounced effect on reduction of Brazil pusley rootstock weight in the greenhouse compared to field conditions.

Several researchers have reported the effects of tank mixing 2,4-D with glyphosate. Flint and Barrett (1989b) noted a synergistic effect on field bindweed control by mixing 2,4-D and glyphosate. The same researchers (Flint and Barrett, 1989a) reported an antagonistic effect on johnsongrass control [*Sorghum halepense* (L.) Pers.] as a result of combining the two herbicides. Sullivan and Donovan (1980) showed reduced phytotoxicity of glyphosate to wheat (*Triticum aestivum* L.) as a result of mixing it with 2,4-D, which was attributed to chemical incompatibility within the tank mixture as opposed to biological interaction within the plant system. Nalewaja and Matysiak (1992) determined that the antagonistic interaction between 2,4-D and glyphosate to wheat was affected by the nature of salts in the tank mixture.

Table 1. Effect of 2,4-D and glyphosate applied alone or in combination in the field on Brazil pusley shoot and rootstock growth.

Treatment	Rate (kg ae/ha)	Study 1: Shoot injury 2 WAT[z]	Study 1: Shoot injury 5 WAT	Study 1: Regrowth from rootstocks[y]	Study 1: Viable rootstocks[x]	Study 2: Shoot injury 2 WAT	Study 2: Shoot injury 5 WAT	Study 2: Regrowth from rootstocks	Study 2: Viable rootstocks
		%		no.		%		no.	
Glyphosate	0.68	63	85	0.8	2.0	29	53	0.5	1.3
2,4-D amine	1.2	54	75	0.3	0.3	82	85	0.0	1.8
2,4-D amine + Glyphosate (PM)[w]	1.2 + 0.68	73	92	0.3	1.3	72	79	0.3	1.8
2,4-D amine + Glyphosate (TM)[w]	1.2 + 0.68	89	95	0.5	0.8	94	94	0.0	0.5
Control	—	0	0	1.0	5.3	0	0	0.8	4.3
L.S.D. ($P = 0.05$)		15	10	NS[v]	1.8	27	25	NS	3.0

[z]Weeks after treatment.
[y]Rootstocks were harvested 6 WAT and were planted in a greenhouse to obtain counts of plants that regenerated subsequently.
[x]Viable rootstocks were counted 6 weeks after transplanting rootstocks into the greenhouse.
[w]PM, prepackage mixture; TM, tank mixture.
[v]Not significant at $P = 0.05$ level.

Table 2. Effect of 2,4-D and glyphosate applied alone or in combination on Brazil pusley shoot and rootstock growth in a greenhouse study.

Treatment	Rate (kg ae/ha)	Injury		Shoot		Rootstock	
		2 WAT[z]	5 WAT	Fresh weight	Regrowth from rootstocks[y]	Viable rootstocks[x]	Fresh weight
		%		g	no.	no.	g
Glyphosate	0.68	27	36	1.22	1.5	1.5	4.82
2,4-D amine	1.2	68	90	0.28	0.8	0.6	0.83
2,4-D amine + Glyphosate (PM)[w]	1.2 + 0.68	77	91	0.7	0.5	0.1	2.07
2,4-D amine + Glyphosate (TM)[w]	1.2 + 0.68	29	44	0.5	0.4	0.6	1.53
Control	—	13	20	1.87	1.3	1.1	4.52
L.S.D. ($P = 0.05$)		22	21	NS[v]	NS	0.8	2.11

[z]Weeks after treatment.
[y]Rootstocks were harvested 6 WAT and were planted in a greenhouse to obtain counts of plants that regenerated subsequently.
[x]Viable rootstocks were counted 6 weeks after transplanting rootstocks into the greenhouse.
[w]PM, prepackage mixture; TM, tank mixture.
[v]Not significant at $P = 0.05$ level.

Extensive surveys throughout Florida and other southeastern United States would be useful to accurately ascertain the distribution of Brazil pusley, an economically important weed species. Our surveys indicate that Brazil pusley dominated Florida pusley in the citrus growing counties of central Florida, contrary to conventional knowledge. Similarity in appearance and growth habits of the two species may have resulted in misidentification of the species, leading to inaccurate estimates of their relative distribution in the past. Another possibility is that Florida pusley may have co-existed with Brazil pusley in small numbers in the past. Subsequent management practices may have favored the proliferation of Brazil pusley. We also noted Brazil pusley as one of the five most prevalent weed species in the major citrus orchards of Florida. Future management efforts should address not only the soil seed bank but also the build-up of rootstocks of Brazil pusley, a perennial weed.

In our experiments to control Brazil pusley, an overall beneficial effect was noticed when 2,4-D and glyphosate were tank-mixed or applied in combination as a prepackage mixture. These results were clear from field studies but greenhouse experiments failed to reproduce them. Differences in herbicide formulations and environmental conditions in the greenhouse vs. field may have attributed to these results. Further studies are required to provide information on the biology of Brazil pusley and its management.

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