Evaluation for the Potential of a New Fungicide 
Pyrimethanil for Postharvest Diplodia Stem-end Rot 
Control on Florida Citrus Fruit

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Postharvest decay is one of the most important factors affecting fresh citrus fruit quality and marketing values. Fungicide application is an important measure to achieve effective citrus postharvest disease control. Pyrimethanil is a newly registered fungicide for postharvest use in citrus. The effectiveness of pyrimethanil for the control of diplodia stem-end rot (caused by Lasiodiplodia theobromae) on Florida citrus fruit was evaluated. Pyrimethanil moderately suppressed the mycelial growth of L. theobromae in vitro on potato dextrose agar. The simulated drench test of pyrimethanil on ‘Fallglo’ tangerine hybrids naturally infected by L. theobromae showed that pyrimethanil at 1000 ppm reduced diplodia stem-end rot incidence by 40.3%, while the standard postharvest fungicides imazalil (1000 ppm) and thiabendazole (1000 ppm) reduced it by 83.1% and 96.7%, respectively. A simulated commercial packingline application test of pyrimethanil (1000 ppm) using ‘Pineapple’ oranges indicated that pyrimethanil reduced diplodia stem-end rot incidence by 29.4%, while imazalil and thiabendazole reduced it by 70.7% and 84.0%, respectively. Overall evaluation results indicated that pyrimethanil actively reduced the diplodia stem-end rot, but it was less effective compared to the existing postharvest fungicides imazalil and thiabendazole.

Postharvest disease control of Florida citrus is accomplished by an integrated procedure using synthetic fungicides as the core component. Postharvest fungicides thiabendazole (TBZ) and imazalil are effective for the control of diplodia stem-end rot (Ritenour et al., 2003). TBZ is more effective than imazalil for diplodia stem-end rot control (Brown and Eckert, 2000). TBZ and imazalil are applied to fruit during postharvest fruit drenching and/or packingline process before or during wax application. Largely due to the need for effective management of Penicillium pathogen resistance to the TBZ and imazalil, two new fungicides, pyrimethanil (Penbotec®, Janssen Pharmaceutica, Titusville, NJ) and fludioxonil (Graduate®, Syngenta Crop Protection Inc., Greensboro, NC), have been registered for citrus postharvest treatments for decay control (US EPA, 2004a, 2004b). Each of the currently registered fungicides has a different mode of action, and provides a chemical tool for controlling the resistant populations of fungal pathogens. The U.S. EPA has also classified pyrimethanil and fludioxonil as reduced-risk compounds (Adaskaveg et al., 2004). The potential of fludioxonil for the control of diplodia stem-end rot and green mold on Florida citrus fruit has been evaluated and reported (Zhang, 2007). Smilanick et al. (2006) and Kanetis et al. (2007) reported that pyrimethanil was effective for green mold control. However, the potential of pyrimethanil for diplodia stem-end rot control is not known and needs to be evaluated.

The objectives of this study were to: 1) test the activity of pyrimethanil against L. theobromae in vitro; 2) determine the efficacy of pyrimethanil for diplodia stem-end rot control using a drenching procedure before fruit ethylene degreening treatment; and 3) evaluate the effect of pyrimethanil on diplodia stem-end rot incidence under simulated commercial packingline application conditions.

Materials and Methods

FUNGICIDES. Pyrimethanil (Penbotec®, 40% w/w) was obtained from Janssen Pharmaceutica Beersel, Belgium and was evaluated in various experiments. Imazalil (Fresguard® 700, 44.6% active ingredient) was obtained from JBT Foodtech (Lakeland, FL). Thiabendazole (TBZ) (Mertect® 340-F, 42.3% active ingredient) was obtained from Syngenta Crop Protection Inc. (Greensboro, NC).

CITRUS FRUIT. Oranges [Citrus sinensis (L.) Osbeck, cv. Pineapple], and tangerine (Citrus reticulata Blanco) hybrids (Fallglo) were used in the tests. Fruit used were obtained from a local citrus packinghouse (Hunt Brothers, Lake Wales, FL). The fruit did not receive any pre- or postharvest treatments before the purchase.

Effect of Pyrimethanil on the Mycelial Growth of L. 
THEOBROMAE. Pyrimethanil was incorporated into autoclaved
Difco™ potato dextrose agar (PDA) at 0, 0.5, 1, 10, 20, 40, 80, and 100 ppm (active ingredient) before pouring the medium into plates. *L. theobromae* isolate D-12 (wild-type) was grown on PDA for 2 d after placing a mycelial plug at the center of the plate. Fungal PDA plugs (0.5-cm diameter) were cut from the edge of *L. theobromae* colonies and transferred onto the centers of pyrimethanil-amended PDA plates. Five plates were used for each concentration of the fungicide. Fungus grown on PDA without pyrimethanil was used as control. The plates with the fungus were placed at 30 °C, and the diameters of radial mycelial growth of the fungus at each pyrimethanil concentration were recorded after 24 h of incubation. The experiment was conducted twice.

**Drench application of pyrimethanil for stem-end rot control.** A simulated drenching system (Zhang and Swingle, 2005) was used in the test. About 40 L of the suspension were applied in a drench cycle. The flow rate was approximately 20 L·min⁻¹. Unwashed ‘Fallglo’ tangerine hybrid fruit were placed into plastic crates (60 cm × 40 cm × 30 cm). Three plastic crates were stacked on top of each other as a treatment to simulate a commercial drench procedure. Each crate contained 70 fruit and represented a replicate. Concentration of pyrimethanil in drench suspension was 1000 ppm. Imazalil and TBZ at 1000 ppm were used as standard postharvest chemical treatments. Fruit were drenched with the aqueous chemicals for 3 min, and drained for 4 min (commercial standard practice) prior to ethylene degreening treatment. After drench treatment, the fruit were subjected to ethylene degreening treatment (10 ppm ethylene, 28 °C and 90% to 95% relative humidity) for 2 d. Drenched and degreened fruit were then stored at 21 °C and about 95% relative humidity for up to 3 weeks. The incidence of diplodia stem-end rot was recorded weekly.

**Packingline application of pyrimethanil for diplodia stem-end rot control.** ‘Pineapple’ sweet oranges were treated with ethylene (10 ppm) at 28 to 30 °C and about 95% relative humidity for 2 d. The fruit were then washed, dried, and randomized through a simulated commercial packingline. The fruit were then treated with an aqueous suspension of pyrimethanil at 1000 ppm by a non-recovery dripping system on the packingline. The delivery rate of the chemical suspension to the fruit was about 50 mL per 60 fruit. TBZ and imazalil at 1000 ppm were used as standard postharvest fungicides. Treated fruit were dried at approximately 52 °C for 1–2 min, and then packed into cartons. Each treatment consisted of three replications (cartons), and each replicate had 60 fruit. Treated fruit were then incubated at 21 °C and about 95% relative humidity. Diplodia stem-end rot incidences were identified and recorded weekly for up to 3 weeks.

**Data analysis.** Analysis of variance of data was performed using the JMP statistical package (SAS Institute Inc., Cary, NC). Percentage decay data were transformed to arcsine values before the analysis. Treatment means were compared using the Tukey–Kramer HSD multiple range test (*P* ≤ 0.05). Actual disease incidences were presented in all cases.

**Results**

**Effect of pyrimethanil on the mycelial growth of *L. theobromae***. Under the test conditions, pyrimethanil at a range of concentration 0.5 to 100 ppm suppressed the mycelial growth of *L. theobromae* with increased suppression as pyrimethanil concentration increased (Fig. 1). The EC₅₀ value against the mycelial of *L. theobromae* was estimated as 2.7 ppm based on a logarithmic model. However, the concentration of pyrimethanil for complete suppression of the pathogen was not observed although a concentration of pyrimethanil up to 1000 ppm was tested (data not shown).

**Drench application of pyrimethanil for stem-end rot control.** For the drenching test, there was a high level (89.2%) of diplodia stem-end rot incidence with control ‘Fallglo’ fruit (Fig. 2). Pyrimethanil at 1000 ppm reduced diplodia stem-end rot incidence by 40.3%, while standard postharvest fungicides imazalil and TBZ at 1000 ppm reduced the decay by 83.1% and 96.7%, respectively. Statistical analysis of the data indicates that pyrimethanil significantly (*P* ≤ 0.05) reduced the incidence of diplodia stem-end rot, but it was significantly less than that of imazalil or TBZ. TBZ was more effective than imazalil for reducing diplodia stem-end rot incidence.

**Packingline application of pyrimethanil for diplodia stem-end rot control.** When aqueous pyrimethanil at 1000 ppm was applied to ‘Pineapple’ oranges that were degreened...
reduce the inoculum levels of Geotrichum and Penicillium species (which cause brown rot), Phytophthora species (which cause brown rot), TBZ-resistant isolates of Penicillium species, and other pathogens. However, other studies (Adaskaveg et al., 2004; Smilanick et al., 2006) indicated that pyrimethanil is not compatible with chlorine in drench solutions. Therefore, pyrimethanil appears to have much less potential as a commercial drencher for diplodia stem-end rot control under the current Florida packinghouse conditions.

Packingline application of fungicides for postharvest decay control is a common commercial practice. Fungicides are usually applied during packing processing by aqueous application before waxing or by incorporating the fungicides into waxes during fruit waxing. TBZ, imazalil or TBZ plus imazalil is normally applied during the packingline processing for stem-end rot and mold control. In the current study, the new fungicide pyrimethanil applied by a non-recovery dripping application system significantly reduced diplodia stem-end rot incidence by 29.4%, but it was significantly less effective compared to imazalil and TBZ, which reduced the disease by 70.7% and 84.0%, respectively. These packingline application test results were similar to those of drenching tests described previously. It appears that pyrimethanil can only provide limited potential for diplodia stem-end rot control by packingline application compared to other postharvest fungicides imazalil and TBZ.

Besides the diplodia stem-end rot, other fungal decays such as green and blue molds are also important postharvest problems on Florida citrus (Ismail and Zhang, 2004; Ritenour et al., 2003). Green mold is one of the most important postharvest diseases on citrus fruit in all citrus growing regions (Zhang and Swingle, 2005). Pyrimethanil has been reported as a new effective postharvest fungicide for green mold control of citrus (Adaskaveg et al., 2004; Kanetis et al., 2007; Smilanick et al., 2006). Our tests with a dipping method also showed that pyrimethanil was effective for green mold control (data not shown). The resistance development of Penicillium species to imazalil and TBZ has been a great limiting factor for effective green and blue mold control using these fungicides (Holmes and Eckert, 1999; Kinay et al., 2007). The mechanism of the new fungicide pyrimethanil against fungal pathogens is to suppress the secretion of cell wall-degrading enzymes by the pathogens. This mode of action of pyrimethanil is different from other existing fungicides. Therefore, the registration of pyrimethanil has provided citrus industry with a new chemical tool to control green mold and the resistant populations of Penicillium species to other fungicides.

In summary, pyrimethanil is a newly registered postharvest fungicide for use in citrus and other fruits. It has been classified as a reduced-risk compound by the EPA, and it has a new mode of action against fungal pathogens compared to other existing postharvest fungicides. Although the current studies showed that pyrimethanil exhibits a moderate activity for diplodia stem-end rot control compared to imazalil and TBZ, other studies have demonstrated that it is effective for green mold control (Adaskaveg et al., 2004; Kanetis et al., 2007; Smilanick et al., 2006). Pyrimethanil could play an important role in the fungicide resistance management of Penicillium species.

**Discussion**

Diplodia stem-end rot is one of the major postharvest diseases on Florida citrus fruit, and it is more prevalent on ethylene-degreased early season fruit (Brown and Eckert, 2000; Zhang, 2004). Ethylene degreening of early season fruit to improve fruit coloration for marketing is a common practice in Florida. The ethylene degreening, especially excessive degreening, can increase the diplodia stem-end rot incidence and severity (Ismail and Zhang, 2004; Zhang, 2004). A more effective approach to control this disease is to treat the fruit with appropriate fungicides before ethylene degreening. Preharvest application of fungicides or postharvest drenching of fruit with fungicides can be a good way for diplodia stem-end rot control before postharvest degreening (Ismail and Zhang, 2004; Zhang and Timmer, 2007). TBZ and imazalil are commercially used in citrus fruit drenching treatments, and they are effective for diplodia stem-end rot and other decay control in Florida (Ritenour et al., 2003). In this study, the drenching test of pyrimethanil for diplodia stem-end rot control showed that pyrimethanil (reduced the disease by 40.3%) exhibited a moderate activity, and was significantly less effective for diplodia stem-end rot control compared to imazalil (reduced the disease by 83.1%) and TBZ (reduced disease by 96.7%). Chlorine is commonly added to TBZ drench solutions to reduce the inoculum levels of Geotrichum citri-aureum (which causes sour rot), Phytophthora species (which cause brown rot), TBZ-resistant isolates of Penicillium species, and other pathogens. However, other studies (Adaskaveg et al., 2004; Smilanick et al., 2006) indicated that pyrimethanil is not compatible with chlorine in drench solutions. Therefore, pyrimethanil appears to have much less potential as a commercial drencher for diplodia stem-end rot control under the current Florida packinghouse conditions.

**Literature Cited**


