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Control of Asian Citrus Psyllid Using *Isaria fumosorosea* and Emulsifiable Oils

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The entomopathogenic fungus *Isaria fumosorosea* (commercially available as PFR-97) and various emulsifiable oils were tested against established infestations of Asian citrus psyllid (*Diphorina citri*) in potted orange jessamine (*Murraya paniculata* L.) and Benton citrange (*Citrus sinensis* L.) in greenhouse cages. Fungal treatments at label rates reduced psyllid populations by approximately 50% over 3 weeks. The combination of PFR-97 with emulsifiable oils did not enhance psyllid mortality compared with either agent alone. Imidacloprid applied as a soil drench killed >99% of psyllids within 3 weeks. Subsequent tests conducted under very humid conditions were hampered by natural dissemination of *I. fumosorosea*, suggesting that this fungus can be spread by air movement or other factors. In addition, a naturally occurring *Cladosporium* spp. rapidly colonized psyllid cadavers and leaf surfaces, but was not pathogenic in laboratory tests. Our studies confirmed the potential of *I. fumosorosea* for control of *D. citri*. Although *I. fumosorosea* or some insecticidal oils may be less effective compared with systemic insecticides in terms of direct toxicity, PFR-97 and some oils are expected to have lower impacts on beneficial species and hence may be suitable for inclusion in integrated management strategies for this pest.

The Asian citrus psyllid, *Diphorina citri* Kuwayama (Hemiptera: Psyllidae), is endemic to Asia and known to have a wide host range within the plant family Rutaceae, specifically citrus and related species including orange jessamine, *Murraya paniculata* L. Jack (Halbert and Manjunath, 2004). *D. citri* was first detected in Florida in 1998 (Knapp et al., 2006) and has since spread throughout the state (Childers and Rogers, 2005; FDACS 2008; Qureshi and Stansly, 2007; Tsai et al., 2000). *D. citri* has also been found in Texas (French et al., 2001), Louisiana, Alabama, Georgia, Mississippi, South Carolina, California, Puerto Rico, and Guam (Hummel and Ferrin, 2010; USDA, 2011) and all of the islands of Hawaii (Conant et al., 2009). Although *D. citri* damages plants directly through its feeding activities, the most serious concern is its ability to vector the phloem-limited bacterium *Candidatus* Liberibacter asiaticus that causes huanglongbing (HLB), also known as citrus greening disease (Hung et al., 2004; Manjunath et al., 2008). HLB is acquired and subsequently transmitted by the probing action of psyllids during feeding in as little as 30 min (Bové, 2006).

Since trees infected with HLB cannot be cured, controlling psyllid populations with petroleum oil and foliar and systemic insecticides (Rogers et al., 2012) and the use of certified disease-free trees (Brlansky et al., 2012) is currently recommended to reduce the spread of HLB. Recent studies have highlighted issues with the wide-scale use of broad-spectrum insecticides to control *D. citri* populations, including evidence of field resistance (Tiwari et al., 2011) and toxicity toxic to psyllid parasitoids released in citrus groves (Hall and Nguyen, 2010). Alternative low risk insecticides would provide resistance management tools and help conserve beneficial arthropods (e.g., parasitic wasps, predatory bugs, spiders, ladybeetles) that help regulate *D. citri* populations (Qureshi and Stansly, 2009).

The insect-killing (entomopathogenic) fungus *Isaria fumosorosea* (Wize) Brown & Smith is a potential alternative tool for use against *D. citri* in citrus groves in Florida. Entomopathogenic fungi penetrate the insect cuticle directly and hence are suitable against phloem-feeding insects. Strains of *I. fumosorosea* are highly active against whiteflies are used as biological pesticide in Europe (Faria and Wraight, 2007). Also, a strain of *I. fumosorosea* was found naturally infecting an adult Asian citrus psyllid in Polk County, Florida, so this pathogen may be considered native (Meyer et al., 2008). In addition, recent studies show that *I. fumosorosea* does not normally infect natural biological control agents of psyllids and thus would most likely be suitable in citrus IPM programs (Avery et al., 2008, 2009). Here we evaluated a commercial strain of *I. fumosorosea* (PFR-97) in greenhouse tests. The fungus was applied with and without emulsifiable oils that were hypothesized to improve the activity of fungal infection against *D. citri,* and was compared with a systemic insecticidal standard.

Materials and Methods

The psyllid colony was obtained from the USDA in Fort Pierce and maintained on 'Marsh' grapefruit. A blastospore formulation of *I. fumosorosea* 'PFR-97' (strain Apopka-97) labeled at 10^9 cfu/g (but found to contain 3×10^9 cfu/g) was obtained from Certis USA, Columbia, MD. The orange jessamine *Murraya paniculata* L. and Benton citrange *Citrus sinensis* L. Osb. × *Poncirus trifoliate* L. Raf plants used in tests were pruned and fertilized (12–5–8 Vigoro®, The Scotts Company LLC, Marysville, OH) 2 weeks

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prior to tests to encourage new "flush" for psyllid oviposition.

In the fall 2010 test, 36 orange jessamine plants (30 cm tall) were placed individually in square nylon mesh cages (60 cm dimensions) and infested with 20 adult *D. citri*. The following treatments were applied after 10 d when nymphs were emerging: control (distilled water), imidacloprid (Merit 2F at 3 mL product/plant), emulsifiable vegetable oil (Addit® 2.5% v/v), PFR-97 (2.1 g/L, equivalent to 28 oz/100 gal), PFR-97 (2.1 g/L) + Addit® (2.5% v/v), a highly refined paraffinic oil (SuffOil-X® @ 2% v/v). All foliar treatments included a wetting agent (Tween80 at 0.025% v/v). The PFR-97 was mixed in approximately 100 mL distilled water for 30 min prior to use. Foliage was sprayed to "runoff" using a 3.8-L (1 gal) hand-held pressurized sprayer. The imidacloprid was applied as a soil drench in 75 mL (2.5 fl oz) water. All foliar treatments were reapplied after 7 d. There were 6 replicate plants per treatment arranged randomly inside a single greenhouse bay. All treatments were applied in the early evening after cooling fans had stopped. To encourage favorable conditions an overhead misting system was operated for 20 s to maintain >95% RH in the greenhouse for 8 h and to encourage fungal spores to germinate, but did not significantly wet the leaves of the plants inside cages. Psyllid populations were counted immediately prior to spray applications and every seventh day over 3 weeks. The number of nymphs and adults were counted from a minimum of three shoot terminals per plant. In the fourth week, a final destructively count was taken. Psyllids showing signs of fungal sporulation were collected, cultured on PDA medium under sterile conditions, and monitored for fungal outgrowths to confirm Koch's postulates.

The study was repeated in the summer and fall of 2011 using Benton citrange plants. As additional modifications, in the fall 2011 test, there were 5 replicate plants per treatment and different oil-combinations were tested. Orocit®, an adjuvant made from citrus oils containing alcohol ethoxylate and labeled for citrus, was evaluated at 0.25% v/v to determine if it would improve fungal pathogenicity of *Isaria*. SuffOil-X® was also tested at 1% v/v (lower label rate) with and without PFR-97. Imidacloprid was omitted in the final test since its efficacy was clearly shown previously. The germination rate of PFR-97 used in these tests was 66% to 77% after 16 h at 25 °C on PDA media. In 2010, temperatures inside greenhouse cages ranged from 16.1 to 41.5 °C (average 25.2 °C) and relative humidity ranged from 15% to 100% (average 77.7%). In 2011, temperatures ranged from 18.5 to 40.9 °C (average 25.0 °C) and relative humidity ranged from 15% to 100% (average 69.1%). The numbers of live psyllid (adults and nymphs) were compared between treatments through repeated measures analysis of variance following log (n+1) transformation. Where appropriate, means were further compared with Fishers protected LSD tests at $P < 0.05$.

Results

In the fall 2010 test, treatments had a significant effect on the number of live *D. citri* adults and nymphs ($F_{5,30} = 13.5$, $P < 0.001$ and $F_{5,30} = 17.1$, $P < 0.001$, respectively). All treatments reduced the number of F1 adult psyllids compared with controls, by days 14 and 21 post treatment (Fig. 1). In the destructive count, Merit 2F was the most effective with 99.9% reduction with respect to

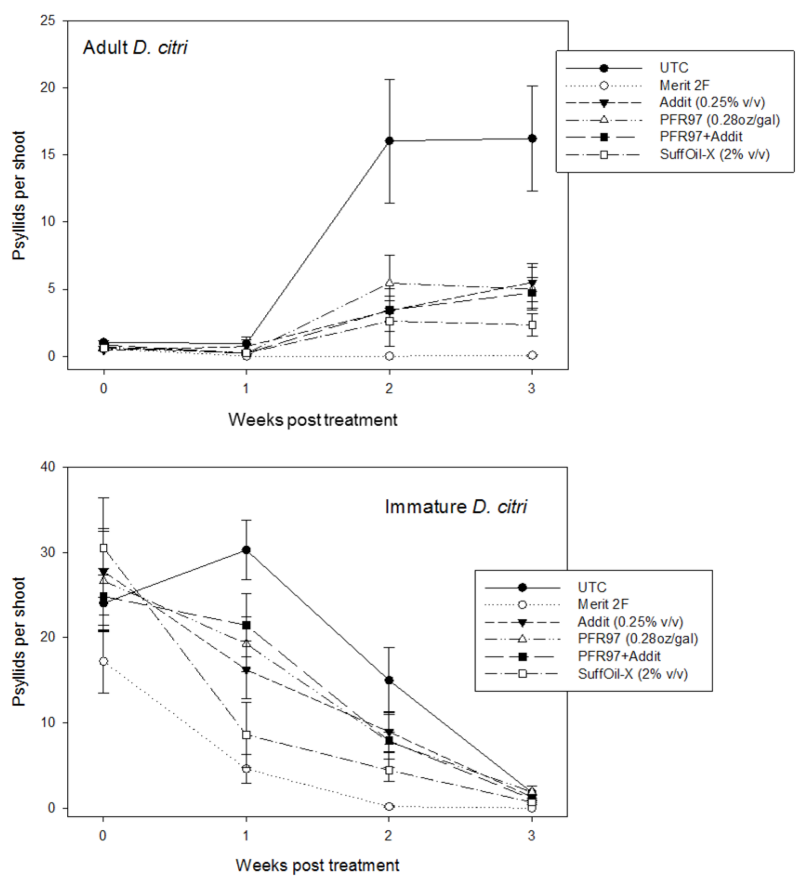

Fig. 1. Number of *Diaphorina citri* (adults and nymphs) recorded on orange jessamine plants following different treatments (Fall 2010).

Table 1. Total mean number of live *D. citri* on orange jessamine plants as determined by a final destructive count (Fall 2010).

Treatment	Rate[z]	Total psyllid/plant[y]	Percent reduction
Control		304.0 a	0
Merit 2F	3 mL/plant	0.2 d	99.9
Addit	0.25% vol/vol	133.8 b	56.0
PFR-97	28 oz/100 gal	145.3 b	52.2
PFR-97+ Addit	28 oz/100 gal + 0.25% vol/vol	152.5 b	49.8
SuffOil-X	2% vol/vol	43.7 c	85.6

[z]All treatments contained 0.05% v/v Tween 80.
[y]Different letters in columns indicate significant differences ($P < 0.5$, Fisher's protected LSD). Data were transformed due to unequal variances [log10 (x+1)] but non-transformed means are shown.

the untreated control followed by SuffOil-X (85.6% reduction), Addit (56% reduction), PFR-97 (52.2% reduction), PFR-97+Addit (49.8% reduction) (Table 1). The combination of PFR-97 and emulsifiable oil did not increase psyllid mortality compared with either agent alone. Only Merit and SuffOil-X significantly reduced the number of infested terminals (data not shown). An average of 20% of psyllids that died following exposure to fungal suspensions produced outgrowths consistent with *I. fumosorosea* symptoms and Koch's postulate tests confirmed that *I. fumosorosea* could be reisolated on healthy psyllids (Fig. 2A).

In the 2011 tests, problems with high control mortality and contamination by *Cladosporium* sp. were observed towards the end of experiments. In the summer test, while all treatments reduced the number of *D. citri* nymphs after 7 d with Merit 2F being the most effective, relatively few F1 adults were subsequently observed in control cages by week 2 (Fig. 3). A similar trend was observed in fall 2011, where foliar treatments significantly reduced psyllids in weeks 1 and 2, but numbers of F1 adults again remained low and *Cladosporium* sp. was observed in all cages (Fig. 2B). Overall, treatments containing oils alone or in combination with PRF were the most effective in the fall 2011 test.

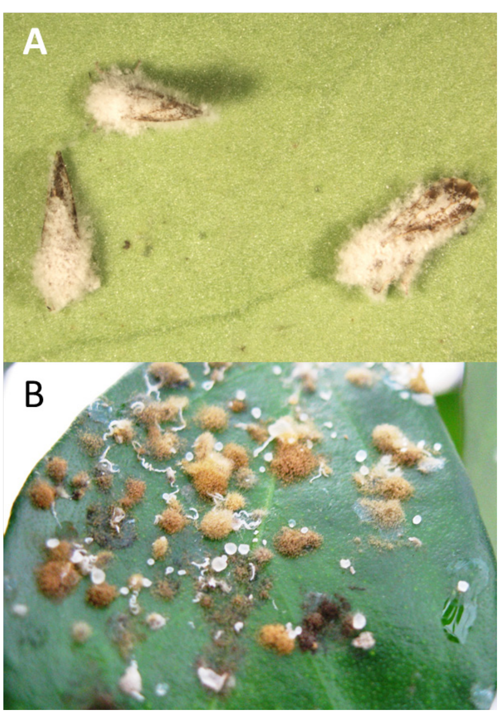

Fig. 2. *Diphorina citri* showing outgrowth of *I. fumosorosea* strain Apopka 97 (**A**) and *Cladosporium* sp. growing on Benton citrange leaves in greenhouse cages (**B**).

Discussion

The Apopka-97 blastospore strain of *I. fumosorosea* was originally isolated from mealybugs at MREC (Osborne and Landa, 1992) and is now produced commercially as 'PFR-97 WDG' registered for control of soft-bodied insects, including aphids, mites and whiteflies on food crops including citrus. Since various petroleum and organic derived oils are used in citrus groves in Florida for control of soft-bodied insects and disease management (Rogers et al., 2012) we chose to include several in this study.

In 2010, PFR-97 reduced psyllid populations by approximately 50% over 3 weeks while oil treatments ranged from 56% to 85% mortality. We hypothesized that combining blastospores with emulsifiable oils might be beneficial. Formulating conidia or blastospores in certain oils has been shown to improve deposition on the insect cuticle, and enhance rain fastness and germination (Inglis et al., 2002). However, in our tests, combining PFR-97 with emulsifiable oils did not increase psyllid mortality compared with either agent alone. The reasons for the lack of synergy are unknown. However one possibility is that certain oils may be toxic or otherwise negatively affect germination of blastospores. Kim et al. (2011) reported differences in germination of *I. fumosorosea* SFP-198 conidia that were maintained in different oils, with corn oil superior in maintain germination rates compared with soybean oil, cottonseed oil, paraffin oil, and methyl oleate. The variability

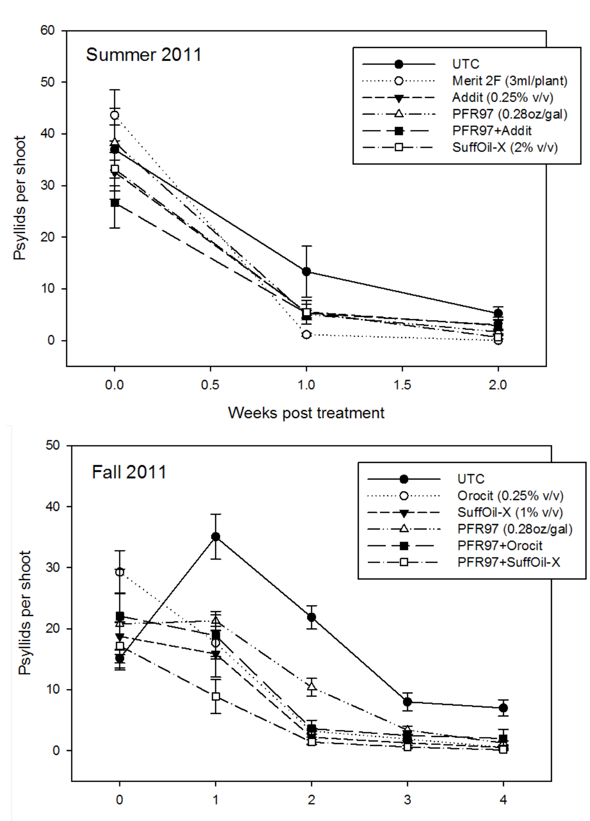

Fig. 3. Number of *Diaphorina citri* (adults and nymphs) recorded on trifoliate orange cv. Benton following different treatments (2011 tests).

of *I. fumosorosea* in different oils needs to be studied further.

In 2011 tests, low survivorship of psyllids and widespread contamination with *Cladosporium* sp. was observed several weeks into the study which made comparisons of treatments problematic. We suspect that psyllids may have been killed by *I. fumosorosea* disseminated inside the greenhouse by wind movement (e.g., cooling fans) or other factors. This is important since it suggests that *I. fumosorosea* can spread under certain conditions. The 2011 tests were conducted during very humid conditions (e.g., in the summer 2011 test, >50% of hourly observations recorded >90% RH) which are known to favor fungal epizootics in psyllids (Avery et al., 2009). The *Cladosporium* growth was thought to be saprophytic (post-mortem) since it did not kill psyllids treated with at 10^7 conidia/mL in laboratory tests (unpublished data). Meyer and Hoy (2008) identified *Cladosporium cladosporioides* and *Penicillium* sp. (amplified through PCR) as natural surface contaminants of *D. citri,* occurring on ventral portion of the abdomen.

There are several factors that can determine how well *Isaria* would work in a commercial grove setting. Important aspects will include ambient air temperatures and relative humidity within the citrus grove, air circulation within the canopy, accurate spray equipment and thorough coverage of the foliage during spray applications. High levels of rainfall and UV-exposure are expected to limit the persistence of the fungus (Ignoffo, 1992). Sprays should be timed early against developing populations and repeated or integrated with other control methods. Another concern is the slower rate of kill (i.e., >4 d in the laboratory (Avery et al., 2011) while *D. citri* can transmit HLB within hours of feeding (Bové 2006). However, Avery et al. (2011) showed that infected psyllids mostly stop feeding within 24 h of infection, suggesting they might not actually transmit disease during this time.

Continued research is needed on effective, environmentally friendly insecticides to help with the fight against *D. citri*. Alternative tools are vital in an integrated crop management program against HLB. Although reduced risk materials such as entomopathogenic fungi and oils may be less effective compared with some broad spectrum insecticides, they need to be weighed against their relative safety to beneficial species and their potential to improve levels of biological control in the groves.

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