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RESEARCH ARTICLE

Evaluation of *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) for control of the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae)

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A laboratory bioassay was developed to evaluate strains of *Isaria fumosorosea* Wize, against *Diaphorina citri*. Up to 100% of adult psyllids were killed at concentrations between 10^6 and 10^7 blastospores/ml after 12 days, with derived LC_{50} values (at 7 days post treatment) between 1.4×10^5 and 2.0×10^6 blastospores/ml for strains ARSEF 3581, FE 9901 and Apopka-97. A significantly higher value (1.5×10^7) was obtained with a conidial formulation of Apopka-97. Average survival times were dosage dependent, i.e. between 10.2 days at 10^3 blastospores/ml and 3.5 days at 10^8 blastospores/ml. Rates of mycosis were also dosage dependent, with up to 100% sporulation on cadavers at 10^8 blastospores/ml but declining at lower concentrations. The Apopka-97 strain (commercially available as PFR-97) was tested against established *D. citri* infestations in potted citrus in greenhouse cages. Treatments at label rates reduced psyllid populations by approximately 50% over 3 weeks. The combination of PFR-97 with emulsifiable oils (0.25% v/v) did not increase psyllid mortality compared with either agent alone. Imidacloprid applied as a drench killed 100% of psyllids within 3 weeks. Subsequent greenhouse tests during humid conditions were hampered by natural dissemination of *I. fumosorosea* to untreated psyllids, suggesting that this fungus is spread by air movement and may be highly effective under very humid conditions. In later tests, a *Cladosporium* sp. rapidly colonised psyllid cadavers and leaf surfaces, but was not pathogenic in laboratory tests. Our studies confirm the potential of *I. fumosorosea* to be used in IPM strategies for *D. citri* that rely on other tactics, such as insecticidal oils and native or introduced biological control agents.

Keywords: blastospore; mycoinsecticide; *Isaria fumosorosea*; *Diaphorina citri*; emulsifiable oil; citrus

1. Introduction

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) has emerged as a major citrus (*Citrus* spp.) pest due to its putative role as vector of the phloem-limited bacterium *Candidatus Liberibacter asiaticus*, which causes citrus huanglongbing (HLB), commonly called citrus greening disease (Bové 2006). Psyllids feed and oviposit on new growth or 'flush' which can also result in distorted, reduced leaf tissue and accumulation of honeydew that promotes the growth of sooty mold

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fungi (Hall and Albrigo 2007). Transmission of HLB is thought to occur through salivary secretions in as little as 30 minutes of feeding (Bové 2006). Native to China, *D. citri* is an exotic pest in North America and has been found in Florida, Texas, Louisiana, Alabama, Georgia, Mississippi, South Carolina, California, Puerto Rico as well as Guam and Hawaii (French, Kahlke, and da Graça 2001; Conant, Hirayama, Kumashiro, Hew, and Young 2009; Hummel and Ferrin 2010; USDA 2011).

Because there is no cure for HLB, controlling psyllid populations with petroleum oil and foliar and broad-spectrum systemic insecticides is currently recommended in Florida and elsewhere (Rogers, Dewdney, and Futch 2011). A variety of chemical pesticides (e.g. imidacloprid, aldicard and chlorpyrifos) have been used against *D. citri* (Qureshi and Stansly 2008, 2010; Sétamou et al. 2010). Although many of these materials are effective, excessive reliance on pesticide programs has resulted in problems such as insecticide resistance (Tiwari, Mann, Rogers, and Stelinski 2011) and toxicity to beneficial species such as exotic parasitoids released in citrus groves for classical biocontrol of *D. citri* (Michaud 2004; Hall and Nguyen 2010). Alternative low risk pesticides would provide resistance management tools and would also help conserve beneficial arthropods (e.g. parasitic wasps, predatory bugs, spiders, ladybeetles) that are highly active during the summer periods in the groves (Qureshi and Stansly 2009).

Entomopathogenic fungi play an important role in the natural regulation of insect populations and are good microbial candidates for control of phloem feeding pests because they penetrate the cuticle directly (Hajek and St. Leger 1994; Goettel, Eilenberg, and Glare 2010). The scientific literature reports several entomopathogenic fungi known to naturally infect or associated with *D. citri*. These include *Isaria fumosorosea* (=*Paecilomyces fumosoroseus*) Wize (Hypocreales: Cordycipitaceae) (Meyer, Hoy, and Boucias 2008), *Cephalosphorium lecanii* Zimm (=*Lecanicillium lecanii*) (Xie, Su, and Lin 1988; Rivero-Aragon and Grillo-Ravelo 2000), *Beauveria bassiana* (Bals.) Vuill. (Rivero-Aragon and Grillo-Ravelo 2000), *Capnodium citri* Berk. and Desm. (Aubert 1987) and *Hirsutella citriformis* Speare (Rivero-Aragon and Grillo-Ravelo 2000; Subandiyah et al. 2000; Meyer, Hoy, and Boucias 2007). There is a previous report of *Cladosporium* sp. nr. *oxysporum* Berk. infecting *D. citri* in Réunion Island (Aubert 1987).

Amongst them, *I. fumosorosea* is a promising candidate for pest management since it is produced commercially and has been recently shown to be pathogenic against *D. citri* and other citrus pests (Avery et al. 2009, 2011; Hoy, Singh, and Rogers 2010; Hunter, Avery, Pick, and Powell 2011). A strain of *I. fumosorosea* was found naturally infecting an adult Asian citrus psyllid collected from the underside of foliage on orange trees in Polk County, Florida (Meyer et al. 2008), so this pathogen may be considered adapted to conditions found inside citrus groves. In addition, *I. fumosorosea* is expected to have a low impact on certain beneficial species (Sterk, Bolckmans, De Jonghe, De Wael, and Vermeulen 1995a; Sterk, Bolckmans, van de Veire, Sels, and Stepman 1995b; Avery, Faull, and Simmonds 2008). This study compared the pathogenicity and virulence of several strains *I. fumosorosea* against *D. citri* in the laboratory. We also assessed psyllid mortality on citrus plants treated with *I. fumosorosea* with and without emulsifiable oil adjuvants under greenhouse conditions. The goal was to investigate whether *I. fumosorosea* might have application under commercial conditions.

2. Materials and methods

2.1. Insects and fungi

Psyllids originated from a colony maintained at the USDA-ARS in Fort Pierce, FL, and were maintained on grapefruit cv. Marsh at MREC. Three strains or formulations of *I. fumosorosea* were assayed: 'PFR-97' (strain Apopka-97) supplied by Mike Dimock of Certis USA (Columbia, MD, USA), ARSEF 3581 supplied by Mark Jackson (USDA-ARS) and FE 9901, produced as 'No-Fly' in Europe, provided by Steve Parker (Natural Industries, Inc., Houston TX). All strains were provided as blastospore formulations sprayed onto bran (Apopka-97) or diatomaceous earth (3581 and FE 9901) and air-dried and vacuum sealed in bags containing between 10^9 and 5×10^9 colony forming units (CFU)/g (ARSEF 3581 and FE 9901). ARSEF 3581 and FE 9901 were originally isolated from whiteflies (*Bemesia* spp.) and Apoka-97 from a mealybug, *Phenacoccus solani*. An experimental conidial formulation of the Apopka-97 strain was included. All products were refrigerated at 4°C until use. Viability tests were performed on each strain by streaking 0.5 ml of a suspension containing 10^5 spores per ml on Sabouraud Dextrose Agar (6.5% w/v, SDA; Becton Dickinson, Sparks MD) and counting germinated spores that produced a clear germ tube after 16 hours at 25°C.

2.2. Laboratory bioassay

A simple and reliable bioassay was developed to screen fungal isolates. Fungal suspensions were prepared in sterile water containing 0.025% v/v Tween and adjusted to different concentrations (between 10^3 and 10^8 CFU/ml) through serial dilutions. Concentrations were adjusted though an improved Neubauer hemocytometer at $\times 100$ magnification. Leaf discs (2 cm-diam) removed from tender leaves of grapefruit cv. Marsh were sprayed with each concentration using a Potter Precision Spray Tower (Burkard Sci, Middx, UK) that allowed a controlled dosage of fungal spores to be applied to leaf discs. Controls were treated with sterile water and Tween only. Each sprayed leaf disc was air-dried and placed in a 1 oz plastic bioassay cup that contained 5 ml of water agar to maintain high humidity during the test. Five adult *D. citri* (< 1 week old) were placed inside cups with a tightly fitting lid used to prevent escape. The cups were maintained in an incubator at 26 ± 1°C, 80% relative humidity and 16 hL: 8 hD photophase. Psyllid mortality and signs of fungal infection (mycosis, hyphal growth and sporulation) were recorded daily for 12 days. There were six replicate leaf discs (30 psyllids) per fungal strain/concentration. Each strain was tested three times.

2.3. Greenhouse studies

We evaluated psyllid mortality on citrus plants that were sprayed with aqueous suspensions of *I. fumosorosea* PFR-97. An additional aim of the greenhouse study was to investigate the impact of various spray adjuvants along with fungal suspensions. PFR-97 was selected because it is commercially available. An insecticidal standard was also included in some of these tests for comparison. Before tests, psyllid infestations were established on potted citrus plants by releasing 20 adults per plant placed inside greenhouse cages fitted with nylon mesh cover. Two

weeks prior to infestation, plants were pruned and fertilised with a slow release citrus fertiliser (12-5-8 Vigoro®, The Scotts Company LLC, Marysville, OH) to encourage new 'flush' for oviposition. The insects were left for 10 days before treatment by which time 1st and 2nd instars (F1 progeny) had emerged.

In the first test (fall 2010), five foliar treatments were tested using orange jasmine, *Murraya paniculata* L. plants (60 cm tall); PFR-97 20% WDG applied at 2.1 g/L (based on label rates), PFR-97 plus an emulsifiable vegetable oil 'Addit'(Koppert Biological Systems, The Netherlands) at 0.25% v/v, Addit alone at the same rate, a highly refined paraffinic-based oil 'SuffOil-X' (BioWorks Inc., Victor, NY) at a high label rate (2% v/v). All treatments included Tween at 0.025% v/v. Control plants were treated with water and Tween only. Foliage was sprayed to 'run off' using a 3.8 litre hand held (FloMaster) sprayer (sprayer output was 3.88 ml/sec). All foliar treatments were reapplied to the same plants after 7 days. Plants were isolated for spraying to prevent drift. Imidacloprid (Merit 2F, Bayer Crop Science, Research Triangle Park, NC) was included as a drench within label rates (3 ml per plant diluted in 75 ml water). There were 6 replicates per treatment arranged in a randomised design inside a greenhouse bay. To increase relative humidity (r.h.) to favour infection, treatments were applied shortly before dusk and an overhead low volume misting system was used to maintain $>95\%$ r.h. in the greenhouse for at least 8 hours post spray. The germination rate was 82% on media after 16 hours at 25°C. Plants were inspected weekly over 3 weeks, starting immediately before treatments were applied. The numbers of infested plant terminals (flush) were counted along with the number of live adults and nymphs from three terminals per plant. Plants were destructively sampled at the end of the observation period. Conditions measured inside cages using a data logger (HOBO U12, Onset Computer Corp., Pocasset, MA) ranged from 16.1 to 41.5°C (average 25.2°C) and 15–100% relative humidity (average 77.7%).

The greenhouse experiment was repeated in summer and fall 2011, using trifoliate orange *Citrus trifoliata* L cv. Benton with 5 replicates per treatment. As additional modifications, in the final study a citrus-oil (Orocit at 0.25% v/v) was included while SuffOil-X was tested at the lower label rate (1% v/v). Imidacloprid was not included in the final test since its high efficacy was clearly shown in the previous two greenhouse tests. The germination rate of PFR-97 used in these tests was 66–77% after 16 hours on artificial media. Since significant contamination from *Cladosporium* sp. was observed in 2011, several psyllids showing signs of sporulation were collected transferred to SDA media under sterile conditions and fungal outgrowths harvested after 2 weeks and applied to new adult psyllids using a Potter spray tower at concentrations of 10^5, 10^6 and 10^7 conidia/ml along with controls in the laboratory (5 replicate cups per treatment). Conditions inside the greenhouse cages ranged from 22.5 to 39.7°C (average 28.8°C) and 42–100% relative humidity (average 83.0%) (Summer test) and 18.5–40.9°C (average 25.0°C) and 15–100% relative humidity (average 69.1%) (Fall test).

2.4. Data analysis

In laboratory bioassays, psyllid mortality and mycosis data were compared for the different treatments. Probit regression analysis was used for determination of the LC_{50} and fiducial limits while survival estimates as a measure of virulence were compared using Kaplan Meier analysis (SPSS for Windows v.19). In greenhouse

tests, the number of infested terminals and live psyllids were compared using repeated measures analysis of variance (ANOVA) following log $(n+1)$ transformation. Where appropriate, means were further compared with Fishers protected LSD tests at $P<0.05$.

3. Results

3.1. Laboratory bioassays

Adult psyllid mortality rates varied over the 12 day observation period (Figure 1). Survival was significantly reduced following exposure to all strains of *I. fumosorosea* with a clear concentration-response in all cases. Overall, 100% psyllid mortality occurred within 12 days at the higher concentrations, while generally $>80\%$ of psyllids died at the lower concentrations for all strains/formulations. Average survival times calculated from the data (censored at day 12 when the study was terminated) showed differences between the strains; notably, the fastest mortality was observed for the ARSEF 3581, followed by FE9901, the Apopka-97 blastospore and then the conidial formulation (Table 1). Probit analysis conducted on the fungus concentration-response data showed that the lethal dose required to kill 50% of the psyllids after 7 days was in the range of $1–2\times10^5$ blastospores/ml for ARSEF 3581 and FE9901 and 2×10^6 blastospores/ml for Apopka-97 (Table 2). A higher value again (based on non-overlapping confidence intervals) was obtained for the conidial formulation of Apopka-97. A 7-day exposure period was selected because control mortality was still relatively low at this time ($<20\%$) and hence results would reflect the effect of the fungal exposure.

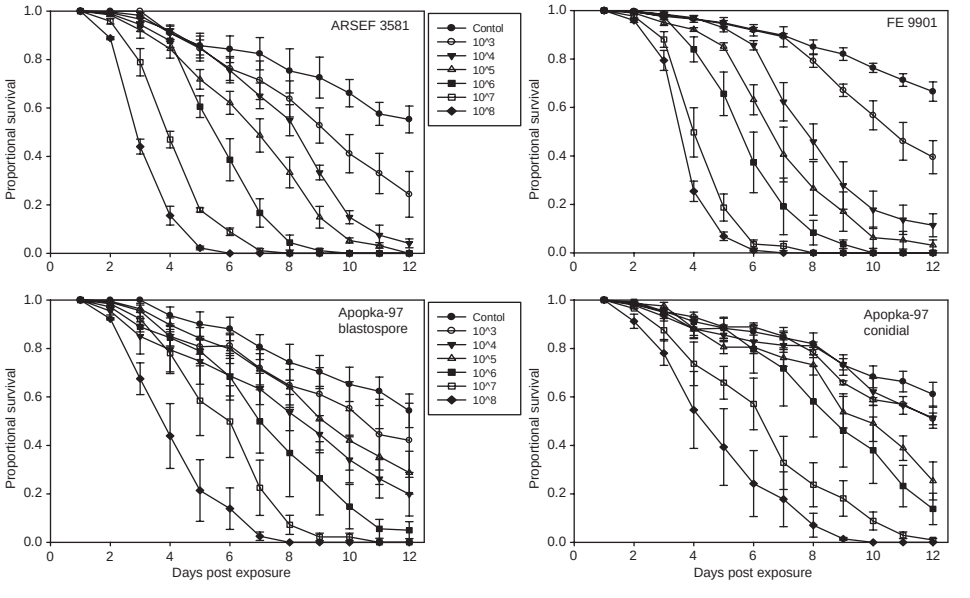

Figure 1. Proportional survival of adult *Diaphorina citri* following exposure to citrus leaves treated with different formulations of *Isaria fumosorosea* at concentrations between 10^3 and 10^8 spores per ml. Data are mean $\pm$ SEM of 3 tests (30 psyllids per test).

Table 1. Average survival times (days) of adult *Diaphorina citri* exposed to 4 strains/formulations of *Isaria fumosorosea* at various concentrations.

	Concentration (spores/ml)						
Strain	0	10^3	10^4	10^5	10^6	10^7	10^8
ARSEF 3581	10.1b	9.1a	8.2a	7.1b	6.0c	4.5c	3.5d
Apopka-97	10.2b	9.4a	8.3a	9.1a	7.5b	6.1b	4.4b
FE 9901	10.9a	10.2a	8.4a	7.3b	6.1c	4.6c	4.1c
Apopka-97 conidial	10.3ab	10.1a	10.0a	9.4a	8.9a	6.7a	5.1a

Note: Data are mean ± SEM of 3 tests (30 psyllids per test). Estimates determined by Kaplan Meier Survival analysis, with data for survivors censored at day 12. Different letters in columns indicate significant differences ($P < 0.05$) between strains according to a log-rank (Mantel-Cox) test.

The proportion of psyllids expressing symptomatic mycosis varied according to the strain and concentration applied to the leaf discs (Figure 2). In general, up to 100% of psyllids sporulated at concentration of 10^8 spores/ml but declined at lower concentrations. This decline was most apparent in the conidial formulation of Apopka-97 strain where < 30% psyllids became symptomatic at a concentration of 10^5 conidia/ml or less. However, the viability of spores grown on SDA after 16 hours at 26°C was also reduced in the conidial formulation (25%), compared with 99% for ARSEF 3581, 79% for Apopka-97 blastospore formulation and 85% for FE9901. Symptoms of inoculated psyllids included twitching of legs and antennae 1–2 days before death. Immediately following death, infected psyllids had fungal hyphae emerging from the tarsi and intersegmental regions of the legs. Within 24–48 hours post-mortem, significant mycelial growth developed on the dead insect, followed by development of phailides and conidiogenesis.

3.2. Greenhouse tests

In the fall 2010 test, large numbers (≥ 17.2 per terminal) of *D. citri* nymphs were present when treatments were first applied, with 2nd generation (F1) adults present by week 2 (Table 3). Overall, treatments had a significant effect on the number of infested terminals ($F_{5,\ 30} = 18.0$, $P < 0.001$) as well as 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) in repeated measure ANOVA. All treatments reduced the number of F1 adults compared with the untreated control (UTC), by weeks 2 and 3 post treatment. Merit 2F was most effective followed by SuffOil-X and PFR-97, Addit and their

Table 2. Estimates of the median lethal concentration (spores/ml) of three entomopathogenic fungi applied against adult *Diaphorina citri* in leaf disc assays at 7 days post-inoculation.

Strain	LC_{50}	95% CL	Slope ± SEM	χ^2
ARSEF 3581	1.37×10^5	$5.1 \times 10^4 - 3.2 \times 10^5$	0.56 ± 0.15	0.21
Apopka-97	2.03×10^6	$7.4 \times 10^5 - 4.8 \times 10^6$	0.54 ± 0.12	1.18
FE 9901	1.36×10^5	$5.4 \times 10^4 - 3.0 \times 10^5$	0.32 ± 0.08	1.25
Apopka-97 conidial	1.47×10^7	$5.6 \times 10^6 - 3.4 \times 10^7$	0.32 ± 0.06	2.35

Note: Data based on 3 tests (30 psyllids per test); all probit estimates were adjusted for control mortality.

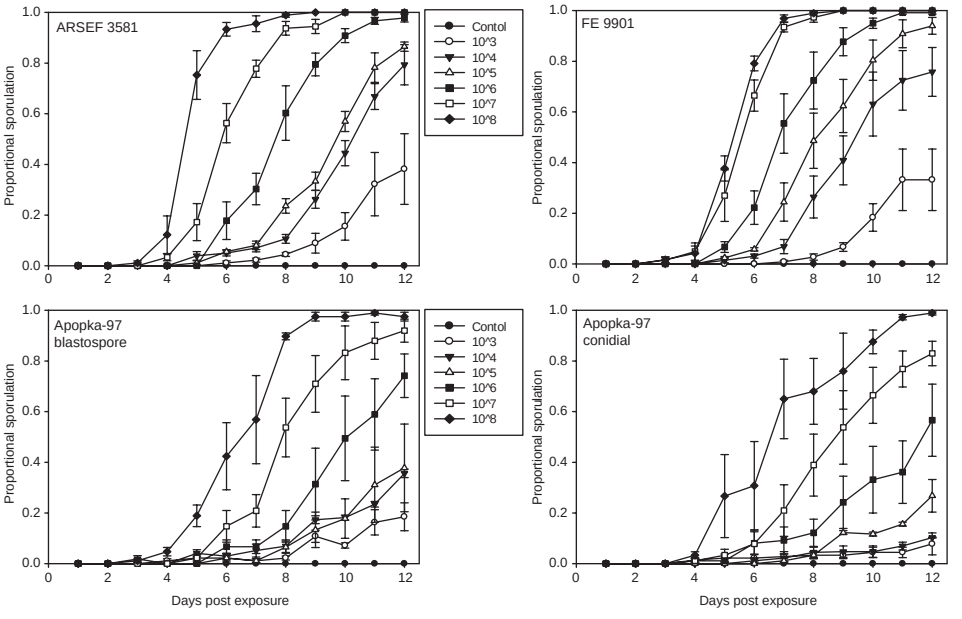

Figure 2. Proportion of mycosis (sporulation) of adult *Diaphorina citri* following exposure to citrus leaves treated with different formulations of *Isaria fumosorosea* at concentrations between 10^3 and 10^8 spores per ml. Data are mean $\pm$ SEM of 3 tests (30 psyllids per test).

combination (Table 3). The combination of PFR-97 and the emulsifiable oil did not increase psyllid mortality compared with either agent alone. Only Merit 2F and SuffOil-X significantly reduced the total number of infested terminals (on enclosed plants) from week 1 to week 3. In the destructive count, Merit 2F was the most effective with 99.9% reduction of psyllids with respect to UTC, followed by SuffOil-X (85.6% reduction), Addit (56% reduction), PFR-97 (52.2% reduction), PFR-97 + Addit (49.8% reduction) (Table 4). Only 20% of psyllids cadavers in fungus treatments produced outgrowth consistent with *I. fumosorosea* symptoms.

In the 2011 tests, problems with high control mortality were observed towards the end of experiments. In the summer test, while all treatments reduced the number of *D. citri* nymphs by week 1 with Merit 2F being the most effective, relatively few F1 adults were subsequently observed in control cages by week 2 (Table 5). Overall, treatments containing oils alone or in combination with PFR were most effective in the earlier part of the fall 2011 test. We also observed widespread contamination of *Cladosporium* sp. in all treatments, which compromised the ability to determine treatment effects when the study was terminated. A similar observation occurred in the fall 2011 test, where foliar treatments significantly reduced live nymph counts on week 1 and 2, but numbers of F1 adults again remained low and widespread fungal contamination was observed in all cages (Table 6). In the Koch's postulate test, no difference in psyllid mortality was observed in cups after 11 days between groups treated with different concentrations of *Cladosporium* sp. or controls ($F_{3,16} = 0.74$, $P = 0.55$) and no mycosis was observed, suggesting a lack of pathogenicity of this fungus.

Table 3. Number of infested terminals and *Diaphorina citri* recorded on orange jasmine plants following different treatments (fall 2010 test).

		Infested terminals/plant				Adults/terminal shoot				Nymphs/terminal shoot			
Treatment	Rate	D0	D7	D14	D21	D0	D7	D14	D21	D0	D7	D14	D21
UTC		7.5a	10.5a	11.8a	16.8a	1.0a	0.9a	16.1a	16.2a	24.1a	30.3a	15.0a	1.8a
Merit 2F	3 ml/plant	10.7a	6.2c	0.5c	0.2c	0.6bc	0.0a	0.0c	0.1d	17.2a	4.6c	0.2c	0.0b
Addit	0.25% v/v	12.0a	10.3a	10.7ab	14.0a	0.4c	0.7a	3.4b	5.5b	27.8a	16.2b	8.9ab	1.3a
PFR-97	2.1 g/L	10.2a	10.3a	10.2ab	15.0a	0.7abc	0.2a	5.4b	5.0b	26.7a	19.3ab	7.9ab	1.9a
PFR + Addit	2.1 g/L + 0.25% v/v	9.0a	9.3ab	8.5ab	11.5ab	0.8ab	0.2a	3.4b	4.7b	24.8a	21.4ab	7.8b	1.1a
SuffOil-X	2% v/v	10.7a	6.8bc	7.0b	7.2b	0.6bc	0.2a	2.6bc	2.3c	30.6a	8.6c	4.4b	0.7ab

Note: Different letters in columns indicate differences ($P < 0.05$, Fisher's protected LSD). Data transformed due to unequal variances [log10 (x + 1)]; non-transformed means presented.

Table 4. Number of *Diaphorina citri* on orange jasmine plants as determined by a final destructive count (fall 2010 test).

Treatment	Rate	Adults/plant	Nymphs/plant	Total/plant
UTC		292.7a	11.3a	304.0a
Merit 2F	3 ml/plant	0.2d	0.0c	0.2d
Addit	0.25% v/v	130.3b	3.5ab	133.8b
PFR-97	2.1 g/L	137.0b	8.3a	145.3b
PFR + Addit	2.1 g/L + 0.25% v/v	144.0b	8.5a	152.5b
SuffOil-X	2% v/v	41.5c	2.2bc	43.7c

Note: Different letters in columns indicate differences ($P < 0.05$, Fisher's protected LSD). Data transformed due to unequal variances (log10 (x + 1)); non-transformed means presented.

4. Discussion

Isaria fumosorosea, known as *P. fumosoroseus* for more than 30 years prior to taxonomic revision, has a worldwide distribution and has been isolated from many insects, particularly Lepidoptera, as well as air, water, plants, other fungi and often from soil (Zimmermann 2008). *Isaria fumosorosea* is regarded as a species complex with at least three monophyletic groups (Zimmermann 2008). The *I. fumosorosea* species complex have gained attention in recent years due to their effectiveness against *Bemisia* species of whiteflies and several commercial strains are sold, particularly in Europe, for biocontrol of whiteflies and other soft-bodied greenhouse pests (Faria and Wraight 2007). In Europe and North America, PFR-97 is registered for greenhouse and nursery use against thrips and other soft-bodied pests, including aphids, mites and whiteflies and was approved by Environmental Protection Agency (EPA) in 2011 for use on food crops in the US. No-Fly is used in Europe with US registration status pending.

Our studies highlight the potential of these *I. fumosorosea* strains for management of *D. citri* in citrus. However, we note that equivalent rates of PFR-97 that killed 100% of adult *D. citri* in laboratory tests only reduced mixed psyllid populations by about 50% in the first greenhouse test. By contrast, imidacloprid applied as a drench killed 100% of psyllids within 3 weeks. Differences in laboratory

Table 5. Number of infested terminals and *Diaphorina citri* recorded on trifoliate orange cv. Benton plants following different treatments (summer 2011 test).

		Infested terminals/plant			Adults/terminal shoot			Nymphs/terminal shoot		
Treatment	Rate	D0	D7	D14	D0	D7	D14	D0	D7	D14
UTC		8.3a	9.5a	7.7ab	0.6a	0.7a	2.4a	36.4a	12.7a	2.8a
Merit2F	3 ml/plant	9.7a	5.6a	1.0c	0.5a	0.0a	0.0d	43.1a	1.1c	0.0c
Addit	0.25% v/v	8.5a	8.2a	9.7a	0.4a	0.7a	0.8bcd	32.1a	4.8b	2.1ab
PFR-97	2.1 g/L	9.0a	7.2a	7.7ab	0.3a	0.8a	1.1abc	38.0a	4.2b	0.6bc
PFR + Addit	2.1 g/L + 0.25% v/v	7.3a	5.2a	6.2b	0.5a	0.7a	1.4ab	26.2a	4.6b	1.6ab
SuffOil-X	2% v/v	8.3a	6.9a	6.8ab	0.3a	0.7a	0.3cd	32.9a	4.8b	0.3bc

Note: Different letters in columns indicate differences ($P < 0.05$, Fisher's protected LSD). Data transformed due to unequal variances [log10 (x + 1)]; non-transformed means presented.

Table 6. Number of infested terminals and *Diaphorina citri* recorded on trifoliate orange cv. Benton plants following different treatments (fall 2011 test).

		Infested terminals/plant				Adults/terminal shoot				Nymphs/terminal shoot			
Treatment	Rate	D0	D7	D14	D21	D0	D7	D14	D21	D0	D7	D14	D21
UTC		7.8a	11.2a	10.4a	9.4a	1.0a	0.9ab	1.2a	1.5ab	14.1a	34.1a	20.7a	6.5a
Orocit	0.25% v/v	7.6a	6.2bc	3.8b	4.0c	0.5a	0.2bc	0.0b	0.2c	28.7a	17.5b	3.2c	1.7b
SuffOil-X	1% v/v	7.0a	5.4c	4.4b	4.8bc	0.5a	0.2bc	0.1b	0.3c	18.3a	15.7b	2.1c	0.9bc
PFR-97	2.1 g/L	8.4a	9.0ab	7.6a	6.6ab	0.7a	1.9a	1.0a	1.7a	20.1a	19.3b	9.4b	1.7b
PFR + Orocit	2.1 g/L + 0.25% v/v	7.6a	7.6abc	4.6b	4.6bc	0.8a	0.0c	0.1b	0.7bc	21.3a	18.9b	3.5c	1.7b
PFR + SuffOil-X	2.1 g/L + 1% v/v	7.8a	6.2bc	3.4b	4.2bc	0.5a	0.0c	0.2b	0.3c	16.7a	8.9c	1.2c	0.3c

Note: Different letters in columns indicate differences ($P < 0.05$, Fisher's protected LSD). Data transformed due to unequal variances [log10 (x + 1)]; non-transformed means presented.

and field results may be related to the more variable environmental conditions in the greenhouse, although it would also be informative to compare the efficacy of *I. fumosorosea* formulations against different life stages of *D. citri*.

In follow up greenhouse tests conducted in 2011, we had difficulty assessing foliar treatments due to low psyllid numbers (i.e. high mortality) in all cages (including controls) in the F1 generation. We hypothesise that, in this case, *I. fumosorosea* might have been disseminated by wind movement (i.e. greenhouse cooling fans) within the greenhouse bay. Although we noted that psyllid cadavers in non-fungus cages did not exhibit sporulation symptomatic of *I. fumosorosea*, the same was also observed fungus-treated cages, where $< 1\%$ of psyllid cadavers were symptomatic. In 2011 tests, an unknown *Cladosporium* sp. rapidly colonised $> 90\%$ psyllid cadavers and honeydew deposits on leaf surfaces. The role of *Cladosporium* in psyllid mortality is unclear; however, we were unable to confirm its direct pathogenicity in laboratory tests at rates up to 10^7conidia/ml, suggesting its growth was saprophytic. The impact of dual infection of *I. fumosorosea* and other fungi has not been studied; however, Avery, Faull, and Simmonds (2004) also noted *Cladosporium* sp. development on whitefly nymphs infected with Trinidadian strains of *I. fumosorosea.* Interestingly, 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. We note that 2011 tests were conducted during warm and very humid conditions (e.g. in the summer 2011 test, $> 50\%$ of hourly observations recorded $> 90\%$ r.h.). We suspect that an initial epizootic of *I. fumosorosea* in the greenhouse which may have infected psyllids, had been outcompeted post-mortem by the *Cladosporium* sp. The inoculum source may have also arisen 'naturally' since the Apopka-97 strain was originally isolated under similar conditions on mealybugs in a greenhouse at MREC (Osborne and Landa 1992). This latter explanation would help explain results of the fall 2011 test, where non-fungal treatments were confined to a adjacent greenhouse bay (maintained under equivalent environmental conditions) in an attempt to reduce contamination.

There are several practical considerations to the use of *I. fumosorosea* for psyllid management. Firstly, like all entomopathogenic fungi, a rapid decline in residual activity may be expected under field conditions, especially in new exposed foliage where psyllids primarily feed (Hajek and St. Leger 1994). Hence, timing populations early and using repeated applications may be necessary to maintain effective control. As a caveat, there is the potential for the fungus to induce epizootics during favourable conditions through recycling (Avery, Queeley, Faull, and Simmonds 2010) as well as spread naturally to surrounding areas, for example, by being vectored by psyllids or else spread via wind (Avery et al. 2009). Another concern is the slower rate of kill (i.e. > 3 days in our laboratory tests) while *D. citri* can transmit HLB within hours of feeding (Bové 2006). However, Avery et al. (2011) showed that adult *D. citri* treated with PFR-97 exhibited a rapid (within 24 h) decline in feeding rates, which remained until death. The role of sub-lethal effects on feeding and the risks of disease transmission by *D. citri* require further study. Lacey et al. (2011) observed a reduction in the incidence of *Candidatus* Liberibacter sp. in field-grown potatoes in Texas, when the potato psyllid vector, *Bactericera cockerelli* Šulc, was treated with PFR-97. However, adult *B. cockerelli* treated with *I. fumosorosea* in the laboratory at 10^7 spores/ml died more quickly, that is within 2–3 days (Lacey, de la Rosa, and Horton 2009) compared with our equivalent estimates for *D. citri* (Table 1).

There may also be additional indirect effects with using a selective microbial pesticide that is compatible with beneficial arthropods. Hoy et al. (2010) noted high rates of *D. citri* predation (e.g. by coccinellids) in citrus blocks treated with a Florida isolate of *I. fumosorosea* (*Ifr* AsCP), which hampered the evaluation of the impact of the fungus under field conditions. Naturally occurring predators and parasitoids can play a vital role in regulating populations of *D. citri*, and it has been argued that their elimination through intensive pesticide use can lead to increased pest pressure and enhance the spread of HLB disease (Michaud 2004; Qureshi, Rogers, Hall, and Stansly 2009; Qureshi and Stansly 2009).

Using a blastospore formulation has certain benefits because they can be produced in submerged culture and germinate rapidly compared to conidia, although they may have reduced environmental persistence compared with conidial formulations (Vidal, Fargues, Lacey, and Jackson 1998; Jackson, Cliquet, and Iten 2003). We observed lower rates of mortality in the conidial formulation used in our tests, although we also noted a reduced germination rate in this sample. Avery et al. (2011) also reported delays in the mortality and onset of sub-lethal feeding reductions (4 days versus 1) in *D. citri* treated with conidial versus a blastospore formulation of *I. fumosorosea*. Formulating conidia or blastospores in certain oils has been shown to improve deposition on the insect cuticle, and enhance rain fastness and germination (Inglis, Jaronski, and Wraight 2002). However, in our tests, combining PFR-97 with emulsifiable oils did not increase psyllid mortality compared with either agent alone. The compatibility of *I. fumosorosea* with various pesticides, such as petroleum and organic derived oils widely used in citrus production (Rogers et al. 2011), requires consideration. Er and Gökce (2004) demonstrated that several common pesticides (especially fungicides) used on tomatoes could interfere with the development of *I. fumosorosea* in vitro.

In conclusion, our studies confirm the potential of *I. fumosorosea* to help manage *D. citri*, although in the short term control may be less effective compared with chemical insecticides. Our studies also suggest that environmental conditions (e.g. high ambient humidity) may be important to the impact and that, under certain conditions, may lead to secondary epizootics. Further field studies are required to evaluate and optimise the biological impact of *I. fumosorosea* formulations applied under different environmental conditions and cropping systems.

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