EFFECT OF *ISARIA FUMOSOROSEA* (HYPOCREALES: CORDYCIPITACEAE) AND *LYSIPHLEBUS TESTACEIPES,* (HYMENOPTERA: BRACONIDAE) ON THE BROWN CITRUS APHID: PRELIMINARY ASSESSMENT OF A COMPATIBILITY STUDY

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Recently, a strain of *Isaria fumosorosea* Wize (*Ifr*) (= *Paecilomyces fumosoroseus* [Luangsa-ard et al. 2005]), (Hypocreales: Cordycipitaceae) was discovered in a Florida citrus grove infecting the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Meyer et al. 2008). Also, laboratory studies have demonstrated that *Ifr* may be effective against several commercially important pests of citrus (Poprawski et al. 1999; Avery et al. 2009; Hoy et al. 2010; Hunter et al. 2011). Therefore, the use of *Ifr* (PFR 97™ 20% WDG, Certis USA, Columbia, Maryland), approved in 2011 by EPA for use in commercial citrus, may offer a potential tool for balancing citrus pest management with consumer demands for organic citrus produce.

The brown citrus aphid (BCA), *Toxoptera citricidus* Kirkaldy (=*T. citricida* [Nieto Nafría et al. 2005]), (Hemiptera: Aphididae) was discovered in Florida in 1995 and is recognized as a serious pest of citrus capable of vectoring *citrus triseza* virus (Halbert & Brown 1996; Michaud 1998; Roy & Brlansky 2009). Under optimal conditions, native natural enemies provide good management of BCA, although these are easily disrupted by insecticide applications (Michaud 1998; 2002). Presently, the aphid's population is managed in part by the parasitoid *Lysiphlebus testaceipes* Cresson (Hymenoptera: Braconidae) (Evans & Strange 1997). This generalist aphid parasitoid *Lysiphlebus testaceipes*, native to Florida and the Americas, is an effective biocontrol agent of BCA (Evans & Strange 1997). The objectives of our study were to assess the impact of *Ifr* on the mortality of BCA populations as well as the parasitism and emergence rates of *L. testaceipes*.

BCA and Carrizo citrus (a trifoliate hybrid: Washington navel x trifoliate orange) were obtained from the Sub-Tropical Insect Research Unit, USDA Horticulture Research Laboratory in Fort Pierce, Florida. For the experiment, 5 carrizo citrus seedlings (25-30 cm tall) were placed in each of four separate nylon mesh-covered cages (35 × 35 × 35 cm) and each infested with 4 alate BCA (20 per cage). A second infestation was made after 9 days. Cages were maintained in a growth room at 24.0 ± 1 °C, 45.0 ± 1 % RH and 16:8 h (L:D) photoperiod. Two weeks later, 10 *L. testaceipes* adults were added to two of the cages. After an additional 4 days, one cage with and without *L. testaceipes* was treated with *Isaria fumosorosea* (*Ifr*) suspension (PFR 97™ 20% WDG) at 10^7 CFU/ml using Nalgene® (Nalge Nunc International, Rochester, NY) aerosol sprayer. The germination of PFR 97™ blastospores was 96% using the technique of Avery et al. 2009. The remaining 2 cages were sprayed with an equal volume of water. Parasitoids were temporarily removed from all cages prior to spraying to eliminate potential drowning and were replaced with an additional 5 to compensate for handling stress. Two replicates of the same study were conducted on different dates.

For the assessments, the number of live aphids on plants, and mummies, were recorded weekly over 6 weeks. Overall, compared with controls, fewer aphids were recorded in *L. testaceipes* but not *Ifr* treatments in the final 2 weeks of the study (Table 1). There was no apparent difference in aphid mortality between treatments *L. testaceipes* only and *L. testaceipes* plus *Ifr*. The number of mummies (both unemerged and emerged) was similar between treatments *L. testaceipes* only and *L. testaceipes* plus *Ifr*, suggesting that the parasitoid was not disrupted by the fungal treatment (Table 2).

The augmentation of entomopathogenic fungi with current IPM management strategies for *T. citricidus* and other insect pests, may offer conventional and organic citrus growers an alternative to chemical insecticides alone. Although *Ifr* caused

Table 1. Recovery of live *Toxoptera citricidus* (BCA) on individual Carrizo citrus treated with and without a parasitoid (*Lysiphlebus testaceipes*) and an entomopathogenic fungus (*Isaria fumosorosea*) weeks post-application[a].

Treatment[b]	Week 1[c]	Week 2	Week 3	Week 4	Week 5	Week 6
Control	22.3 a	21.2 a	40.3 a	55.4 a	60.8 a	179.8 a
L. testaceipes	21.4 a	20.5 a	28.4 a	31.0 a	6.3 b	2.8 b
Ifr	9.9 a	8.7 a	22.2 a	35.4 a	58.0 a	167.5 a
L. testaceipes + *Ifr*	15.2 a	13.3 a	18.5 a	16.9 a	1.8 b	0.3 b

[a]Data are the average per plant from 2 tests (5 plants per replicate).
[b]Treatments: C = control (water only); *L. testaceipes* = parasitoid exposed to BCA populations; *Ifr* = *I. fumosorosea* blastospores exposed to BCA populations; *L. testaceipes* + *Ifr* = parasitoid exposed to BCA populations and sprayed 4 days post-release with *I. fumosorosea* blastospores.
[c]Values with the different letters in a column represent significant differences between treatments ($P < 0.05$, Fishers LSD).

Table 2. Number of *Toxoptera citricidus* aphid mummies (emerged and unemerged) recovered from individual carrizo citrus treated with and without the entomopathogenic fungus *Isaria fumosorosea* (*Ifr*) weeks post-application[a].

Aphid mummies/treatment[b]	Week 1[c]	Week 2	Week 3	Week 4	Week 5	Week 6
Unemerged						
L. testaceipes	0 a	0 a	8.3 a	13.3 a	32.0 a	18.7 a
L. testaceipes + *Ifr*	0 a	0 a	14.0 a	18.0 a	26.2 a	17.5 a
Emerged						
L. testaceipes	0 a	0 a	0.1 a	3.5 a	11.0 a	15.5 a
L. testaceipes + *Ifr*	0 a	0 a	0.4 a	4.6 a	9.6 a	13.4 a

[a]Data are the average per plant from 2 tests (5 plants per replicate).
[b]Treatments: *L. testaceipes* = *Lysiphlebus testaceipes* parasitoid exposed to BCA populations; *Lysiphlebus testaceipes* + *Ifr* = *Lysiphlebus testaceipes* parasitoid exposed to BCA populations and sprayed 4 days post-release with *I. fumosorosea* blastospores.
[c]Values with the same letters in a column for each category of aphid mummies (unemerged and emerged) represent no significant differences between treatments ($P > 0.05$, Student's t-test).

some aphid mortality (based on symptoms of mycosis observed in aphids that dropped from the plant), its impact was low based on similar numbers of live aphids remaining on the plant compared with controls and lack of mycosis symptoms observed in aphids that remained on the plants. However, during the first 2 weeks post-application, it appears that as infected aphids fell off the plant, the *Ifr* inoculum may have been removed with them, potentially slowing down possible epizootics. Data also suggests that *L. testaceipes,* and *Ifr* are compatible, since *Ifr* treatments did not negatively impact parasitism or emergence. Compatibility of *Ifr* with *L. testaceipes* may be useful for managing aphid pests in citrus and other crops, especially under conditions that may promote higher rates of pest infection. Results of other studies involving pest-parasitoid-fungus interactions conducted with *Ifr* or other species of entomopathogenic fungi concur with our findings; that in an IPM program, a parasitoid and a fungus can be compatible when utilized together (Fransen & van Lenteren 1993, 1994; Sterk et al. 1995a, b; Mesquita et al. 1997; Askary & Brodeur 1999; de la Rosa et al. 2000; Mesquita & Lacey 2001; Jaronski et al. 2003; Kim et al. 2005; Avery et al. 2008).

Summary

Treatments with *Ifr* alone were not effective for managing BCA populations in these cage trials. However, *Ifr* did not inhibit parasitism or emergence, and *L. testaceipes* was highly effective at parasitizing the aphids even in the presence of *Ifr*. The compatibility of *Ifr* with *L. testaceipes* demonstrated potential for managing brown citrus aphid pests in citrus.

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References Cited

Askary, H., and Brodeur, J. 1999. Susceptibility of larval stages of the aphid parasitoid *Aphidius nigripes* to the entomopathogenic fungus *Verticillium lecanii*. J. Invertebr. Pathol. 73:129-132.

Avery, P. B., Faull, J., and M. S. J. Simmonds. 2008. Effects of *Paecilomyces fumosoroseus* and *Encarsia formosa* on the control of the greenhouse whitefly: preliminary assessment of a compatibility study. BioControl 53: 303-316.

Avery, P. B., Hunter, W. B., Hall, D. G., Jackson, M. A., Powell, C. A., and Rogers, M. A. 2009. *Diaphorina citri* (Hemiptera: Psyllidae) infection and dissemination of the entomopathogenic fungus *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) under laboratory conditions. Florida Entomol. 92: 608-618.

De La Rosa, W., Segura, H. R., Barrera, J. F., and Williams, T. 2000. Laboratory evaluation of the impact of entomopathogenic fungi on *Prorops nasuta* (Hymenoptera: Bethylidae) a parasitoid of the coffee berry borer. Environ. Entomol. 29:126-131.

Evans, G. A., and Strange, L. A. 1997. Parasitoids associated with the brown citrus aphid, *Toxoptera citricida* in Florida (Insecta: Hymenoptera). Florida Dept. Agric. Cons. Serv., Div. Plant Indus., Entomol. Cir. No. 384.

Fransen, J. J., and Van Lenteren, J. C. 1993. Host selection and survival of the parasitoid *Encarsia formosa* on greenhouse whitefly, *Trialeurodes vaporariorum*, in the presence of hosts infected with the fungus *Aschersonia aleyrodis*. Entomol. Exp. Appl. 69:239-249.

Fransen, J. J., and Van Lenteren, J. C. 1994. Survival of the parasitoid *Encarsia formosa* after treatment of parasitized greenhouse whitefly larvae with fungal spores of *Aschersonia aleyrodis*. Entomol. Exp. Appl. 71:235-243.

Halbert, S. E., and Brown, L. G. 1996. *Toxoptera citricida* (Kirkaldy), brown citrus aphid - identification, biology and management strategies. Florida Dept. Agric. Cons. Serv., Div. Plant Indus., Entomol. Cir. No. 374.

Hoy, M. A., Singh, R., and Rogers, M. E. 2010. Evaluations of a novel isolate of *Isaria fumosorosea* for control of the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae). Florida Entomol. 93: 24-32.

Hunter, W. B., Avery, P. B., Pick, D., and Powell, C. A. 2011. Broad spectrum potential of *Isaria fumosorosea* on insect pests of citrus. Florida Entomol. 94: 1051-1054.

Jaronski, S. T, Goettel, and Lomer, C. J. 2003. Regulatory requirements for ecotoxological assessments of microbial insecticides – how relevant are they?, pp. 237-260 *In* H. M. T. Hokkanen and A. E. Hajek [eds.], Environmental Impacts of Microbial Insecticides: Needs and Methods for Risk Assessment. Kulwer Academic Publishers, Dordrecht, The Netherlands.

Kim, J. J., Kim, K. C., and Roberts, D. W. 2005. Impact of the entomopathogenic fungus *Verticillium lecanii* on development of an aphid parasitoid, *Aphidius colemani*. J. Invertebr. Pathol. 88: 254-256

Luangsa-Ard, J. J., Hywel-Jones, N. L., Manoch, L., and Samson, R. A. 2005. On the relationships of *Paecilomyces* sect. *Isarioidea* species. Mycol. Res. 109: 581-589.

Mesquita, A. L. M., Lacey, L. A., and Leclant, F. 1997. Individual and combined effects of the fungus, *Paecilomyces fumosoroseus* and parasitoid, *Aphelinus asychis* Walker (Hym., Aphelinidae) on confined populations of Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Hom., Aphididae) under field conditions. J. Appl. Entomol. 121: 155-163.

Mesquita, A. L. M., and Lacey, L. A. 2001. Interactions among the entomopathogenic fungus, *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes), the parasitoid, *Aphelinus asychis* (Hymenoptera: Aphelinidae), and their aphid host. Biol. Control 22: 51-59.

Meyer, J. M., Hoy, M. A., and Boucias, D. G. 2008. Isolation and characterization of an *Isaria fumosorosea* isolate infecting the Asian citrus psyllid in Florida. J. Invertebr. Pathol. 99: 96-102.

Michaud, J. P. 1998. A review of the literature on *Toxoptera citricidus* (Kirkaldy) Homoptera: Aphididae). Florida Entomol. 81: 37-61.

Michaud, J. P. 2002. Classical biological control: a critical review of recent programs against citrus pests in Florida. Ann. Entomol. Soc. Am. 94: 531-540.

Nieto Nafría, J. M., Alonso-Zarazaga, M. A., and Pérez Hidalgo, N. 2005. *Toxoptera citricida* or *Toxoptera citricidus*? The validity of a specific name (Hemiptera, Aphididae, Aphidini). Graellsia 61: 141-142.

Poprawski, T. J., Parker, P. E., and Tsai, J. H. 1999. Laboratory and field evaluation of hyphomycete insect pathogenic fungi for control of brown citrus aphid (Homoptera: Aphididae). Environ. Entomol. 28: 315-321.

Roy, A., and Brlansky, R. H. 2009. Population dynamics of a Florida *Citrus tristeza virus* isolate and aphid-transmitted subisolates: Identification of three genotypic groups and recombinants after aphid transmission. Phytopathology 99: 1297-1306.

Sterk, G., Bolckmans, K., De Jonghe, R., De Wael, L., and Vermeulen, J. 1995a. Side-effects of the microbial insecticide PreFeRal WG (*Paecilomyces fumosoroseus*, strain Apopka 97) on *Bombus terrestris*. Meded. Fac. Landbouww. Rijksuniv. 60: 713-717.

Sterk, G., Bolckmans, K., Van De Veire, M., Sels, B., and Stepman, W. 1995b. Side-effects of the microbial insecticide PreFeRal WG (*Paecilomyces fumosoroseus*, strain Apopka 97) on different species of beneficial arthropods. Meded. Fac. Landbouww. Rijksuniv. 60: 719-724.