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EFFECT OF *ISARIA FUMOSOROSEA* (HYPOCREALES: CORDYCIPTACEAE)
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 Nafria 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 trifoliolate hybrid: Washington navel x trifoliolate orange) were obtained from the Sub-Tropical Insect Research Unit, USDA Horticulture Research Laboratory in Fort Pierce, Florida. For the experiment, 5 car-

rizo 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 10⁷ 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
<i>L. testaceipes</i>	21.4 a	20.5 a	28.4 a	31.0 a	6.3 b	2.8 b
<i>Ifr</i>	9.9 a	8.7 a	22.2 a	35.4 a	58.0 a	167.5 a
<i>L. testaceipes</i> + <i>Ifr</i>	15.2 a	13.3 a	18.5 a	16.9 a	1.8 b	0.3 b

^aData are the average per plant from 2 tests (5 plants per replicate).

^bTreatments: 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.

^cValues 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						
<i>L. testaceipes</i>	0 a	0 a	8.3 a	13.3 a	32.0 a	18.7 a
<i>L. testaceipes</i> + <i>Ifr</i>	0 a	0 a	14.0 a	18.0 a	26.2 a	17.5 a
Emergед						
<i>L. testaceipes</i>	0 a	0 a	0.1 a	3.5 a	11.0 a	15.5 a
<i>L. testaceipes</i> + <i>Ifr</i>	0 a	0 a	0.4 a	4.6 a	9.6 a	13.4 a

^aData are the average per plant from 2 tests (5 plants per replicate).

^bTreatments: *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.

^cValues 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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