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BROAD SPECTRUM POTENTIAL OF *ISARIA FUMOSOROSEA* AGAINST INSECT PESTS OF CITRUS

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Renewed interest in the use of entomopathogenic fungi in the management of insect pests led to the evaluation of *Isaria fumosorosea* Wize *(Ifr)* (= *Paecilomyces fumosoroseus* (Wize) A.H.s. Br. & G. Sm.) on citrus pests due to finding the invasive, Asian citrus psyllid, *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllidae) infected with a strain of this fungus in a citrus grove (Meyer et al. 2008). The fungus was isolated from mycosed *D. citri* collected from the underside of foliage on orange trees in Polk County, Florida. In this study, 3 hemipteran insects (psyllid, leafhopper, aphid) and 1 coleopteran (*Diaprepes* citrus root weevil) were evaluated for susceptibility to *Ifr* under laboratory conditions. The Asian citrus psyllid, *D. citri*, is the vector of the citrus-infecting bacteria associated with citrus yellow shoot disease, known as Huanglongbing (Bové, 2006; 2008), and the glassy-winged sharpshooter, *Homalodisca vitripennis* Germar (Hemiptera: Cicadellidae) is a vector for the plant-infecting bacteria, *Xylella fastidiosa* which causes citrus variegated chlorosis, scorch-like diseases of fruit crops, and Pierce's disease of grapevine (Daugherty & Almeida 2009). the brown citrus aphid, *Toxoptera citricidus* (Kirkaldy) (Hemiptera: Aphididae), is a vector of citrus tristeza virus, CTV (Roy & Brlansky 2009) and the root weevil, *Diaprepes abbreviatus* L., is a common pest of citrus which has a broad host range and damages the roots of trees which permits phytopthora infections (Graham et al. 2003). Thus these insect pests of citrus trees were evaluated for susceptibility to strains of the entomopathogenic fungus, *Ifr*.

Presently, 3 *Ifr* strains are available for research as blastospore formulations in the U.S.A. There is the *Ifr* APOPKA strain, in *PFR* 97 20% WDG® (Certis, Columbia, Maryland, USA), labeled for controlling psyllids, leafhoppers, and aphids; the *Ifr* 9901 strain, in NoFly™ WP (Natural Industries, Houston, Texas labeled for controlling whiteflies, aphids and thrips and lastly, the non-commercial strain, *Ifr* 3581 available from the USDA/ARS, NCAUR, Peoria, Illinois, USA (Jackson et al. 1997). *Ifr* has several characteristics that favor evaluation for controlling insect pests of citrus: 1) it is native to Florida, 2) it is compatible with non-target arthropods and 3) it can be horizontally transferred and infect conspecifics (Sterk et al. 1995a, b; Avery et al. 2008, 2009, 2010). In our experiments only *Ifr* strain 3581 *and PFR* 97 were tested on the citrus pest insects. This is the first report indicating that *H. vitripennis*, *T. citricidus* and *D. abbreviatus* were susceptible to *Ifr* strains under laboratory conditions.

Effects on insects were assessed under laboratory conditions. Plants used in all these experiments were either Duncan grapefruit (*Citrus paradisi* Macf.) or 'carrizo' citrus seedlings grown in Premier Pro-mix General Purpose Growing Medium in size C10 "Cone-tainers"™ (Stuewe & Sons, Inc., Corvalis, Oregon) from seed for approximately 6 months. Experiments that used leaf disks, leaves or seedlings were sprayed at the same rate (10^7 blastospores / ml) for all insects. Insects were all kept at 25 °C under a 16:8 h L: h D photoperiod. *Diaphorina citri* and *Homalodisca vitripennis*: Leaf disks (~962 mm^2) cut to fit snugly in a 35 × 10 mm Petri dish, were sprayed with *Ifr* (*PFR* 97) to runoff (1,313 ± 257.7 blastospores mm^2) using a Nalgene® aerosol sprayer (Nalge Nunc International, Rochester, New York) or water and then allowed to air dry in a fume hood. Disks were then placed on top of warm water agar and allowed to stick to the agar after the leaf was embedded, then 1 adult psyllid was allowed to walk off the camel hair brush onto the leaf disk in each dish. Sixty dishes (control and treated) were sealed with Parafilm™ for 24 h and then left unsealed thereafter, but held together by a rubber band (RH was not measured inside the dish) (Lietze et al. 2011). Control leaf discs were treated similarly using only water, and insects were added as with treatments with corresponding numbers of insects. Insects were monitored daily until death occurred and mycosis of *Ifr* was verified by characteristic morphology of the fungus inside each dish (Fig. 1 A-B). Glassy winged sharpshooter nymphs (2 - 3) or adults (2) per dish were monitored as described above (Fig. 1 C-D). Leafhopper egg masses were also treated and observed (Fig. 1 E-F). *Toxoptera citricidus*: Two bug dorm cages (35 cm^3), were set up with 2 cages per light fixture (four 40W Philips fluorescent lights) under a 16 h L: 8 h D photoperiod at 24 ± 0.03 °C with 45 ± 0.29% RH. Five carrizo citrus seedlings (25-30 cm tall) grown in 4.9 cm^3 yellow Cone-tain-

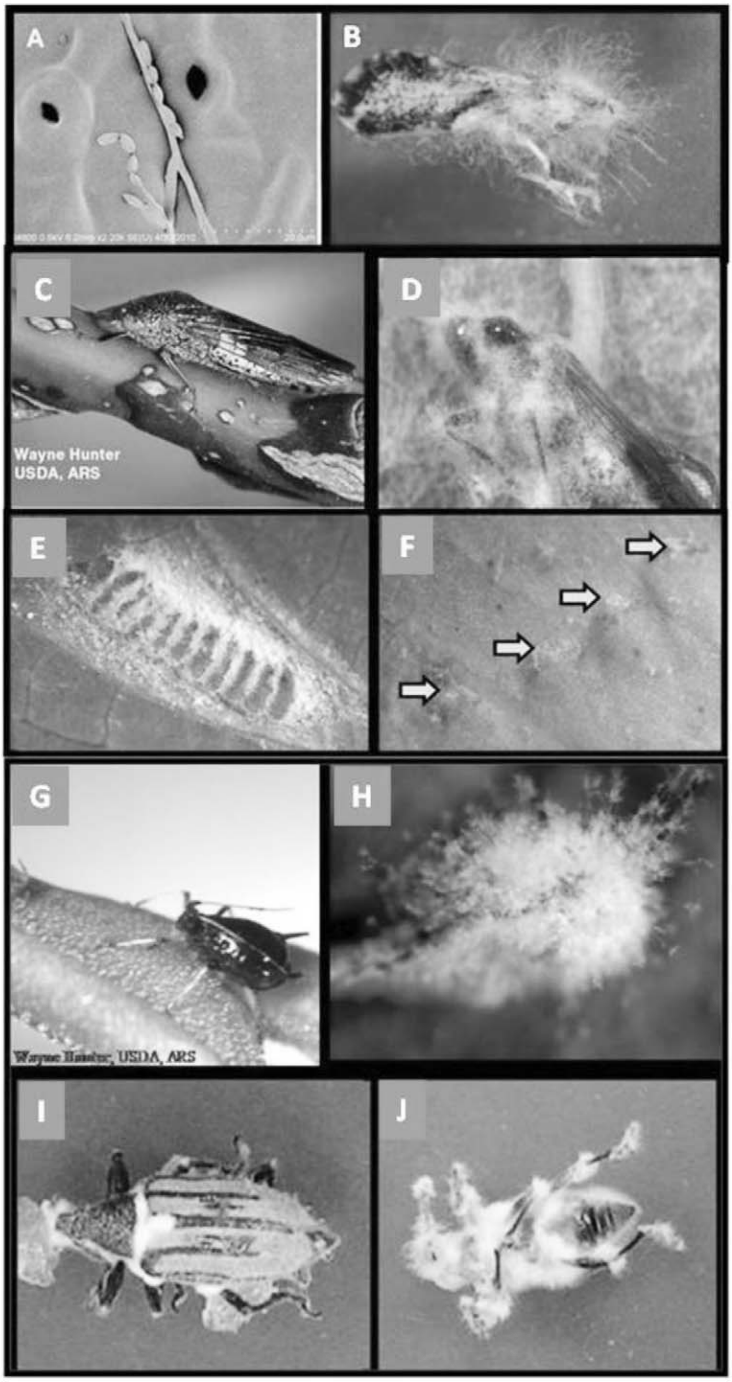

Figure 1. Citrus insect pests exhibiting mycosis of *Isaria fumosorosea*: A) Fungi growing on leaf surface, scanning electron micrograph; B) Asian citrus psyllid showing extrusion of fungal hyphae; C) Adult glassy-winged sharpshooter, *Homalodisca vitripennis* (Hemiptera: Cicadellidae); D) Leafhopper showing extrusion of fungal hyphae. Nymphs were also susceptible to fungal spray treatments (not shown). Leafhoppers died while feeding on citrus seedling 6-7 d post treatment sprays of *PFR* 97™. E) Healthy leafhopper egg mass covered in whitish powder of brocosomes. F) Treated leafhopper egg mass in citrus leaf showed fungi hyphae at oviposition sites (arrows); nymphs did not emerge. G). Citrus aphid, *Toxoptera citricidus*, apterous adult when given access to citrus leaf treated with *PFR* 97™, H) become comatose, slowly stops feeding and are colonized by fungi. I) *Diaprepes* citrus root weevil, showing signs of *Ifr* colonization at day six post treatment. J) Fully mycosed weevil adult.

ers® held in a rack were added to each cage, and watered as needed. Citrus seedlings were sprayed with *Ifr* or water, allowed to air dry, and then 20 adult aphids were added to roam freely (Fig. 1G). Dead aphids were removed daily from cages, and/or leaves, and placed on water agar plates to determine if they were infected with *Ifr*, verified by characteristic morphology of the fungus (Fig. 1H) (Poprawski et al. 1999; Pick et al. 2010).

Diaprepes abbreviatus adult weevils were fed single grapefruit leaves of similar size that were sprayed with either *Ifr* or water, and allowed to air dry in a fume hood. One adult was placed in a 100 × 15 mm Petri dish containing a single leaf; 40 adults per treatment. High humidity (~100% RH) was provided by a moistened filter paper on the bottom of the dish. Dish chambers were sealed with Parafilm™ and placed in a growth chamber for several weeks at 25 °C under a 16 h photophase. Once a leaf was consumed after 24 h, an untreated leaf was provided to the adult weevils and replaced thereafter as needed until death occurred. Adults were monitored daily for 1-6 weeks for symptoms of the fungus (Fig. 1 I-J).

The percentage mortality for the treated insects is summarized in Table 1. Psyllids appeared most susceptible to *PFR* 97™ with the earliest mortality observed 2 d post-exposure, with 100% mortality by the eighth d. During this time period, psyllid feeding was reduced as evidenced by the reduction of honeydew droplets (Avery et al. 2011). Leafhopper adults showed increasing mortalities beginning 3-4 d up to 100% at 15 d post exposure. The glassy-winged sharpshooter leafhopper is significantly larger than the psyllid (~4 *), which may account for the delayed mortality rates, plus leafhoppers continuously groom with their spiky hind legs which could dislodge spores from penetrating the cuticle. Leafhopper nymphs showed similar rates of mortalities during the same time period, with 100% still occurring at 15 d post exposure. Aphids showed 100% mortality at 15-20 d post exposure. Within all hemipteran control groups, mortality was 3% or less over the same time period. For the coleopterans, weevils have a much larger body size, with a thicker cuticular layer that could potentially increase the difficulty for fungi to penetrate. In weevil control groups, 13% mortality was observed 15 d post-exposure reaching a maximum of 33% at 35 d, while fungal treated weevils showed 100% mortality at 35 d post exposure.

The *Ifr* spray product, i.e. *PFR* 97™, demonstrated that applications of these strains have potential to reduce citrus insect pests and may provide an additional biological control agent; however, these results need to be confirmed under field conditions. Furthermore, the role of environmental conditions, such as early morning humidity, rainy seasons, or temperatures are being investigated for their impact on efficacy (Avery - unpublished observations) to establish best management practices and integration with currently implemented IPM practices in citrus production.

Summary

The insects evaluated were susceptible to infection by the Hypocreales fungus, *Ifr,* in the product PFR 97™ and *Ifr* 3581 when applied as a spray to seedlings, leaves or leaf discs. This is the first report indicating that *H. vitripennis*, *T. citricidus* and *D. abbreviatus* were susceptible to *Ifr* strains under laboratory conditions. The results suggest that *Ifr* provides a potential biological control agent for these and other pests of agricultural crops, and may provide a product for organic growers, as well as an additional tool to augment standard IPM programs.

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Table 1. Percent mortality (range) and time of death of various insects after being exposed to *Isaria fumosorosea*.

		% Mortality in range/days post exposure					
Insects	Stage	1-2	3-4	6-8	9-15	22	35
Asian citrus psyllid	adult	0-8	28-53	91-100	100		
Glassy winged sharpshooter	adult	0	10-12	22-60	60-100		
Brown citrus aphid	adult	0	6-10	30-70	70-80	100	
Diaprepes root weevil	adult	0	0	0-7	31-47	87-93	100

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References Cited

AVERY, P. B., WEKESA, V. W., HUNTER, W. B, HALL, D. G., MCKENZIE, C. L., OSBORNE, L. S., POWELL, C. A., AND ROGERS, M. E. 2011. Effects of the fungus *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) on reduced feeding and mortality of the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae). Biocontrol Sci. Technol. 21: 1065-1078.

AVERY, P. B., QUEELEY, G., FAULL, J., AND SIMMONDS, M. S. J. 2010. Effect of photoperiod and host distribution on the horizontal transmission of *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) in greenhouse whitefly assessed using a novel leaf model bioassay. Biocontrol Sci. Technol. 20: 1097-1111.

AVERY, P. B., HUNTER, W. B, HALL, D. G., JACKSON, M. A., POWELL, C. A., AND ROGERS, M. E. 2009. *Diaphorina citri* (Hemiptera: Psyllidae) infection and dissemination of the entomopathogenic fungus *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) under laboratory conditions. Florida Entomol. 92: 608-618.

AVERY, P. B., FAULL, J., AND SIMMONDS, M. S. J. 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.

BOVÉ, J. M. 2006. Huanglongbing: a destructive, newly emerging, century old disease of citrus. J. Plant Pathol. 88: 7-37.

BOVÉ, J. M. 2008. Huanglongbing. IOCV Citrus Diseases, http://www.ivia.es/iocv/

DAUGHERTY, M. P., AND ALMEIDA, R. P. P. 2009. Estimating *Xylella fastidiosa* transmission parameters: decoupling sharpshooter number and feeding period. Entomol. Exp. Appl. 132: 84-92.

GRAHAM, J. H., BRIGHT, D. B., AND MCCOY, C. W. 2003. *Phytophthora-Diaprepes* weevil complex: *Phytophthora* spp. relationship with citrus rootstocks. Plant Dis. 87: 85-90.

JACKSON, M. A., MCGUIRE, M. R., LACEY, L. A., AND WRAIGHT, S. P. 1997. Liquid culture production of desiccation-tolerant blastospores of the bioinsecticidal fungus *Paecilomyces fumosoroseus*. Mycol. Res. 101: 35-41.

LIETZE, V-U., MIZELL III, R. F., AND BOUCIAS, D. G. 2011. Transmission of the mycopathogen, *Hirsutella* spp., to nymphs and adults of the glassy-winged sharpshooter, *Homalodisca vitripennis* (= *coagulata*), in the greenhouse. Florida Entomol. 94: 106-108.

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.

PICK, D. A., AVERY, P. B., HUNTER, W. B., POWELL, C. A., AND HALL, D. G. 2010. Entomopathogenic fungus, *Isaria fumosorosea*, and aphid parasitoid, *Lysiphlebus testaceipes*, for managing infestations of brown citrus aphid, *Toxoptera citricida*. Florida Sci. 73: 5.

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. Phytopathol. 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.