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RESEARCH ARTICLE

Effects of the fungus *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) on reduced feeding and mortality of the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae)

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The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), is an important citrus pest primarily because it transmits bacteria putatively responsible for huanglongbing, a serious disease of citrus. We present a study on the effects of blastospore and conidial formulations of *Isaria fumosorosea* Wize on feeding rates and mortality of adult psyllids in laboratory bioassays. Information on quantities of honeydew droplets was used to make inferences on feeding rates. Psyllids treated with the blastospore formulation of *I. fumosorosea* produced fewer honeydew droplets compared to the conidial treatment and control beginning within the first 24 h after treatment. The highest daily mean number of droplets thereafter never exceeded 2.4 drops compared to 4 and 8 for the conidial treatment and control, respectively. The mean number (± SEM) of honeydew droplets produced per psyllid per day over 7 days was significantly higher in the control (5.5 ± 0.5) compared to the blastospore treatment; however, there were no significant differences between the treatments. Psyllids treated with the conidial formulation of the pathogen showed no significant reduction in feeding activity until 4 days after treatment. One and 2 day's post-exposure, mortality of psyllids in the blastospore treatment ranged from 8 to 25% compared to 0% in the conidial and control treatments. By 7 days post-exposure, psyllid mortality reached 100% under both fungal treatments compared to none in the controls. This study documented that adult psyllids infected by *I. fumosorosea* (PFR 97) produce less honeydew than healthy psyllids and suggests that they may feed less, which could potentially reduce the spread of huanglongbing.

**Keywords:** citrus greening disease; blastospores; honeydew droplets; entomopathogenic fungi; antifeedants; Hypocreales
1. Introduction

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), a vector of the putative phloem-limited bacteria responsible for huanglongbing (citrus greening disease, HLB), was discovered in Florida in 1998 (Halbert 1998). Management of *D. citri* populations (and consequently HLB) is currently based on intensive use of insecticides (Stansly and Rogers 2006). However, the integration of biological control using both native and imported natural enemies along with compatible insecticides may be an alternative long-term approach (Hoy and Nguyen 2000; Michaud 2004; Meyer, Hoy, and Boucias 2008). Pathogenic fungi have been reported to suppress *D. citri* populations during humid conditions in some citrus growing areas (Rivero-Aragon and Grillo-Ravelo 2000; Subandiyah, Nikoh, Sato, Wagiman, Tsuyumyu, and Fakatsu 2000; Étienne, Quilici, Marival, and Franck 2001). The entomopathogenic fungi *Hirsutella citriformis* and *Isaria fumosorosea* Wize (=*Paecilomyces fumosoroseus*) have been observed infecting *D. citri* in Florida citrus groves (Meyer, Hoy, and Boucias 2007; Meyer et al. 2008). *I. fumosorosea* infects all stages (eggs and motile) of *D. citri* and is actively being investigated for its potential use in sustainable management of this pest (Avery, Hunter, Hall, Jackson, Powell, and Rogers 2009; Hoy, Singh, and Rogers 2010).

Fungal pathogens require time to germinate, penetrate and infect their hosts. During this process, prior to death, host insects normally continue feeding and may inflict physical plant damage or even vector diseases (Mohamed, Brewer, Bell, and Hamale 1982; Lacey, Liu, Buchman, Munyaneza, Goolsby, and Horton 2011). The use of fungal pathogens has been of concern to agronomists and other insect pest managers because fungal pathogens may not kill insects as rapidly as insecticides. However, once insect pests are infected, fungal pathogens can alter their behavior resulting in reduced feeding (Mohamed et al. 1982), reproduction (Noma and Strickler 2000) and transmission of bacterial diseases (Lacey et al. 2011). Most recently, Ali, Huang, and Ren (2010) showed that culture filtrates of *I. fumosorosea* containing cuticle degrading enzymes were potent antifeedants against the diamondback moth, causing reductions in feeding rates and body weight of the larvae. However in contrast, there are also examples of no sub-lethal effects of infection by entomopathogenic fungi on feeding and fecundity of some insect pests (Noma and Strickler 2000).

Reduced rates of feeding by insects would be advantageous to growers because less damage occurs to a crop and subsequently pest control expenses may be reduced (Crawley 1989). In ecological terms, infected insects that remain in the environment may be useful for the dissemination of the fungal pathogens (Avery et al. 2009) and horizontal transmission to the healthy conspecifics (Long, Groden, and Drummond 2000; Avery, Queeley, Faull, and Simmonds 2010).

Two formulations of the pathogen *I. fumosorosea* (*Ifr*) were studied, one containing blastospores and one containing conidia. The different spore forms (blastospores and conidia) of *Ifr* were chosen to determine if the conidial formulation was as effective as the blastospores and whether it could be a viable option as a new commercial biopesticide product to be applied in the field. The blastospore formulation is now readily available as a commercial product in the USA (*PFR 97™*, Certis USA); however, since a recently developed conidial formulation (*CX 2105*), considered more environmentally tolerant was now available for experimentation, we decided to compare the two formulations in order to determine
which product would ultimately be more efficacious for managing *D. citri*. This research was the first laboratory study initiated to better understand the dynamics of an important citrus pest and its fungal pathogen infection process and the subsequent potential for field application for managing this pest. Therefore, the objectives of this initial study were to evaluate the effect of *Ifr* infectivity of both formulations (blastospore or conidial) on adult *D. citri* feeding rates under laboratory conditions. Information on quantities of honeydew droplets were used to make inferences on the effect of the fungus on psyllid feeding rates.

2. Materials and methods

2.1 Insects

*Diaphorina citri* used in these experiments were obtained from the USDA-ARS laboratory colony established during early 2000 at the U.S. Horticultural Research Laboratory, Fort Pierce, FL. Originally collected from citrus, the psyllids have been continuously reared on orange jasmine, *Murraya paniculata* (L.), housed in Plexiglas (0.6 × 0.6 × 0.6 m) or BugDorm-2 cages (MegaView Science Education Services Co., Ltd, Taichung, Taiwan) (Hall, Lapointe, and Wenninger 2007) under the following environmental conditions: 20–28°C, 40–80% RH and a 14 h L:10 h D photoperiod. The original colony has not had field collected psyllids added since establishment.

2.2 Fungi

*Ifr* used in this study was licensed to Certis USA, LLC. (Columbia, MD) and has been developed as a biopesticide under the trade name *PFR 97™*. Two *PFR 97™* formulations were studied, one comprised of blastospores and one of conidia (CX 2105). Fungal blastospores are asexual spores produced by budding in liquid culture media or in the insect hemocoel, and conidia are the asexual stage produced either on solid culture media or from germinated blastospores. The suspensions were prepared by mixing 1 g of the spore-containing powder in 100 mL of sterile distilled water, stirring the suspension with a magnetic bar for 30 min, and then 50 mL was pipetted to a Nalgene® aerosol sprayer (Nalge Nunc International, Rochester, NY). Two aliquots were taken from the suspension prior to spraying, and the concentration of *Ifr* blastospores and conidia mL⁻¹ was determined using a hemocytometer. The concentration for the blastospore and conidial formulation was adjusted to 4 × 10⁷ spores mL⁻¹ by adding water which is comparable to field rates for other fungal biopesticide products (Puterka 1999).

The viabilities of *Ifr* blastospores and conidia were assessed with two potato dextrose agar plates sprayed at a rate of 4 × 10⁷ spores mL⁻¹. After the plates had been incubated for 12 h at 25 ± 1°C, 100% RH, the percent viability was determined by viewing a total of 200 spores. Spores were considered to have germinated if a germ tube had formed. This procedure was repeated for each repetition of the experiment, and the percent viability for all three repetitions ranged from 87 to 89% for both blastospore and conidial formulations.

To determine the deposition of *Ifr* blastospores or conidia mm⁻², six plastic microscope cover slips (Fisherbrand® 22 × 22 mm; Fisher Scientific, Pittsburgh, PA) were placed randomly on brown paper towels among the leaf disks during spraying.
After drying, the cover slips were stained with acid fuschin and blastospore and conidia density was assessed using a compound light microscope (400 ×) with a 10-mm reticulated grid (Hunt Optic and Imaging, Pittsburg, PA). Mean number (±SEM) of viable spores deposited mm⁻² for blastospore and conidial treatments for all repetitions was 1313 ± 257.7 and 1389 ± 265.4, respectively.

### 2.3 STEM photomicrograph
Preparation of fungal treated surfaces was by fixation for 2 h in a solution of 2% glutaraldehyde, 3% paraformaldehyde, in sodium cacodylate buffer 0.2 M, pH 7.4 (#11650 Electron Microscopy Sciences, Hatfield, PA), followed by dehydration in an ethanol series, 35, 50, 70, 95, and three changes in 100%, at 1 h each. Dehydrated samples were freeze-dried through lyophilization, sputtercoated and viewed with a Hitachi 4800, STEM, electron microscope (U.S. Horticultural Research Lab, Ft. Pierce, FL, Figure 1a).

### 2.4 Bioassay arena chamber preparation
Adult psyllids (mixed population of males and females), 7–14 days old, were aspirated into small screw cap glass vials without food and kept on the lab bench.

![Figure 1.](image)

Figure 1. (A) SEM of *Isaria fumosorosea* conidial chain (letter c) growing out of an extended stylet (letter s) of an infected adult *Diaphorina citri* while feeding on a leaf disk; (B) honeydew droplet (see arrow) produced by an adult *D. citri* after feeding on a leaf disk; (C) two *D. citri* adults mycosed and colonized by *I. fumosorosea* after feeding on treated leaf disks.
Duncan grapefruit (Citrus paradisi Macf.) seedlings were grown in Premier Pro-mix General Purpose Growing Medium from seed in size C10 ‘Cone-tainers’™ (Stuewe & Sons, Inc., Corvalis, OR) for approximately 6 months. Leaves of similar age and size were excised from seedlings grown in the U.S. Horticultural Research Laboratory greenhouse, Fort Pierce, FL, placed in resealable plastic bags, and brought back to the laboratory at University of Florida, Indian River Research and Education Center, in Ft. Pierce, FL. The leaves were washed gently in tap water containing liquid soap (1 drop of soap/11.4 L of water) to remove any possible saprophytic fungi present on the surface, then rinsed three times with water to remove any soap residue, and placed on brown paper towels in a fume hood to air dry. Leaf disks (~962 mm²) were punched using a cork borer from clean citrus leaves and the abaxial surface (underside) was sprayed with a 4.0 × 10⁷ spores ml⁻¹ of either Ir blastospores or conidia of PFR 97™ using separate Nalgene® aerosol sprayers. The control disks were sprayed with sterile water in a separate room to prevent contamination with fungal spores. Twenty Petri dishes (35 × 10 mm) containing 1% water agar were prepared as described by Hall and Nguyen (2010) for each formulation and the control to preserve the leaf disks and maintain a semi-constant percent RH for the duration of the experiment. The experiments were repeated on three separate dates for a total of 60 disks per treatment (all three experiments combined together).

Spray residues on leaf disks were allowed to air dry prior to being placed into the Petri dishes containing semi-solidified warm water agar with the abaxial side of the leaf facing up. One randomly chosen psyllid was transferred using a camel hair brush and allowed to walk off of the brush onto the embedded leaf disk. Separate brushes were used for each treatment when transferring the psyllid to prevent cross contamination. The bioassay arenas were sealed with Parafilm®, inverted and incubated in a growth chamber at 25°C under a photoperiod of 16 h L:8 h D for 1 week. All arena chambers were sealed for 24 h to maintain a high relative humidity (~100%) to promote germination of the fungus. Chambers were then unsealed (% RH unknown). Honeydew droplets (spherical balls; Figure 1b) were quantified every 24 h using a dissecting microscope (40×) for 7 days; old droplets were cleaned off the Petri dish lids with a new KimTECH Science Kimwipe® (Kimberly-Clark, Roswell, GA) and replaced. Each chamber lid was carefully removed to prevent the psyllid from escaping and the inverted dish was placed on the laboratory table while the honeydew droplets were cleaned off. The chambers were stacked in groups of 5 for each treatment. Each stack was held together using a rubber band and then randomly placed back into two separate open plastic boxes (Sterlite® 29.2 × 18.7 × 8.3 cm; Sterlite Corporation, Townsend, MA). All treatments in a box were placed back into the growth chamber and incubated for another 24 h before the next assessment.

2.5 Statistical analyses
The mean number of honeydew droplets produced per day (total honeydew droplets/days lived) by one adult psyllid in each of the treatments was analyzed by ANOVA (α = 0.05) for 7 days post-exposure. ANOVA was conducted on the arcsine square
Table 1. Mean number (±SEM) of honeydew deposits for one adult psyllid per day after feeding on a leaf disk sprayed with blastospores or conidia of *Isaria fumosorosea.*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastospores</td>
<td>0.5 ± 0.3a</td>
<td>1.4 ± 0.6a</td>
<td>2.4 ± 0.4a*</td>
<td>2.0 ± 0.4a</td>
<td>0.8 ± 0.7a</td>
<td>2.6 ± 0.0a</td>
<td>0.8 ± 0.0a^c</td>
</tr>
<tr>
<td></td>
<td>(60)</td>
<td>(55)</td>
<td>(43)</td>
<td>(28)</td>
<td>(9)</td>
<td>(5)</td>
<td>(0)</td>
</tr>
<tr>
<td>Conidia</td>
<td>2.4 ± 1.3b</td>
<td>4.0 ± 1.8b</td>
<td>3.1 ± 1.0ab</td>
<td>1.8 ± 0.6a</td>
<td>0.6 ± 0.2a</td>
<td>0.6 ± 0.6a</td>
<td>0.0 ± 0.0a</td>
</tr>
<tr>
<td></td>
<td>(60)</td>
<td>(60)</td>
<td>(55)</td>
<td>(29)</td>
<td>(9)</td>
<td>(2)</td>
<td>(0)</td>
</tr>
<tr>
<td>Control</td>
<td>3.9 ± 1.1b</td>
<td>3.9 ± 0.9b</td>
<td>4.1 ± 0.8b</td>
<td>8.0 ± 1.6b</td>
<td>6.7 ± 2.2b</td>
<td>6.5 ± 2.3b</td>
<td>5.3 ± 0.8b</td>
</tr>
<tr>
<td></td>
<td>(60)</td>
<td>(60)</td>
<td>(60)</td>
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<td>(60)</td>
</tr>
</tbody>
</table>

^aDisks were sprayed with blastospores or conidia of *Isaria fumosorosea* and control with water and allowed to dry before being placed on 1% water agar inside the Petri dish and then exposed to the adult psyllid.

^bData was transformed by log (n + 1) prior to analysis; untransformed data means are shown (n = 60/treatment – # psyllids dying). Means (±SEM) in a column not labeled by the same letter are significantly different from another according to the Tukey’s test (P < 0.05). Only live psyllids were used for the analysis on a daily basis.

^cSignificant at P = 0.07.

^dAdult psyllids that were found dead the next sampling day deposited the honeydew during the previous night.
root transformed percentage mortality, and log \((n+1)\) transformed daily droplet production data. Each disk (Petri dish) was counted as an independent replicate with in a treatment group (20 disks/treatment) and each of the three treatments were assessed on three separate dates \((n=60/\text{treatment})\) until the insects start dying \((n=60/\text{treatment} – \# \text{dying})\). \textit{Post-hoc} multiple comparisons were made on live adult psyllids that had deposited honeydew/treatment with in 24 h of the previous reading using a Tukey’s HSD test \((\alpha=0.05)\). A repeated measures ANOVA (RMANOVA) was conducted to determine the treatment effect on the droplet production over time. All statistical analyses were conducted using SAS Proc GLM procedures and executed on a WIN_PRO platform (SAS 1999–2001).

3. Results

3.1 Number of honeydew droplets

The mean number of honeydew droplets ± SEM produced by the psyllids per day in the \textit{Ifr} blastospore treatment \((0.5 \pm 0.3 \text{ and } 1.4 \pm 0.6 \text{ at } 24 \text{ and } 48 \text{ h, respectively})\) was significantly lower at 24 h \((F=10.68; \text{df} = 2, 179; P<0.0001)\) and 48 h \((F=7.21; \text{df} = 2, 174; P=0.0010)\) post-exposure compared to the conidial \((2.4 \pm 1.3 \text{ and } 4.0 \pm 1.8)\) and control \((3.9 \pm 1.1 \text{ and } 3.9 \pm 0.9)\) treatments which were similar (Table 1). The mean number of honeydew droplets observed 3 days post-exposure in the blastospore treatment was significantly lower \((F=2.70; \text{df} = 2, 157; P=0.0699)\) compared to the control, but the number of droplets between psyllids treated with

![Figure 2](image)

Figure 2. Mean number of honeydew droplets produced by one \textit{Diaphorina citri} adult per day after feeding for 7 days on a leaf disk embedded in water agar inside of an unsealed Petri dish. Leaf disks were sprayed with either blastospores or conidia of \textit{Isaria fumosorosea} and control with water. Data were transformed by log \((n+1)\) prior to analysis; untransformed data means are shown in the graph \((n=20 \text{ adult psyllids/day/treatment} – \# \text{psyllids dying})\) for each experiment. Error bar means \((\pm \text{SEM})\) labeled by the same letter are not significantly different from another (Tukey’s HSD test; \(P<0.05\)). These data only include living psyllids and experiments were repeated on three separate dates.
Table 2. Cumulative mean percent mortality for adult psyllids after feeding on a leaf disk sprayed with blastospores or conidia of *Isaria fumosorosea* over time.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1</th>
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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastospores</td>
<td>0.0 ± 0.0a</td>
<td>8.3 ± 8.3a</td>
<td>28.3 ± 9.3b</td>
<td>53.3 ± 14.5b</td>
<td>85.0 ± 15.0b</td>
<td>91.7 ± 8.3b</td>
<td>100 ± 0.0b</td>
</tr>
<tr>
<td>Conidia</td>
<td>0.0 ± 0.0a</td>
<td>0.0 ± 0.0a</td>
<td>8.3 ± 8.3a</td>
<td>43.3 ± 3.3b</td>
<td>85.0 ± 7.6b</td>
<td>96.7 ± 3.3b</td>
<td>100 ± 0.0b</td>
</tr>
<tr>
<td>Control</td>
<td>0.0 ± 0.0a</td>
<td>0.0 ± 0.0a</td>
<td>0.0 ± 0.0a</td>
<td>0.0 ± 0.0a</td>
<td>0.0 ± 0.0a</td>
<td>0.0 ± 0.0a</td>
<td>0.0 ± 0.0a</td>
</tr>
</tbody>
</table>

Disks were sprayed with blastospores or conidia of *Isaria fumosorosea* and control with water and allowed to dry before being placed on water agar inside the Petri dish and then exposed to the adult psyllid.

Data was arc sine transformed prior to analysis; untransformed data means are shown (*n* = 20/treatment). Means (±SEM) in a column not labeled by the same letter are significantly different from another according to the Tukey’s test (*P* < 0.05).
blastospores and conidia were similar. The number of honeydew droplets in the control was significantly higher ($3 \pm 0.0$) compared to both blastospore and conidia treatments at 4 ($F=10.76; \text{df}=2, 116; P<0.0001$), 5 ($F=24.05; \text{df}=2, 78; P<0.0001$), and 6 days ($F=9.07; \text{df}=2, 67; P=0.0003$) post-exposure. After 7 day post-exposure, the mean number of droplets produced by the psyllids in the blastospore and conidial treatments was only $0.8 \pm 0.0$ and 0 compared to $5.3 \pm 0.8$ in the control, respectively. Over the 7-day observation period, the highest mean number $\pm \text{SEM}$ of droplets produced by psyllids daily in the blastospore, conidial and control treatments was $2.6 \pm 0.0$, $4 \pm 1.8$, and $8 \pm 1.6$, respectively. The mean number ($\pm \text{SEM}$) of honeydew droplets produced by one $D. \ citri$ adult psyllid per day over all 7 days post-exposure was significantly higher ($F=8.70; \text{df}=2, 8; P=0.0350$) in the control ($5.5 \pm 0.5$) than the blastospore treatments, but there was no significant difference in numbers of honeydew droplets between the blastospore ($1.3 \pm 0.1$) and conidial ($2.4 \pm 0.9$) treatments (Figure 2). Both fungal treatments had a significant positive effect (RMANOVA: $F=64.40; \text{df}=2, 62; P<0.0001$) in reducing the production of honeydew droplets by $D. \ citri$ over the 7-day observation period (RMANOVA: $F=17.58; \text{df}=6, 62; P<0.0001$) compared to the control.

3.2 Percent psyllid mortality
The cumulative mean percent psyllid mortality was $8.3\%$ ($8-25\%$) in the Ifr blastospore treatment compared to $0\%$ in the conidial or water treatments 48 h post-exposure (Table 2). Mortality in the conidial treatment reached $8.3\%$ by the third day post-exposure compared to $28.3\%$ in the blastospore treatment; the latter which was significantly higher ($F=14.63; \text{df}=2, 8; P=0.0145$). After 4 days post-exposure, the mean percent mortality of psyllids infected was significant ($F=7.83; \text{df}=2, 8; P=0.0414$) for both the blastospore and conidia treatments, and mortality reached $91-97\%$ at day 6 for each of these treatments compared to $0\%$ mortality in the controls. All psyllids treated with either Ifr blastospore or conidia were dead 7 days post-exposure.

4. Discussion
This report is the first investigation evaluating the effects of the Ifr infection on adult $D. \ citri$ feeding rates as assessed by counts of honeydew droplets on a daily basis. Considering that adult $D. \ citri$ produce honeydew within 24 h after feeding is initiated (Hall, Shatters, Carpenter, and Shapiro 2010), and the psyllids were starved for 24 h prior to being placed on a new leaf disk, the honeydew drops counted 1 day post-exposure would be the result of feeding on the new leaf. Honeydew production as a proxy for ingestion has been used successfully as a technique to measure different aspects of insect biology such as: feeding resistance between plant genotypes (Jiang and Walker 2007); efficacy of chemical pesticides (Boina, Onagbola, Salyani, and Stelinski 2009); and evaluating population density and biology (Vassiliou and Drees 2008; Hall et al. 2010). However, this is the first report using this technique to evaluate feeding behavior of psyllids infected with an entomopathogenic fungus.

Adult psyllids treated with Ifr blastospores produced significantly fewer honeydew droplets than those treated with Ifr conidia or with plain water 1 and 2
days after exposure to the fungus, and death occurred in 8–25% of the population treated with blastospores within 2 days post-exposure. We suspect that the blastospore formulation had a faster effect than the conidia formulation due to differences in germination rates. After assessing the germination rates of conidia and blastospores of Ifr ARSEF strain 3581 on the cuticle of the silverleaf whitefly using SEM micrographs, Vega, Jackson, and McGuire (1999) found that 4 h after treatment applications, more than 37% of blastospores had germinated compared to none for the conidia on the whitefly cuticle at 24°C. When comparing Ifr blastospore and conidial formulations against the greenhouse whitefly, Trialeurodes vaporariorum, Avery, Faull, and Simmonds (2004) found that blastospore treatments caused the highest mortality (86–100%) before adult eclosion under optimum conditions for fungal growth (25°C and ~100% RH) and a 16 h L:8 h D photoperiod light regime. In addition, conidial treatments were observed to be less virulent than blastospore treatments at both photoperiod regimes (16 h L:8 h D and 24 h L:0 h D). In our study, Ifr blastospores were shown to reduce the number of honeydew droplets significantly faster compared to the conidial treatment 24 h post-exposure at 25°C under a 16 h L:8 h D photoperiod. In contrast, the results of some studies on other insect species have indicated that conidia of hypocrealean fungi can be as effective as blastospores (Vandenburg, Jackson, and Lacey 1998) or less virulent (Lane, Trinci, and Gillespie 1991). Although slower to affect the psyllid in our study, ultimately the conidia formulation provided high levels of mortality. Osborne and Landa (1992) observed that once the conidia of Ifr attached to the dorsum of whiteflies, hyphae were present in the hemocoel within 24 h.

Fungal spores of Ifr that are pathogenic (blastospores and conidia) germinate at different rates on the exoskeleton producing hyphae which will eventually form a penetration peg and eventually penetrate the insect cuticle (Vega et al. 1999; Gökçe and Er 2005). In this study, the progression of the disease observed in adult D. citri was consistent with that characterized by others as being due to the infection and subsequent colonization by Ifr (Meyer et al. 2008; Avery et al. 2009; Figure 1c). Ifr inoculated psyllids displayed disease symptoms including twitching of legs and antennae, 2–3 days post exposure for both blastospore and conidial formulations. Also, immediately prior to death, most infected psyllids had fungal hyphae emerging from the tarsi and inter-segmental regions of the legs. Under high humidity (RH >80%), infected insects were observed to mycose and form a sporulating cadaver cemented in a feeding position to the leaf surface by hyphae growing from their tarsi. Based on the STEM micrograph (Figure 1a), infected adult psyllids can succumb to the fungus, become moribund and attached to the leaf while feeding with its stylet fully extended into the leaf tissue. Whether this moribund feeding state will promote the spread of HLB is unknown and warrants further research. The infection process and progress of different Ifr isolates or strains for other hemipteran species has been well summarized by many authors and is consistent with our observations (Osborne and Landa 1992; Landa, Osborne, Lopez, and Eyal 1994; Gökçe and Er 2005; Wraight, Lacey, Kabaluk, and Goettel 2009; Goettel, Eilenberg, and Glare 2010).

The mean number of honeydew droplets produced daily by adult psyllids was significantly reduced 1–3 days post-exposure in the blastospore treatments. Reduced feeding rates associated with infected adults may have been related to fungal colonization possibly in conjunction with the production of toxins by the fungus Ifr (Assaf, Cerda-Garcia-Rojas, and de la Torre 2005). The antifeedant effect of the
fungus could have been increased due to an avoidance feeding behavior by the psyllid on leaves covered with fungal spores. Several authors have indicated that entomopathogenic fungal spores produce bioactive metabolites and cuticle degrading enzymes which may cause avoidance and antifeedant behavior in insects (Meyling and Pell 2006; Quesada-Moraga, Carrasco-Díaz, and Santiago-Alvarez 2006; Ali et al. 2010; Baverstock, Roy, and Pell 2010). Most recently, Ali et al. (2010) demonstrated that the culture filtrates containing cuticle degrading enzymes of Ifr were potent antifeedants against Plutella xylostella. Considering that Ifr blastospores are produced in a similar way to the culture filtrates, there may have been bioactive metabolites and cuticle degrading enzymes produced by Ifr that contributed to the antifeedant effect and resulting reduction of honeydew droplets observed in the blastospore treatment. Therefore, the possible effect of cuticle degrading enzymes produced by Ifr on the antifeedant behavior of the psyllid warrants further in depth research.

Psyllid adults and nymphs cause mechanical damage due to their feeding activities (Hall and Albrigo 2007) and can transmit the plant pathogen which is associated with causing the citrus greening disease (HLB) (Halbert 1998); therefore, any reduction in phloem feeding behavior on susceptible citrus plants could subsequently reduce transmission of the plant pathogen. Bonani, Fereres, Garzo, Miranda, Appezzato-Da-Gloria, and Lopes (2010) using the electrical penetration graph technique, discovered that prior to the initiation of phloem ingestion, psyllids must first penetrate the phloem with their stylets and then perform phloem salivation behaviors which is the putative stage for HLB pathogen inoculation. Furthermore, Bonani et al. (2010) demonstrated that not all phloem salivation events lead to phloem ingestion and thus multiple phloem salivation events can occur before successful phloem ingestion takes place. Thus, considering that it sometimes requires multiple phloem events throughout the year by the adult psyllid to potentially transmit the bacteria associated with HLB (Boina et al. 2010), the use of Ifr against D. citri in an IPM program has much potential. Further research is needed to assess fungus-infected psyllids ability to acquire/transmit the HLB pathogen and especially also for their ability to disperse to other plants, now that reduced feeding has been shown.

Although Ifr may not kill psyllids as rapidly as a traditional insecticide (Boina et al. 2009), the net effect of the fungus may be similar because infected individuals feed less. Our results are corroborated with similar reports that show reduced feeding in insects infected with entomopathogenic fungi (Mohamed et al. 1982; Hajek 1989; Ali et al. 2010). In contrast, some studies have shown that fungus-infected insects may actually increase their feeding rates in response to nutrient depletion because fungi thrive on the insect’s hemolymph (Noma and Strickler 2000).

Any reduction in feeding activity on susceptible citrus plants could subsequently reduce transmission of the plant pathogen; however, it remains to be determined if the observed reductions in feeding activity of psyllids treated with Ifr would be enough to reduce transmission rates of the HLB pathogen. In support of this hypothesis, after testing candidate entomopathogenic fungi, Lacey, de la Rosa, and Horton (2009) found that the transmission of a similar pathogen (Candidatus Liberbacter solanacearum) responsible for ‘zebra chip’ disease of potatoes vectored by the potato psyllid Bactericera cockerelli was significantly reduced when the psyllids were infected with Ifr (Lacey et al. 2011).
Our study documented that adult psyllids infected by Ifr (PFR 97) produce less honeydew than healthy psyllids and suggests that they may feed less, which could potentially reduce the spread of HLB. This study further documented that, under laboratory conditions, Ifr (PFR 97) can be effective against psyllids causing up to 100% mortality within 7 days. Blastospores caused significantly higher mortality of D. citri than conidia within the first 2 days probably due to faster germination rates and successive colonization by blastospores, but ultimately the two formulations caused the same level of mortality. However, reduced feeding by fungus-infected psyllids could account for only part of the equation; the ability of fungus-infected psyllids to acquire the HLB pathogen (while displaying reduced feeding), disperse to a new plant, and vector HLB-causing bacteria are needed to substantiate that the rates of HLB transmission could be reduced in fungus-infected psyllids. Also, based on the results of this laboratory study, the use and replacement of an Ifr conidial formulation for the commercially available blastospore formulation for potential field management of this psyllid pest is not justified.

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