Effect of Mexican Hirsutella citriformis (Hypocreales: Ophiocordycipitaceae) Strains on Diaphorina citri (Hemiptera: Liviidae) and the Predators Chrysoperla rufilabris (Neuroptera: Chrysopidae) and Hippodamia convergens (Coleoptera: Coccinellidae)

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Effect of Mexican *Hirsutella citriformis* (Hypocreales: Ophiocordycipitaceae) strains on *Diaphorina citri* (Hemiptera: Liviidae) and the predators *Chrysoperla rufilabris* (Neuroptera: Chrysopidae) and *Hippodamia convergens* (Coleoptera: Coccinellidae)

Orquídea Pérez-González, Raúl Rodríguez-Guerra, J. Isabel López-Arroyo, Carlos Francisco Sandoval-Coronado, and María Guadalupe Maldonado-Blanco

Abstract

Due to its role in the transmission of *Candidatus Liberibacter* asiaticus, a pathogen associated with huanglongbing, a catastrophic disease of citrus in the world, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) has become a very dangerous invasive pest. To contribute to increasing alternatives for its management, we evaluated against *D. citri* adults the pathogenicity of conidia and blastospores of 8 Mexican strains of the entomopathogenic fungus *Hirsutella citriformis* Speare (Hypocreales: Ophiocordycipitaceae). Furthermore, we conducted tests with non-target insects that included the predators *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) and *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae). Experiments in the laboratory included the application of the fungus by contact and through liquid sprays. Strains were collected from citrus areas of Mexico. They were grown in PDAY media and applied at 1 × 10⁶ conidia per mL. All bioassays were conducted under controlled conditions at 26 ± 1 °C, 16:8 h L:D photoperiod, and 76 ± 4% RH and maintained during 26 d after inoculation. Tests with *D. citri* were performed 3 times at different dates. Mean mortality by *H. citriformis* strains on *D. citri* adults ranged from 82 to 92%; INIFAP-Hir-1 strain produced the highest rate. Sprayed conidia produced 69% mortality. Use of sprayed blastospores caused 32 to 49 % mortality. LC₅₀ obtained for INIFAP-Hir-1 strain was 3.4 × 10⁵ conidia per mL. IB-Hir-1, IB-Hir-2, INIFAP-Hir-1, and INIFAP-Hir-2 strains inoculated by contact on *H. convergens* adults caused 9 to 11% mortality; in larvae of *C. rufilabris*, mortality ranged from 19 to 25%. In both tests, there were no statistical differences when compared with the untreated control. Unlike *D. citri*, all dead predator specimens showed absence of *H. citriformis* mycosis. The results suggest potential for the integration of this fungus in the management of *D. citri*. Data obtained from the predators could support safe use of this biological control agent.

Key Words: huanglongbing; Asian citrus psyllid; biological control; non-target insect

Resumen

Debido a su función en la transmisión de *Candidatus Liberibacter* asiaticus, un patógeno asociado al Huanglongbing, una enfermedad catastrófica de los citricos en el mundo, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) se ha convertido en una plaga invasiva muy peligrosa. Para contribuir a incrementar las alternativas para su manejo, evaluamos la patogenicidad de conidias y blastosporas de 8 cepas mexicanas del hongo entomopatógeno *Hirsutella citriformis* Speare (Hypocreales: Ophiocordycipitaceae) contra adultos de *D. citri*; además realizamos pruebas contra insectos no blancos, los depredadores *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) y *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae). Los experimentos en laboratorio incluyeron aplicación del hongo por contacto y mediante aspersiones. Las cepas fueron colectadas en diferentes áreas cítricas de México, cultivadas en medio PDAY y asperjadas a 1 × 10⁶ conidias/mL; todos los bioensayos fueron condujidos bajo condiciones controladas a 26 ± 1 °C, 16:8 L:D, 76 ± 4% HR y manteniendo durante 26 días después de la inoculación; las pruebas contra *D. citri* fueron realizadas 3 veces en diferentes fechas. La mortalidad causada por *H. citriformis* contra adultos de *D. citri* fue 82-92%; la cepa INIFAP-Hir-1 registró el mayor porcentaje. Las conidias asperjadas produjeron 69% de mortalidad; mientras que el uso de blastosporas causó 32-49%. La LC₅₀ obtenida para la cepa INIFAP-Hir-1 fue 3.4 × 10⁵ conidias/mL. Las cepas IB-Hir-1, IB-Hir-2, INIFAP-Hir-1 e INIFAP-Hir-2 inoculadas por contacto a adultos de *H. convergens* causaron 9-11% de mortalidad; en larvas de *C. rufilabris* fue de 19-25%; en ambas pruebas no existieron diferencias estadísticas con respecto al testigo. A diferencia de *D. citri*, todos los especímenes de depredadores muertos mostraron ausencia de micosis por *H. citriformis*. Los resultados sugieren potencial para la integración de este hongo en el manejo de *D. citri*; datos obtenidos de los depredadores podrían respaldar el uso seguro de este agente de control biológico.

Palabras Clave: huanglongbing; psilídio asiático de los citricos; control biológico; insectos no blanco

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Diaporhina citri Kuwayama (Hemiptera: Livididae) is one of the most important invasive pests in the citrus industry of the world; this qualification has been assigned mainly by its role as a vector of Candidatus Liberibacter, a bacterium associated with huanglongbing (Bové 2006; Yang et al. 2006). Nowadays, huanglongbing is considered as one of the most devastating diseases that plague the crop worldwide; its management has been achieved basically through the elimination of infected plants, use of certified plant material, and vector control (Bové 2006; Gottwald et al. 2007; National Research Council 2010; da Graça et al. 2015). Presently, effective eradication methods against the rapidly dispersing vector are unavailable (Bové 2006). Management of D. citri has been constrained to the use of chemical control (Yang et al. 2006; Belasque et al. 2010; Stansly et al. 2013; Qureshi et al. 2014). However, intensive use of pesticides has begun to produce collateral effects that are noticed in resistance selection to insecticides in the vector (Yang et al. 2006; Tiwari et al. 2011), resurgence of controlled key pests, and outbreaks of secondary pests (Yang et al. 2006; Monzó et al. 2012).

Vector management needs to be addressed toward the integration of methods that allow a reduction in the use of insecticides as well as in insect population density and huanglongbing infection and dispersion rate (Yang et al. 2006; Stansly et al. 2013). Some beneficial insects (Aubert 1990; Høy & Nguyen 2001; Michaud 2004; López-Arroyo et al. 2009; Qureshi & Stansly 2009; Cortez-Mondaca et al. 2010) and entomopathogens have been considered as biological control agents of D. citri (Subandiay et al. 2000; Yang et al. 2006; Meyer et al. 2007; Avery et al. 2009). Easy integration of fungi with pesticides has motivated the exploration of diverse fungal species, e.g., Beauveria bassiana (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) (Padulla & Alves 2009; Gandarilla-Pacheco et al. 2013; Casique-Valdes et al. 2015), Hirsutella citriformis Speare (Hypocreales: Ophiocordycipitaceae) (Subandiay et al. 2000; Meyer et al. 2007; Casique-Valdes et al. 2011; Hall et al. 2012; Romero-Rangel et al. 2012; Cortez-Madrigal et al. 2014; Orduño-Cruz et al. 2015; Perez-Gonzalez 2015a), Isaria fumosorosea (Wailes) (Hypocreales: Fumosoroseaceae) (Yang et al. 2006; Stansly et al. 2013; Bové et al. 2010; Qureshi & Stansly 2009; Cortez-Mondaca et al. 2010) and entomopathogenic fungi, even in those tested against D. citri (Mena et al. 2003; Høy et al. 2010). Thus, this factor may allow identifying more aggressive strains with the potential to cause increased mortality in D. citri while it proves to be harmless to other non-target insects.

The objective of this study was to evaluate 8 native strains of H. citriformis, obtained from diverse areas of the Mexican citiculture, against D. citri adults, and to test non-target effects in the predators Chrysoperla rufilabris (Burmeister) (Neuroptera: Chrysopidae) and Hippodamia convergens Guérin-Méneville (Coleoptera: Coccinellidae), beneficial insects that attack D. citri in areas where the fungus has been found in Mexico (Cortez-Mondaca et al. 2010).

Materials and Methods

Fungal Strains

Eight H. citriformis strains obtained from 8 citrus-producing areas of Mexico were used (Table 1). Six strains were deposited in the National Institute of Forestry, Agriculture, and Livestock (INIFAP) and 2 in the Biotechnology Institute at the Biological Sciences Department, Nuevo León Autonomous University (IB-UNAL). Strains were grown in Petri dishes containing potato dextrose agar medium (PDA) with 0.5% yeast extract (PDAY); they were incubated for 6 to 7 wk at 25 ± 1 °C until conidia production.

Experimental Insects

Adults of D. citri were collected with a buccal plastic aspirator from shoots of Valencia orange trees from a 3-yr-old commercial orchard. Collected insects were transported to the laboratory and those that appeared healthy were selected for the bioassays. They were anesthetized using a cotton ball impregnated with 80 μL chloroform before being subjected to the treatments.

Adults of H. convergens were collected in the field and transported to the laboratory, where they were maintained in separate cages to eliminate those that were parasitized or damaged. For feeding, they received frozen eggs of Sitotroga cerealella Olivier (Lepidoptera: Gelechiidae) and a source of water mixed with honey. Larvae of C. rufilabris were the 3rd generation of field-collected females maintained in the laboratory with water and a volumetric mixture of yeast, sugar, honey, and powdered milk used as artificial diet. Larvae were fed with S. cerealella frozen eggs. All the insects were maintained under laboratory conditions at 26 ± 1 °C, 60 ± 4% RH, and a 16:8 h L:D photoperiod.

Bioassays Against D. Citri Adults

Test of Conidia Inoculated by Contact

Anesthetized D. citri adults were transferred to Petri dishes containing sporulating cultures of the 8 strains, where they remained for

### Table 1. Hirsutella citriformis monoclonal strains isolated from Diaporhina citri obtained from 8 citrus-growing areas of Mexico.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Locality</th>
<th>Date of collection</th>
<th>Host plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>INIFAP-Hir-1</td>
<td>Huimanguillo, Tabasco</td>
<td>Aug 2008</td>
<td>Citrus sinensis (L) Osbeck</td>
</tr>
<tr>
<td>INIFAP-Hir-2</td>
<td>Mocochá, Yucatán</td>
<td>Sep 2011</td>
<td>Citrus tangerina Tanaka</td>
</tr>
<tr>
<td>INIFAP-Hir-4</td>
<td>Xolol, San Luis Potosí</td>
<td>Oct 2009</td>
<td>Citrus sinensis (L) Osbeck</td>
</tr>
<tr>
<td>INIFAP-Hir-5</td>
<td>Nuevo Israel, Quintana Roo</td>
<td>Jan 2010</td>
<td>Murraya paniculata (L) Jack</td>
</tr>
<tr>
<td>INIFAP-Hir-6</td>
<td>Edzná, Campeche</td>
<td>Sep 2011</td>
<td>Murraya paniculata (L) Jack</td>
</tr>
<tr>
<td>INIFAP-Hir-7</td>
<td>Tapachula, Chiapas</td>
<td>Dec 2011</td>
<td>Murraya paniculata (L) Jack</td>
</tr>
<tr>
<td>IB-Hir-1</td>
<td>Tapacoyan, Veracruz</td>
<td>Sep 2011</td>
<td>Citrus latifolia Tan.</td>
</tr>
</tbody>
</table>

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1 cm² were grown on PDA in Petri dishes at 25 ± 1 °C for 2 wk. Thereafter, four insects were sprayed against the same procedure as in the spray of conidia that was described previously. Sterile water was used for the untreated control. To avoid cannibalism, we added in each cage a zig-zag folded paper strip (length: 10 cm; width: 1 cm) that acted as a barrier between the larvae. There were 5 replications per treatment for H. convergens and 8 replications per treatment for C. rufilabris. After inoculation, insects were maintained during 24 d for H. convergens and 30 d for C. rufilabris under the environmental conditions described before for D. citri. Every other day, we examined all the insects, removed dead specimens, changed the Petri dish and the paper strip, and provided the food indicated before. Retrieved dead insects were placed in a humidity chamber to promote or verify mycelial growth from the evaluated fungal strains.

### BIOASSAY AGAINST INSECT PREDATORS

Strains INIFAP-Hir-1, INIFAP-Hir-2, IB-Hir-1, and IB-Hir-2 were used for bioassays against H. convergens adults and C. rufilabris 1st instar larvae. Both predator species were inoculated in the same way as D. citri in the infection by contact test. In the case of H. convergens, for each experimental replication there were cohorts of 10 specimens kept in a Petri dish. For C. rufilabris, we used a pair of larvae per Petri dish; to avoid cannibalism, we added in each cage a zig-zag folded paper strip (length: 10 cm; width: 1 cm) that acted as a barrier between the larvae. There were 5 replications per treatment for H. convergens and 8 replications per treatment for C. rufilabris. After inoculation, insects were maintained during 24 d for H. convergens and 30 d for C. rufilabris under the environmental conditions described before for D. citri. Every other day, we examined all the insects, removed dead specimens, changed the Petri dish and the paper strip, and provided the food indicated before. Retrieved dead insects were placed in a humidity chamber to promote or verify mycelial growth from the evaluated fungal strains.

### DATA ANALYSES

One-way ANOVA was performed to analyze mortality data from the bioassays. Before analysis, data were transformed by using arcsin (sin⁻¹√x + 1). Treatment means were compared using Tukey’s test (α = 0.05) (SPSS 2008). We used a computerized program (US Applied and Environmental Health, 1989) to run probit analysis to determine LC50 and LC90 for the INIFAP-Hir-1 strain; for this analysis, data were transformed by using arcsin (sin⁻¹√x + 1).

### Results

#### VIRULENCE AGAINST D. CITRI

All H. citriformis strains were able to kill D. citri adults in the contact bioassays (Fig. 1; Table 2). Mortality was observed beginning 6 d post exposure to conidia in a range from 1.8 to 8.2%, with the highest mortality in insects exposed to strain INIFAP-Hir-1 and the lowest in

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**Fig. 1.** Overall mean mortality of Diaphorina citri adults caused by conidia of 8 Hirsutella citriformis strains applied by contact (3 separate bioassays) under controlled conditions (26 ± 1 °C, 76 ± 4% RH, 16:8 h L:D photoperiod). Error bars represent the standard error (n = 7).
mortality in the untreated control was 4.1% (Fig. 3). At 12 d post application and ranged from 6.2% (INIFAP-Hir-2) to 13.9% (INIFAP-Hir-1), whereas Diaphorina citri, 20.5 to 62.7%.

We observed 23.48; df = 2, 18; significance higher than that recorded in the untreated control (F(17.6%) (Table 3). Observed mycosis by H. citriformis ranged from 9% (INIFAP-Hir-1) to 11% (INIFAP-Hir-2) after 24 d of conidia application; these percentages did not differ significantly from that recorded in the control (P = 0.953) (Table 3). Dead specimens did not develop mycosis after being placed in a humidity chamber. In C. rufilabris, overall mortality by H. citriformis 30 d after application in larvae and recorded in emerged adults ranged

### Table 2. Mortality of Diaphorina citri caused by conidia of 8 Hirsutella citriformis strains applied by contact (3 separate bioassays) under controlled conditions (26 ± 1 °C, 76 ± 4% RH, 16:8 h L:D photoperiod).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Bioassay 1</th>
<th>Bioassay 2</th>
<th>Bioassay 3</th>
<th>% ± 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.08 ± 1.02e</td>
<td>12.00 ± 0.89d</td>
<td>18.50 ± 0.93c</td>
<td>0.0</td>
</tr>
<tr>
<td>INIFAP-Hir-1</td>
<td>98.33 ± 3.13a</td>
<td>89.20 ± 5.89a</td>
<td>88.07 ± 2.56a</td>
<td>80.6 ± 29.6</td>
</tr>
<tr>
<td>INIFAP-Hir-2</td>
<td>82.48 ± 3.46abc</td>
<td>83.55 ± 3.43a</td>
<td>81.70 ± 3.53a</td>
<td>80.5 ± 29.3</td>
</tr>
<tr>
<td>INIFAP-Hir-4</td>
<td>73.78 ± 6.90bc</td>
<td>80.55 ± 5.12ab</td>
<td>80.00 ± 3.93a</td>
<td>78.3 ± 30.5</td>
</tr>
<tr>
<td>INIFAP-Hir-5</td>
<td>64.88 ± 6.26cd</td>
<td>52.12 ± 3.92c</td>
<td>58.50 ± 3.19b</td>
<td>48.7 ± 37.0</td>
</tr>
<tr>
<td>INIFAP-Hir-6</td>
<td>50.78 ± 4.05d</td>
<td>70.63 ± 3.14ab</td>
<td>43.00 ± 2.40b</td>
<td>61.3 ± 36.1</td>
</tr>
<tr>
<td>INIFAP-Hir-7</td>
<td>85.76 ± 2.23ab</td>
<td>69.10 ± 2.21b</td>
<td>30.31 ± 3.10b</td>
<td>54.6 ± 36.9</td>
</tr>
<tr>
<td>IB-Hir-1</td>
<td>79.73 ± 6.64bc</td>
<td>89.20 ± 5.89a</td>
<td>85.62 ± 4.06a</td>
<td>73.0 ± 32.9</td>
</tr>
<tr>
<td>IB-Hir-2</td>
<td>87.43 ± 3.87ab</td>
<td>89.30 ± 2.17a</td>
<td>87.68 ± 2.53a</td>
<td>81.2 ± 28.9</td>
</tr>
</tbody>
</table>

Different letters within columns indicate significant differences (Tukey’s test, α = 0.05). SE = Standard error. CI= Confidence interval.

When conidia were applied by spraying, INIFAP-Hir-1 and IB-Hir-2 strains caused 69% overall mortality in D. citri; this value was statistically different from that recorded in the control (17.6%) (F(17.6%) = 4.68; df = 5.36; P = 0.002; Tukey’s test, α = 0.05) (Fig. 3). Occurrence in humid chambers of mycosis by H. citriformis in D. citri dead specimens from the treatments with blastospore application was very low, approximately 10%; in the case of cadavers from the control, mycosis was null. Probit analysis showed that LC50 for strain INIFAP-Hir-1 was 3.40 × 10⁸ conidia per mL, with 95% confidence interval 1.38 to 8.51 × 10⁸ conidia per mL. LC90 was 2.67 × 10⁹ conidia per mL, with 95% confidence interval 2.18 × 10⁸ to 2.44 × 10⁹ conidia per mL. The regression equation obtained was y = 4.31 + 0.44x (R² = 0.7055; P = 0.05).

### PATHOGENICITY AGAINST PREDATORS

Overall mortality by H. citriformis in H. convergens adults ranged from 9% (INIFAP-Hir-1) to 11% (INIFAP-Hir-2) after 24 d of conidia application; these percentages did not differ significantly from that recorded in the untreated control (F(17.6%) = 4.68; df = 5.36; P = 0.002; Tukey’s test, α = 0.05) (Fig. 3). This test, we observed H. citriformis mycosis in dead specimens ranging from 20.5 to 62.7%.

In the test of H. citriformis blastospores in spray inoculation against D. citri, initial mortality was observed 6 d after the fungus application and ranged from 6.2% (INIFAP-Hir-2) to 13.9% (INIFAP-Hir-1), whereas mortality in the untreated control was 4.1% (Fig. 3). At 12 d post application, H. citriformis blastospores caused 20.4 to 38.0% mortality of D. citri (Fig. 3). After 26 d, the INIFAP-Hir-2 and INIFAP-Hir-5 strains sprayed as blastospores produced the highest overall mortality (48.7%) in D. citri; this value was statistically different from that recorded in the control (17.6%) (F(17.6%) = 4.68; df = 5.36; P = 0.002; Tukey’s test, α = 0.05) (Fig. 3). Occurrence in humid chambers of mycosis by H. citriformis in D. citri dead specimens from the treatments with blastospore application was very low, approximately 10%; in the case of cadavers from the control, mycosis was null. Probit analysis showed that LC50 for strain INIFAP-Hir-1 was 3.40 × 10⁸ conidia per mL, with 95% confidence interval 1.38 to 8.51 × 10⁸ conidia per mL. LC90 was 2.67 × 10⁹ conidia per mL, with 95% confidence interval 2.18 × 10⁸ to 2.44 × 10⁹ conidia per mL. The regression equation obtained was y = 4.31 + 0.44x (R² = 0.7055; P = 0.05).

![Fig. 2. Mean mortality of Diaphorina citri caused by conidia sprays of 2 Hirsutella citriformis strains under controlled conditions (26 ± 1 °C, 76 ± 4% RH, 16:8 h L:D photoperiod). Different letters indicate significant differences (Tukey’s test, α = 0.05). Error bars represent the standard error (n = 7).](https://bioone.org/journals/Florida-Entomologist/article-pdf/10.1623/fe-99-3-512-521/44955/941290bd5f51c07d97a3b0b40d92e489e5f137e0/10.1623/fe-99-3-512-521.pdf)

![Fig. 3. Mean mortality of Diaphorina citri caused by blastospores of 5 Hirsutella citriformis strains under controlled conditions (26 ± 1 °C, 76 ± 4% RH, 16:8 h L:D photoperiod) during 26 d post inoculation. Different letters indicate significant differences (Tukey’s test, α = 0.05). Error bars represent the standard error (n = 7).](https://bioone.org/journals/Florida-Entomologist/article-pdf/10.1623/fe-99-3-512-521/44955/941290bd5f51c07d97a3b0b40d92e489e5f137e0/10.1623/fe-99-3-512-521.pdf)
Control 18.7 ± 0.5 (5) 10.6 ± 0.6 (8)

**Discussion**

In our study, all the tested Mexican *H. citriformis* strains showed potential as biological control agents of *D. citri* as they performed well in the bioassays using inoculation by conidia contact, by which they caused high mortality rates that were close to 90% (Table 2). Similar to the results of Meyer et al. (2007), we observed that mortality of *D. citri* specimens began 6 d after exposure to the fungus (Fig. 1); occurrence of characteristic *H. citriformis* synnemata on *D. citri* cadavers was observed 10 d after inoculation, which agreed with results by Casique-Valdés et al. (2011); however, the highest accumulated mortality (90%; Fig. 1; Table 2) in our study was recorded until 26 d after the application of the fungal strains, whereas Casique-Valdés et al. (2011) and Orduño-Cruz et al. (2015) reported 100% mortality just 6 d after inoculation, and Meyer et al. (2007) obtained the same rate at 9 d post inoculation. These differences in achieving the highest mortality could be due to genetic variability of the evaluated strains that could be associated with high pathogenicity as observed in many pathogens (see Mena et al. 2003; Hoy et al. 2010); hence, we were expecting some strain variability.

In addition, variations in bioassay procedures such as the use of different fungal conidia doses could explain the above differences; in Meyer et al. (2007) and Casique-Valdés et al. 2011, doses were unreported, and we realized based on the size of conidia (see Speare 1920; Perez-Gonzalez et al. 2015b) that Orduño-Cruz et al. (2015) could have overestimated the number of conidia attached to the insect and consequently used a very high dose of conidia, probably far beyond to the LC90 of 2.6 × 10^7 conidia per mL estimated in our bioassay with sprayed conidia. Meyer et al. (2007) and Casique-Valdés et al. (2011) might have used a dose in these amounts. It is also possible that the first dead specimens observed in our trial were those that were infected with a greater number of conidia and that those that died later barely had received a lethal dose. Such differences in the number of conidia per insect could be due mainly to the inoculation method by not providing a homogeneous presence of conidia on the insect body.

Nonetheless, we emphasize that the observed mortality curves (Fig. 1) may resemble a possible pattern for infected *D. citri* by *H. citriformis* in nature. Because the *H. citriformis* conidia are characteristically sticky (Speare 1920; Meyer et al. 2007; Perez-Gonzalez et al. 2015b), they would not spread readily as seen in other entomopathogenic fungal genera such as *Beauveria* or *Metarrhizium* (Mena et al. 2003; Padulla & Alves 2009). Thus, it is possible that *H. citriformis* would exhibit a behavioral manipulation of the host as it has been documented for *Ophiocordyceps unilateralis* (Tul. & C. Tul.) Petch (Hypocreales: Ophiocordycipitaceae) in ants (Andersen et al. 2009; Pontoppidan et al. 2009). *Hirsutella citriformis* could use the host as a vehicle for dispersion. For instance, numerous healthy hosts could be infected with a lethal, sublethal, or innocuous concentration of conidia that eventually will kill the insect through time and on several host plants, possibly far away from the initial site of infection. This scenario would reflect a possible strategy or adaptation of the fungus to spread and survive as suggested by Casique-Valdés et al. (2011) and Hall et al. (2012).

Research on *H. citriformis* has been neglected, and there are many uncovered areas of study. From the stated above, an essential need is to demonstrate the mechanisms involved in the possible host manipulation, as well as to understand completely its role at the insect population level and, therefore, the settings and development of epidemics.

In the bioassays in which conidia were applied by spraying, mortality caused by the 8 strains was remarkably reduced (Fig. 2). Such low values when conidia in suspension were used for inoculation was also observed by Orduño-Cruz et al. (2015) in *H. citriformis*. A possible explanation for these results could be the damage or removal of the water-soluble muclaginous envelope of conidia during the preparation of inoculum (Sánchez-Peña et al. 2011). These changes eventually could result in poor adherence to the cuticle of the host, as well as in low germination, decreased virulence, and consequently reduced host mortality as the one that we obtained in our tests (Fig. 2). We used acacia gum to overcome such factor; however, it was ineffective and it has prompted the need for further research in application techniques of the fungus that could support its successful use in the field.

The LC50 and LC90 determined for the strain INIFAP-Hir-1 in our study (3.4 × 10^6 and 2.6 × 10^7 conidia per mL respectively), applied by spraying of conidia, are the first reported for *H. citriformis*. Hoy et al. (2010) determined for *I. fumosorosea* against *D. citri* LC50 and LC95 values of 6.8 × 10^6 and 2.2 × 10^7 conidia per mL, respectively. Although the LC values are relatively similar for both fungal species, *H. citriformis* due to its slow development would be more expensive to use in an extensive way. For example, the conidia of the *H. citriformis* strains used herein germinated after 48 to 72 h (O. P. G., unpublished data) and growth is reportedly slow (radial growth = 0.083 to 0.114 cm/d) (Meyer et al. 2007) and growth is reportedly slow (radial growth = 0.083 to 0.114 cm/d) (Meyer et al. 2007; Pérez-González et al. 2015a). For *I. fumosorosea*, Vu et al. (2009) indicated conidia germination within 12 to 24 h, and according to Cabanillas & Jones (2009), this fungus showed fast radial growth (0.33 cm/d); both traits were at least 3 times greater than in *H. citriformis*. With the estimated LC50 and LC90 for *H. citriformis* against *D. citri* as reference, its potential use for biological control of this insect requires investigation to facilitate the commercial production of the fungus and make it economically viable.

In the evaluation of 5 *H. citriformis* Mexican strains as blastospores, we found that they induced low mortality against *D. citri*. Similar results were obtained previously by Pérez-González et al. (2015a). In our bioassays, the average mortality ranged from 31 to 48% (Fig. 3), and mycosis occurred only in a small number of insects. A higher mortality in *D. citri* with the use of blastospores was reported by Orduño-Cruz et al. (2015); they observed 61 to 65% mortality caused by 2 strains of *H. citriformis* isolated from unidentified species of insects; mycosis data were unavailable. Low values of mortality and mycosis produced by blastospores recorded in this study may have been caused in part by toxins released during *H. citriformis* liquid-culture growth, as reported by Liu et al. (1995) and Rosas-Acevedo et al. (2003) for strains of *Hirsutella thompsonii* F. E. Fisher. Our mortality data suggest that the application of conidia for control of *D. citri* is preferable over the use of blastospores; however, it is important to continue determining conditions under which this fungus develops infective and virulent blastospores against *D. citri* as their use could be more practical and...
they could be produced in high numbers in liquid culture in a short time (Avery et al. 2009; Romero-Rangel et al. 2012).

Tests of *H. citriformis* conidia inoculation by contact against *H. convergens* adults and *C. rufilabris* larvae produced low mortality without significant differences among treatments, including the untreated controls (Table 3). Data concerning *H. convergens* adults were similar to those reported by Gandarilla-Pacheco et al. (2012) in tests with *B. bassiana* and *I. fumosorosea*, in which they recorded 6 to 25% mortality. In contrast, the effect on *C. rufilabris* (18 to 25% mortality; Table 3) was lower than that reported by these authors for *Ceraeochrysa valida* (Banks) and *Eremochrysa punctinervis* (McLachlan) (Neuroptera: Chrysopidae) larvae, for which they recorded 50% mortality caused by a local strain of *I. fumosorosea* (Gandarilla-Pacheco et al. 2012).

Our study confirms the value of including tests for non-target effects. We here evaluated 4 *H. citriformis* strains under a hypothetical scenario of causing high mortality in diverse organisms different to our target. Hence, we would have the opportunity to select a possible fungal strain that produces no harmful effects for other organisms, thereby evading antagonistic interactions (Thomas & Lynch 2003) and allowing a relatively safe use for our evaluated agent for biological control. As there are many species of arthropods associated with citrus (Monzó et al. 2012), and as we tested the fungus in only 2 beneficial entomophagous insect species, it would be imprecise to state that *H. citriformis* appears to be a specific natural enemy for *D. citri*. Instead, we suggest further studies of this valuable natural enemy aimed to generate in the short term knowledge for its successful and safe use as a biological control agent. Plans should be made for its integration with other beneficial arthropods for a sustainable management of *D. citri* (Thomas & Lynch 2003), generating in this way alternatives to the present and intense use of pesticides against the vector of huanglongbing.

We conclude that the 8 Mexican fungal strains of *H. citriformis* tested against *D. citri* were pathogenic to the Asian citrus psyllid adults, with variability in performance associated with strain diversity. Among the strains, INIFAP-Hir-1 showed constantly favorable results. At the present time, use of conidia seems to be the best method for application of this fungus; the use of blastospores produced in liquid culture requires further investigation to determine optimal conditions to develop infective and virulent propagules against *D. citri*. Our data obtained from tests against the predators *C. rufilabris* and *H. convergens* could support the use of *H. citriformis* as a biological control agent of *D. citri*.

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