The Consumption and Survival Rate of Lilioceris cheni (Coleoptera: Chrysomelidae) on Air Potato Leaves Exposed to Cordyceps javanica (Hypocreales: Cordycipitaceae)

Authors: Kariuki, Eutychus M., Lovo, Erika E., Price, Terri, Parikh, Vraj, Duren, Emily B., et al.

Source: Florida Entomologist, 105(3) : 258-261

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.105.0313

The consumption and survival rate of *Lilioceris cheni* (Coleoptera: Chrysomelidae) on air potato leaves exposed to *Cordyceps javanica* (Hypocreales: Cordycipitaceae)

Eutychus M. Kariuki[1], Erika E. Lovo[1], Terri Price[1], Vraj Parikh[1], Emily B. Duren[1], Pasco B. Avery[1], and Carey R. Minteer[1,]*

Lilioceris cheni Gressitt and Kimoto (Coleoptera: Chrysomelidae) is a highly effective biological control agent for the invasive vine *Dioscorea bulbifera* L. (Dioscoreaceae) in Florida (Rayamajhi et al. 2019). The vine has invaded most of Florida and many other southeastern US states, and can displace native vegetation in the absence of control measures (Overholt et al. 2016; EDDMapS 2020). *Lilioceris cheni* is established in more than 70% of Florida counties; both adults and larvae skeletonize the leaves and occasionally feed on the bulbils of *D. bulbifera* (Rayamajhi et al. 2019).

Cordyceps javanica (Frieder. & Bally) Kepler, B. Shrestha & Spatafora (Hypocreales: Cordycipitaceae) is a fungus contained in biopesticides labeled for control of aphids, psyllids, spider mites, thrips, and whiteflies (Mascarin et al. 2019). The fungus is a potential pest control tool in citrus groves in Florida (Hunter et al. 2011), ornamentals (Hussain et al. 2009), and soybeans (Correa et al. 2020). However, *Cordyceps* species also can infect certain chrysomelid species (Demir et al. 2013; Hussein et al. 2016), but its effect on *L. cheni* is unknown.

Pesticides can drift extensively during application (Woods et al. 2001; Garcerá et al. 2017), for instance up to 195 m downwind in citrus groves (Salyani & Cromwell 1992), and adversely affect weed biological control agents (Wheeler et al. 2020). Therefore, the present study investigated the effects of *C. javanica* on *L. cheni* that might occur via spray drift or overspray. Specifically, leaf consumption and survival of *L. cheni* provisioned with air potato leaves exposed to *C. javanica* under laboratory-controlled conditions were measured.

A stock suspension of *C. javanica* was prepared by mixing 1g of the biopesticide PFR-97™ 20% WDG product (20% *C. javanica* Apopka strain 97; Certis USA, Columbia, Maryland, USA) to 100 mL of sterile distilled water and allowed to rest for 30 min to precipitate. The supernatant was diluted serially with sterile distilled water to concentrations (treatments) of 10^6, 10^5, 10^3, and 10^2 blastospores mL^{-1}. The treatments simulated overspray or spray drift as 10^6 blastospores mL^{-1} as the label application rate. The concentrations were verified using a disposable plastic C-Chip™ DHC Neubauer hemocytometer (Incyto Co., Ltd., Chungnam-do, Seoul, South Korea). *Dioscorea bulbifera* leaves were obtained from an outdoor screenhouse and adults of *L. cheni* were obtained from a laboratory colony that was located at the University of Florida, Indian River Research and Education Center, Fort Pierce, Florida, USA.

A randomized complete block design was used with 6 repetitions for each treatment per block. Five blocks were run over the course of 15 Jul to 15 Oct 2020, for a total of 30 replications per treatment. In each experimental unit, a randomly chosen adult (male or female) of *L. cheni* (1- to 14-d old and starved for 24 h) was placed individually in a bioassay arena, a 150 mm × 15 mm plastic Petri dish, inside an environmental chamber and provisioned with a *D. bulbifera* leaf treated with 1 of the 4 *C. javanica* treatments or sterile distilled water (control) treatment.

To prepare the leaves, 11 freshly detached leaves (6 for the bioassays, 5 for determining spore coverage) were placed adaxial side up along with 4 plastic coverslips. Each leaf and coverslip on the tray were sprayed with the suspension or distilled water (control) to the point of runoff using a Nalgene® hand-pump aerosol sprayer (Nalgene Nunc Intl., Rochester, New York, USA). The sprayed leaves and coverslips were allowed about 3 h to air-dry on the laboratory bench, and then the leaves were transferred into the bioassay arenas. Coverslips were placed individually in separate Petri dishes (100 × 15 mm) containing dry filter paper (90 mm diam) for later processing. Bioassays for the control treatment always were processed first to prevent risk of cross contamination.

The bioassay arena chambers for each treatment received a treated *D. bulbifera* leaf placed on top of a filter paper that was moistened with 8 mL sterile distilled water and a *L. cheni* adult. The bioassay arenas were covered with a lid, sealed with Parafilm® M (Bemis Company, Inc., Neenah, Wisconsin, USA) for the first 24 h to maintain about 100% RH required for the blastospores of *C. javanica* to germinate. The Parafilm was then removed for the remainder of the study. The bioassay arenas were held in an environmental chamber that was set to 26 °C, with 70% RH, under a 14:10 h (L:D) photoperiod. Beetles were exposed to *D. bulbifera* treated leaves for 72 h, then the treated leaf was replaced with a fresh untreated leaf. Leaves were replaced every 72 h for an additional 18 d for a total observational period of 21 d to allow sufficient time for *C. javanica* to infect the beetles (Avery et al. 2016). Beetle survival was monitored daily. The leaf area consumed by the beetles was measured after every 72 h; leaves were retrieved from the bioassay arenas, scanned into digital formats, and analyzed for area of leaf consumed using an imaging processing program, ImageJ Fiji™ (National Institutes of Health, http://imagej.net) (Schindelin et al. 2015).

The blastospores of *C. javanica* deposited per unit area were calculated by examining 10 random swaths per coverslip under a microscope at the 400× magnification as described by Avery et al. (2019). The colony forming units per treated leaf were determined for 5 ad-

[1]University of Florida, Indian River Research and Education Center, 2199 South Rock Road, Fort Pierce, Florida 34945-3138, USA; eutychusmukure@gmail.com (E. M. K.), elovozelaya@gmail.com (E. E. L.), terriprice@ufl.edu (T. P.), vparikh@ufl.edu (V. P.), bliss@ufl.edu (E. B. D.), pbavery@ufl.edu (P. B. A.), c.minteerkillian@ufl.edu (C. R. M.)
*Corresponding author; E-mail: c.minteerkillian@ufl.edu

ditional leaves per treatment using a leaf press technique. Sprayed leaves were pressed on selective potato dextrose agar (PDA-dodine) in plastic Petri dishes (100 × 15 mm) containing 2 bactericides (streptomycin and chloramphenicol at 0.1%) and 0.5% dodine (Dodin Pestanal; Sigma Aldrich, Inc., Saint Louis, Missouri, USA). Leaves were pressed on the adaxial side for 1 to 2 min on the agar, then removed and immediately placed into a resealable plastic bag for scanning the surface area using a flat-top Epson Perfection V19 scanner (Epson America, Inc., Los Alamitos, California, USA). Scanning of leaves was performed by placing a ruler next to each leaf in preparation for using ImageJ Fuji™. Plates were sealed using Parafilm and placed into the growth chamber set at 25 °C under a 14:10 h (L:D) photophase for 14 d to allow colony forming units to grow and be counted.

After ensuring that statistical test assumptions were satisfied, mean survival times (d) for the beetles were compared using an ANOVA, and if significant, post hoc mean values were compared using a Tukey's HSD test ($\alpha = 0.05$). All statistical analyses for mean survival times were conducted using SAS Proc GLM procedures executed on a PRO_WIN 6.1 platform (SAS 2002–2012; SAS Institute Inc., Cary, North Carolina, USA). Data for mean leaf consumption were analyzed by d and compared using an ANOVA with the SAS Proc GLIMMIX program (Version 9.4; SAS Institute Inc., Cary, North Carolina, USA).

The mean ± SEM of *C. javanica* blastospores deposited on the coverslips for 10^2, 10^3, 10^5, and 10^6 blastospores mL^{-1} treatments were 15 ± 2.5, 67 ± 7.3, 213 ± 16.9, and 534 ± 60.0 blastospores mm^{-2}, respectively. No blastospores of *C. javanica* were observed on the coverslips of the control treatment. The mean ± SEM of colony forming units mm^{-2} on leaves of 10^2, 10^3, 10^5, and 10^6 blastospores mL^{-1} treatments were 0.041 ± 0.03, 0.044 ± 0.01, 0.700 ± 0.21, and 3.27 ± 0.83, respectively. No *C. javanica* colony forming units were found on the leaves in the control treatment. Leaf consumption (ANOVA: $F_{4, 1067} = 061$; $P = 0.6949$) (Fig. 1a) and mean survival time (ANOVA: $F_{4, 117} = 1.22$; $P = 0.2123$) (Fig. 1b) of *L. cheni* adults over the 21-d observation period did not differ statistically among treatments of *C. javanica* compared to the control treatment. The mean survival time of the beetles among the treatments ranged from 18.24 to 20.93 d (Fig. 1b). Results indicated the *L. cheni* adults could feed on air potato leaves sprayed with *C. javanica* at a concentration of 10^6 blastospores mL^{-1} (label rate) or lower without any significant effect on the survival time or feeding rate of the beetle.

Cordyceps javanica pathogenicity varies based on host species, host developmental stage, and dosage. For example, *C. javanica* at 10^6 blastospores mL^{-1} or lower caused a significant mortality to *Phalacrococcus howertoni* Hodges and Hodgson (Hemiptera: Coccidae) (Barahona et al. 2018), and larvae of *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae), (Montemayor et al. 2016), but not to adults of *M. ochroloma* (Gámez Herrera et al. 2016). Adults of beneficial insects such as *Thalassa montezumae* Mulsant (Coleoptera: Coccinellidae) (Barahona et al. 2018), *Delphastus catalinae* Horn (Coleoptera: Coccinellidae) (Avery et al. 2020), and *Dicyphus hesperus* Knight (Hemiptera: Miridae) (Alma et al. 2007) were compatible and not affected significantly by exposure to *C. javanica*-contaminated leaf residues.

Together, previous and present studies indicate *C. javanica* may be compatible with certain beneficial insects, an outcome that requires a case-by-case assessment. Specifically, the present study demonstrated that any inadvertent exposure of *L. cheni* adults to label or lower application rates of *C. javanica* via spray drift will not negatively impact the survival or leaf consumption of the adult beetles, and thus will unlikely interfere with the biological control of *D. bulbifera*. Future studies should examine the effects of *C. javanica* on the larvae of *L. cheni.*

The authors would like to thank the staff at the University of Florida's Biological Control Research and Containment Laboratory at Fort Pierce for their assistance with data collection. We also would like to thank Edzard van Santen for his assistance with data analysis. This research was funded by the Florida Department of Agriculture and Consumer Services and the NIFA/USDA Multi-State Hatch Research Project #FLA-IRC-005729; S1070: The Working Group on Improving Microbial Control of Arthropod Pests.

Summary

PFR-97 20% WDG (PFR-97) is a broad-spectrum entomopathogenic fungal biopesticide that contains the active ingredient *Cordyceps javanica* (Frieder. & Bally) Kepler, B. Shrestha & Spatafora (Hypocreales: Cordycipitaceae). This biopesticide is labelled for controlling aphids, psyllids, spider mites, thrips, and whiteflies in various crops, but also can infect certain chrysomelid species. Pest control activities applying PFR-97 in 1 agroecosystem may inadvertently drift and affect biological control agents purposefully released in another ecosystem. Therefore, our goal was to determine if PFR-97 was compatible when applied near *Lilioceris cheni* Gressitt and Kimoto (Coleoptera: Chrysomelidae), a chrysomelid biological control agent for the invasive air potato. The consumption and survival rates of adult beetles after exposure to PFR-97 were investigated via leaf bioassays. Thirty *L. cheni* adults were provisioned individually with a *Dioscorea bulbifera* L. (Dioscoreaceae) leaf sprayed with either a *C. javanica* suspension at the concentration of 10^6, 10^5, 10^3, or 10^2 blastospores per mL^{-1} or sterile distilled water (control) for 72 h. Untreated leaves were provided every 3 d for an additional 18 d and leaf consumption was determined. Results showed the survival and leaf consumption of the *L. cheni* adults did not differ among treatments and control. Overall, our study demonstrated that any inadvertent exposure of *L. cheni* adults to *C. javanica* via spray drift is unlikely to impact the performance of the adult biological control agents.

Key Words: air potato leaf beetle; entomopathogenic fungus; biological control; spray drift

Sumario

PFR-97 20% WDG (PFR-97) es un bioplaguicida fungifero entomopatógeno de amplio espectro que contiene el ingrediente activo *Cordyceps javanica* (Frieder. & Bally) Kepler, B. Shrestha & Spatafora (Hypocreales: Cordycipitaceae). Este biopesticida está etiquetado para el control de áfidos, psílidos, araña rojas, trips y mosca blancas en varios cultivos, pero también puede infectar a ciertas especies de crisomélidos. Las actividades de control de plagas que aplican PFR-97 en 1 agroecosistema pueden derivar inadvertidamente y afectar a los agentes de control biológico liberados intencionalmente en otro ecosistema. Por lo tanto, nuestro objetivo fue determinar si PFR-97 era compatible cuando se aplicaba cerca de *Lilioceris cheni* Gressitt and Kimoto (Coleoptera: Chrysomelidae), un agente de control biológico de crisomélidos para la papa aérea invasora, *Dioscorea bulbifera* L. (Dioscoreaceae). Se investigaron mediante bioensayos en hojas las tasas de consumo y sobrevivencia de escarabajos adultos después de la exposición a PFR-97. Se suministraron treinta adultos de *L. cheni* individualmente con una hoja de *D. bulbifera* rociada con una suspensión de *C. javanica* a una concentración de 10^6, 10^5, 10^3, or 10^2 blastosporas por mL^{-1} o agua destilada estéril (control) durante 72 h. Se proporcionaron hojas sin tratar cada 3 días durante 18 días adicionales y se determinó el consumo de hojas. Los resultados mostraron que la sobrevivencia y el consumo de hojas de los adultos de *L. cheni* no difirieron entre los tratamientos y el control. En general, nuestro estudio demostró que es poco probable que cualquier exposición inadvertida de adultos de *L. cheni* a *C. javanica* a través de la dispersión del rocío afecte el desempeño de los agentes de control biológico para adultos.

Palabras Claves: escarabajo de la papa aérea invasora; hongo entomopatógeno; control biológico; deriva del rociado

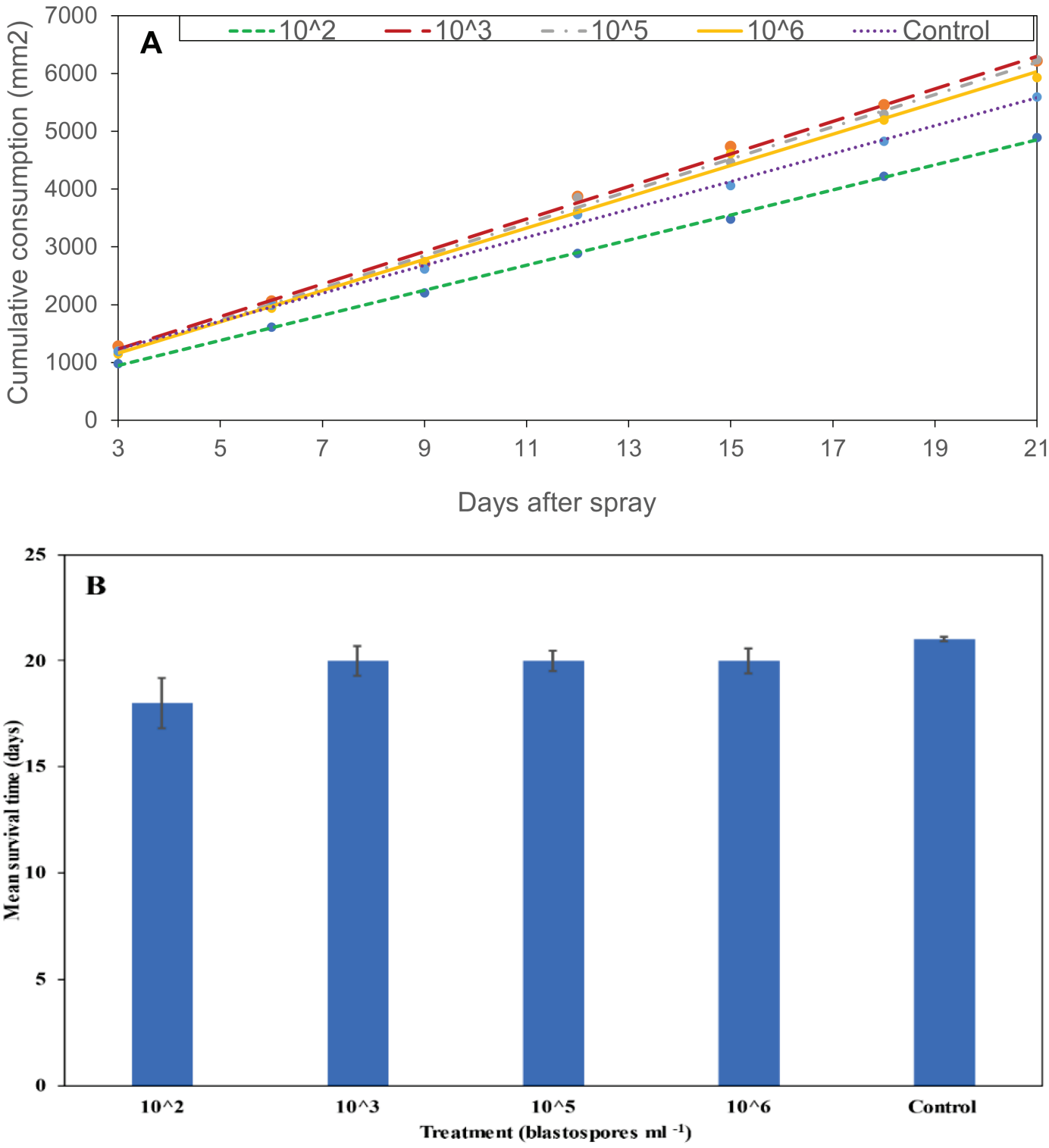

Fig. 1. Performance of *Lilioceris cheni* adult beetles after exposure for 72 h to leaves sprayed with different concentrations (blastospores mL^{-1}) of *Cordyceps javanica* over a 21-d observation period. (A) Cumulative mean leaf consumption (mm^2) (ANOVA: $F_{5, 1067} = 0.61$; $P = 0.6949$), and (B) mean survival time ± SEM of *Lilioceris cheni* adult beetles (ANOVA: $F_{4, 117} = 1.22$; P 0.2123). No significant differences were detected amongst the treatments.

References Cited

Alma CR, Goettel MS, Roitberg BD, Gillespie DR. 2007. Combined effects of the entomopathogenic fungus, *Paecilomyces fumosoroseus* Apopka-97, and the generalist predator, *Dicyphus hesperus*, on whitefly populations. BioControl 52: 669–681.

Avery PB, Hunter WB, Hall DG, Jackson MA, Powell CA. 2016. Efficacy of topical application, leaf residue or soil drench of blastospores of *Isaria fumosorosea* for citrus root weevil management: laboratory and greenhouse investigations. Insects 7: 66. doi:10.3390/insects7040066

Avery PB, Kumar V, Francis A, McKenzie CL, Osborne LS. 2020. Compatibility of the predatory beetle, *Delphastus catalinae*, with an entomopathogenic fungus, *Cordyceps fumosorosea*, for biocontrol of invasive pepper white-

fly, *Aleurothrixus trachoides*, in Florida. Insects 11: 590. doi.org/10.3390/insects11090590

Avery PB, Kumar V, Skvarch EA, Mannion CM, Powell CA, McKenzie CL, Osborne LS. 2019. An ecological assessment of *Isaria fumosorosea* applications compared to a neonicotinoid treatment for regulating invasive ficus whitefly. Journal of Fungi 5: 36. doi:10.3390/jof5020036

Barahona CFS, Threlkeld BC, Avery PB, Francis AW, Cave RD. 2018. Compatibility and efficacy of the lady beetle *Thalassa montezumae* and the entomopathogenic fungus *Isaria fumosorosea* for biological control of the green croton scale: laboratory and greenhouse investigations. Arthropod-Plant Interactions 12: 715–723.

Correa B, Duarte VD, Silva DM, Mascarin GM, Delalibera I. 2020. Comparative analysis of blastospore production and virulence of *Beauveria bassiana* and *Cordyceps fumosorosea* against soybean pests. BioControl 65: 323–337.

Demir I, Kocaccedil S, Souml E, Demirbağ Z, Sevim A. 2013. Virulence of entomopathogenic fungi against *Plagiodera versicolora* (Laicharting, 1781) (Coleoptera: Chrysomelidae). African Journal of Agricultural Research 8: 2016–2021.

EDDMapS. 2020. Early Detection & Distribution Mapping System. The University of Georgia, Center for Invasive Species and Ecosystem and Ecosystem Health, Tifton, Georgia, USA.

Gámez Herrera C, Niño AA, Avery PB, Cave RD. 2016. Effect of the fungus *Isaria fumosorosea* Wize on herbivory by adult yellowmargined leaf beetles, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae). Ceiba 54: 118–126.

Garcerá C, Moltó E, Chueca P. 2017. Spray pesticide applications in Mediterranean citrus orchards: canopy deposition and off-target losses. Science of the Total Environment 599: 1344–1362.

Hunter WB, Avery PB, Pick D, Powell CA. 2011. Broad spectrum potential of *Isaria fumosorosea* against insect pests of citrus. Florida Entomologist 94: 1051–1054.

Hussain A, Tian MY, He YR, Ahmed S. 2009. Entomopathogenic fungi disturbed the larval growth and feeding performance of *Ocinara varians* (Lepidoptera: Bombycidae) larvae. Insect Science 16: 511–517.

Hussein H, Habustova O, Puza V, Zemek R. 2016. Laboratory evaluation of *Isaria fumosorosea* CCM 8367 and *Steinernema feltiae* Ustinov against immature stages of the Colorado potato beetle. PLoS One 11 e0152399. doi: 10.1371/journal.pone.0152399

Mascarin GM, Lopes RB, Delalibera I, Fernandes EKK, Luz C, Faria M. 2019. Current status and perspectives of fungal entomopathogens used for microbial control of arthropod pests in Brazil. Journal of Invertebrate Pathology 165: 46–53.

Montemayor C, Avery PB, Cave RD. 2016. Infection and mortality of *Microtheca ochroloma* (Coleoptera: Chrysomelidae) by *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) under laboratory conditions. Biocontrol Science and Technology 26: 605–616.

Overholt WA, Rayamajhi M, Rohrig E, Hight S, Dray FA, Lake EC, Smith MC, Hibbard K, Bhattarai GP, Bowers K. 2016. Release and distribution of *Lilioceris cheni* (Coleoptera: Chrysomelidae), a biological control agent of air potato (*Dioscorea bulbifera*: Dioscoreaceae), in Florida. Biocontrol Science and Technology 26: 1087–1099.

Rayamajhi MB, Rohrig E, Leidi J, Kerr C, Salcedo E, Poffenberger R, Smith MC, Lake EC, Dray Jr FA, Pratt P. 2019. Herbivory by the biocontrol agent *Lilioceris cheni* suppresses propagule production and smothering ability of the invasive vine *Dioscorea bulbifera*. Biological Control 130: 1–8.

Salyani M, Cromwell RP. 1992. Spray drift from ground and aerial applications. Transactions of the American Society of Agricultural Engineers 35: 1113–1120.

Schindelin J, Rueden CT, Hiner MC, Eliceiri KW. 2015. The ImageJ ecosystem: an open platform for biomedical image analysis. Molecular Reproduction and Development 82: 518–529.

Wheeler GS, Lake EC, Rayamajhi MB, Smith MC. 2020. Acute toxicity of mosquito pesticides on weed biological control agents in south Florida, USA. Biocontrol Science and Technology 30: 855–861.

Woods N, Craig IP, Dorr G, Young B. 2001. Spray drift of pesticides arising from aerial application in cotton. Journal of Environmental Quality 30: 697–701.