BioOne COMPLETE

Pathogenicity and Virulence of Purpureocillium lilacinum (Hypocreales: Ophiocordycipitaceae) on Mexican Fruit Fly Adults

Authors: Ricardo A. Toledo-Hernández, Jorge Toledo, Javier Valle-Mora, Francisco Holguín-Meléndez, Pablo Liedo, et. al.

Source: Florida Entomologist, 102(2) : 309-314

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.102.0204

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://bioone.org/journals/Florida-Entomologist on 21 Sep 2019
Terms of Use: https://bioone.org/terms-of-use

Pathogenicity and virulence of *Purpureocillium lilacinum* (Hypocreales: Ophiocordycipitaceae) on Mexican fruit fly adults

Ricardo A. Toledo-Hernández[1], Jorge Toledo[1], Javier Valle-Mora[1], Francisco Holguín-Meléndez[1], Pablo Liedo[1], and Graciela Huerta-Palacios[1,]*

Abstract

Purpureocillium lilacinum (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson (Hypocreales: Ophiocordycipitaceae) is a fungus commonly used for controlling nematodes, and also has been reported as an insect pathogen. However, little is known about its effects on insects. Here, the pathogenicity of 9 isolates and the virulence and sublethal effects of 2 isolates were evaluated to control adult *Anastrepha ludens* (Loew) (Diptera: Tephritidae). The pathogenicity assays demonstrated that the 9 isolates were pathogenic, with mortality percentages ranging from 28.8 to 52.4% and LT_{50} values were 18 d or more. The strain CFFSUR-A53 was more virulent than CFFSUR-A60, with LC_{50} values of 7.62×10^6 and 5.2×10^9 conidia per mL, respectively. The isolates reduced the life expectancy of the flies by 65 and 37%, decreased fecundity by 78 and 36%, and reduced egg hatching by 31.4 and 18.5%, respectively.

Key Words: entomopathogenic fungi; biological control; *Anastrepha ludens*, fecundity and fertility

Resumen

Purpureocillium lilacinum (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson (Hypocreales: Ophiocordycipitaceae) es un hongo comúnmente utilizado en el control de nematodos y ha sido reportado como patógeno de insectos. Sin embargo, son poco conocidos los efectos que causa sobre los insectos. Aquí se evaluó la patogenicidad de nueve aislamientos y la virulencia y efectos subletales de dos aislamientos en adultos de *Anastrepha ludens* (Loew) (Diptera: Tephritidae). Los ensayos demostraron que los nueve aislamientos fueron patogénicos, y causaron porcentajes de mortalidad que oscilaron entre 28.8 y 52.4% y un TL_{50} de 18 d o más. La cepa CFFSUR-A53 fue más virulenta que la cepa CFFSUR-A60, con valores de CL_{50} de 7.62×10^6 y 5.2×10^9 conidios por mL, respectivamente. Ambos aislamientos redujeron en 65 y 37% la esperanza de vida de las moscas, disminuyeron en 78 y 36% la fecundidad de las hembras, y en 31.4 y 18.5% la eclosión de los huevos, respectivamente.

Palabras Clave: hongos entomopatógenos; control biologico; *Anastrepha ludens*; fecundidad y fertilidad

Purpureocillium lilacinum (Thom.) Luangsa-ard, Hou-braken, Hywel-Jones & Samson (Hypocreales: Ophiocordycipitaceae) is commonly used for the biological control of plant parasitic nematode eggs (Atkins et al. 2005). There are reports that *P. lilacinum* causes the death of thrips, aphids, white flies, bugs, beetles, mosquitoes, and some flies (Tigano-Milani et al. 1995; Posada et al. 1998; Gökçe et al. 2005; Fiedler & Sosnowska 2007; Luz et al. 2007; Marti et al. 2007; Panyasiri et al. 2007; Rambadan et al. 2011; Amala et al. 2013; Fernandes et al. 2013; Goffré & Folgarait 2015). This suggests that this species has the potential for use in biological control for white flies and nematodes in tropical and subtropical zones, because it can grow extensively over the humid surface of leaves and in the plant rhizosphere.

The Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae), is an important pest in Mexico because of the direct damage it causes to citrus (*Citrus* spp., except *C. lemon*; Rutaceae) and mango (*Mangifera indica* L.; Anacardiaceae) fruits (Aluja 1994). The presence of this insect in Mexico has led to strict quarantine measures for both national and international markets (Aluja & Mangan 2008; Santiago-Martínez 2010). The integrated management of fruit flies employs environmentally benign techniques that reduce insecticide use, such as augmentative biological control and the sterile insect technique (Gutierrez 2010). An additional control method could be the use of entomopathogenic fungi, because they can infect and kill, or produce sublethal effects such as alteration on the mating behavior of the pest populations (Castillo et al. 2000; Tefera & Pringle 2003; Quesada-Moraga et al. 2006; Hajek et al. 2008; Dimbi et al. 2009). *Metarhizium anisopliae* (Metsch.) Sorokin (Hypocreales: Clavicipitaceae) caused 37.5 to 98.5% mortality in *A. ludens* third instar larvae (Lezama-Gutierrez et al. 2000), and 86.5 to 97.3% mortality in *A. ludens* adults (Campos-Carbajal 2000). *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) caused 82 to 100% mortality in *A. ludens* adults (De la Rosa et al. 2002; Toledo et al. 2007). It also has been reported that *Isaria fumosorosea* (Wize) (Hypocreales: Cordycipitaceae), formerly identified as *Paecilomyces fumosoroseus*, causes 10 to 100% mortality in *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) adults (Castillo et al. 2000), more than 90% in *Rhagoletis cerasi* (L.) (Diptera: Tephritidae)

[1]El Colegio de la Frontera Sur, Carretera Antiguo Aeropuerto Km. 2.5, Apdo. Postal 36. 30700, Tapachula, Chiapas, Mexico; E-mail: rtoledo@ecosur.edu.mx (R. A. T. H.), jtoledo@ecosur.mx (J. T.), jvalle@ecosur.mx (J. V. M.), fholguin@ecosur.mx (F. H. M.), pliedo@ecosur.mx (P. L.), ghuerta@ecosur.mx (G. H. P.)
* Corresponding author e-mail: ghuerta@ecosur.mx

Downloaded From: https://bioone.org/journals/Florida-Entomologist on 21 Sep 2019
Terms of Use: https://bioone.org/terms-of-use

flies (Daniel & Wyss 2009), and less than 48% in *Bactrocera cucurbitae* (Coquillet) (Diptera: Tephritidae) (Sookar et al. 2008). This suggests that numerous species may be pathogenic or affect the reproductive potential of tephritid fruit flies. Our aims in this study were to (1) assess the pathogenicity of 9 isolates of *P. lilacinum* on *A. ludens* adults, (2) determine the virulence of 2 selected isolates, and (3) estimate the effects of *P. lilacinum* on the reproduction by females.

Material and Methods

ISOLATE ORIGIN AND IDENTIFICATION

The evaluated *P. lilacinum* isolates CFFSUR-A53, CFFSUR-A54, CFFSUR-A60, CFFSUR-A62, CFFSUR-A63, CFFSUR-A65, CFFSUR-A66, CFFSUR-A67, and CFFSUR-A68 were obtained from different mycosed instars of *Antiteuchus innocens* Englemand & Rolston (Hemiptera: Pentatomidae) collected in Altamirano, Chiapas, Mexico (16.725833°N, 92.030833°W) during 2008 and 2009. These isolates were deposited in the plant pathology collection at El Colegio de la Frontera Sur (Tapachula, Chiapas). The fungi were first identified to genus level using their morphological characteristics (Barnett & Hunter 1998), and to species by comparing the sequence of the rDNA ITS region (ITS1-5.8S-ITS2) of 2 isolates, with sequences in the GenBank, using the BlastN program of the National Center for Biotechnology Information, Bethesda, Maryland, USA (http://www.ncbi.nlm.nih.gov). CFFSUR-A53 and CFFSUR-A62 ITS sequences were deposited at GenBank under the accession numbers KM273262 and KM273263, respectively. We assume that all isolates were the same species.

INOCULA PREPARATION

Prior to each bioassay to test pathogenicity, virulence, and sublethal effects, the isolates were activated and multiplied in potato dextrose agar consisting of 15 g agar, 20 g dextrose, 4 g potato extract, and 1 L distilled water. The isolates were incubated for 15 d at 26 ± 2 °C. The conidia of each isolate were suspended in 0.1% Tween® 80, and the concentration was determined using an improved Neubauer brightline chamber (Hausser Scientific, Horsham, Pennsylvania, USA) (Goettel & Inglis 1997). The number of conidia in each suspension was adjusted to the required concentration for each bioassay.

To estimate the viability of each isolate, 250 µL of each suspension was placed in a Petri dish with 1.5% agar in water, dispersed with a spatula under sterile conditions, and incubated at 26 ± 2 °C for 24 h with a 12:12 h (L:D) photoperiod. The percentage of germinated conidia in 5 microscope fields at 40× magnification was determined (Model Dialux 20 EB, Leitz, Wetzlar, Germany). A conidium was considered germinated when the length of the germ tube was at least twice the diam of the conidia (Wraight et al. 2007). An isolate was considered viable when > 90% conidia germinated.

FRUIT FLY ADULTS

Anastrepha ludens pupae were provided by the MOSCAFRUT mass-rearing facility located in Metapa de Dominguez, Chiapas, Mexico (SAGARPA–SENASICA–IICA). Four cohorts from the 17th generation under mass-rearing conditions were provided. After emergence, the flies were sorted by sex and placed in emergence glass cages (30 × 30 × 30 cm). The flies were maintained in the laboratory at 26 ± 2 °C and 70 ± 10% relative humidity (RH) until they were used for the different tests. The flies were fed with a 1:3 (based on w/w) mixture of enzymatically hydrolyzed yeast (MP Biomedicals LLC, Irvine, California, USA) and sucrose. Water was supplied in vials with a cotton stopper.

PATHOGENICITY BIOASSAYS

The pathogenicity of 9 isolates of *P. lilacinum* was tested on 2,600 flies at 8 d of age. Groups of 52 flies (26 males, 26 females) were placed in test tubes (25 cm height × 2.2 cm diam), and were cooled at 0 °C for 5 min to induce lethargy. Each group of flies was placed on a folder, and the dorsal and ventral sides of the flies were sprayed with 1.5 mL of a 10^8 conidia per mL suspension (Home Depot all-purpose spray bottle F-80HD2-24). This was made separately for each isolate. Subsequently, each group of inoculated flies were placed in a plastic box (23.5 cm height × 12.5 cm diam) covered with a nylon cap. The control flies were chilled in the same way as described above and sprayed with 1.5 mL of 0.1% Tween® 80 only. The plastic boxes containing the different treatments were randomly distributed, and maintained under laboratory conditions at 27 ± 2 °C, 70 ± 10% RH, and a 12:12 h (L:D) photoperiod for 23 d. Five replicates per treatment were used for this assay.

We removed dead flies daily; surviving flies remained in the containers. To confirm that the inoculated fungus caused death, the flies were disinfected with 2% sodium hypochlorite for 20 s, rinsed twice with sterile water, and then placed into a moist chamber (Petri dishes with filter paper moistened with sterile distilled water) to stimulate the development of the inoculated fungus (Butt & Goettel 2000). The mortality observed in each treatment was quantified by recording the number of dead flies per d. The data were used to estimate the percentage of mortality produced by mycosis and the median lethal time required to kill 50% of the treated population (LT_{50}). These data were analyzed by 1-way ANOVA, and the mean mortality values were compared using the Tukey test ($P \leq 0.05$).

The inoculation efficiency was estimated by quantifying the number of conidia attached to the body of the inoculated flies. Two flies removed from each replicate and treatment were placed in vials, and rinsed with 1 mL of 0.1% Tween® 80. The vials were then individually shaken for 2 min in a vortex mixer (Fisher Scientific Industries, Inc., Bohemia, New York, USA) at 500 rpm, so the conidia attached to the body of the flies could enter into the suspension. The number of conidia was quantified using a Neubauer chamber. Averages of 1.21×10^6 (SE ± 0.79) conidia were attached to the body of the flies.

VIRULENCE BIOASSAY

Two isolates were evaluated at 5 concentrations of conidia suspension: (1) CFFSUR-A53 (10^4, 10^6, 10^8, 10^{10}, 10^{11} conidia per mL), and (2) CFFSUR-A60 (10^4, 10^6, 10^8, 10^{10}, 5×10^{10} conidia per mL). For this assay, each conidia concentration of each isolate was inoculated on a group of 52 (26 males, 26 females) 8-d-old flies, with 1.5 mL of a conidia suspension in 0.1% Tween® 80. The inoculation process was similar to that described for the pathogenicity assay in the previous section. A random experimental design with 5 replicates was used, and each experimental unit had 50 flies from different cohorts. The lethal concentration required to kill 50% of the flies (LC_{50}) and the LT_{50} were used to determine the virulence of the isolates.

A probit analysis of the mortality observed at 30 d was performed to calculate the LC_{50} and the LT_{50}. Values were estimated by the proportional Cox model using survival data with the statistical package R (R Development Core Team 2010). To determine the significance of the observed differences between the isolates and concentrations, the mean was compared using 99.5% fiducial limits (Agresti 2006).

EFFECT OF *PURPUREOCILLIUM LILACINUM* ON FECUNDITY AND FERTILITY

The effects of the CFFSUR-A53 and CFFSUR-A60 isolates on the fecundity and fertility of *A. ludens* adults were evaluated applying a

Downloaded From: https://bioone.org/journals/Florida-Entomologist on 21 Sep 2019
Terms of Use: https://bioone.org/terms-of-use

suspension of 10^{10} conidia per mL over a group of 52 (26 males, 26 females) 8-d-old flies per isolate per repetition. From the treated flies, 2 flies were selected and analyzed in order to check the inoculation efficiency. The control group was sprayed with 0.1% Tween® 80. Each experimental unit had 50 flies. A random experimental design with 3 treatments and 5 replicates (15 experimental units) was used. Dead flies were removed daily. An oviposition device was placed at 1 end of each experimental unit to facilitate egg collection. The oviposition device was constructed with a plastic ring (4 cm height × 10 cm diam) covered with a black cloth, then covered with a thin layer of silicone on 1 side. To prevent dehydration of the eggs, 50 mL of water was added to the device. Every 24 h, the oviposition device with the eggs was removed, and a new oviposition device was placed. The effect of treatments with *P. lilacinum* infection on fly fecundity was quantified by recording the number of eggs laid in the oviposition device daily during 40 d. The eggs were removed from the device with a dropper, then placed on a black cloth that was placed over a sponge saturated with water on a tray. The eggs were placed in a row with a brush for counting. The number of eggs laid by a female per d was then recorded.

To estimate the effect of the treatments on fertility, a sample of 50 eggs per replicate was collected daily over 40 d. To avoid dehydration, the eggs were arranged on a black cloth and placed over a sponge saturated with water, which was subsequently placed in a Petri dish (FAO/IAEA/USDA 2003). Hatching was recorded after 5 d of incubation. The laboratory conditions during the experiment were 24 ± 2 °C, 70 ± 10% RH, and a 12:12 h (L:D) photoperiod.

To analyze the differences in *A. ludens* survival, fecundity, and fertility caused by *P. lilacinum* infection, a demographic analysis of the data (Carey 1993), 1-way ANOVA, and a Tukey test ($P \leq 0.05$) were performed. To estimate the percentage reduction of fecundity and fertility, the Abbott formula was applied (Abbott 1925).

Results

PATHOGENICITY AND VIRULENCE OF *PURPUREOCILLIUM LILACINUM* ISOLATES ON *ANASTREPHA LUDENS* ADULTS

Purpureocillium lilacinum isolates showed different abilities to infect and kill *A. ludens* adults, according to Tukey multiple comparisons ($F = 61.1$; df = 9, 40; $P < 0.05$). The highest mortality was produced by isolate CFFSUR-A53 (52.4%), and the lowest mortality was observed for isolate CFFSUR-A65 with 28.8% (Table 1). Isolates CFFSUR-A53 and CFFSUR-A62 required 18 d to kill 50% of the treated flies, whereas the remaining isolates required more than 20 d.

The virulence of isolate CFFSUR-A53 was higher than that of isolate CFFSUR-A60, because the former required a lower concentration of conidia (7.62×10^6 conidia per mL) to kill 50% of the flies, whereas the latter required 682 times more conidia to produce the same level of mortality (5.20×10^9 conidia per mL), and this was statistically significant (Table 2). The slopes of the regression equations between concentrations and mortality were 0.1842 ± 0.017 for CFFSUR-A53, and 0.2358 ± 0.023 for CFFSUR-A60 (Table 2). The χ^2 test showed that the data fit the Probit model ($\chi^2 = 0.0239$; df = 3; $P = 0.988$; and $\chi^2 = 0.334$; df = 3; $P = 0.846$).

EFFECT OF *PURPUREOCILLIUM LILACINUM* ISOLATES ON SURVIVAL, FECUNDITY, AND FERTILITY OF *ANASTREPHA LUDENS*

When females were infected with *P. lilacinum*, the survival rates of adult males and females of *A. ludens* were reduced, as were the fecundity and fertility of the females. Survival curves are shown in Figure 1. Life expectancy of flies treated with CFFSUR-A53 and CFFSUR-A60 strains were 22 and 39 d, respectively, whereas for the untreated flies, it was 62 d (Table 3). These results indicate that the survival of flies infected by isolates of *P. lilacinum* was reduced by 65 and 37%, respectively.

Purpureocillium lilacinum infection produced 3 different effects on female fecundity: (a) CFFSUR-A53 isolate reduced the mean net fecundity (lifetime egg production per newborn female [Carey 1993]) by 78%, where the inoculated flies laid 247 eggs per female, whereas the non-treated flies laid an average of 1,131 eggs per female (Table 3); (b) infection delayed the time to maximal egg laying per female by 3 d; and (c) infection shortened the oviposition period as a result of mortality (Fig. 2).

Purpureocillium lilacinum infection also reduced egg fertility. On average, only 52.6 and 62.5% of the eggs hatched from flies infected with isolates CFFSUR-A53 and CFFSUR-A60, respectively, whereas eggs from the non-treated flies had an average hatching of 76.7%, showing a reduction in hatching of 31.4 and 18.5%, respectively.

Table 1. Average mortality (± SE) produced by infection of 9 *Purpureocillium lilacinum* isolates on *Anastrepha ludens* adults (Concentration inoculated 10^8 conidia per mL; N = 250 flies per treatment).

Strain code	Mortality by mycosis (% ± SE)*
CFFSUR-A53	52.4 ± 2.1 a**
CFFSUR-A62	51.6 ± 2.0 a
CFFSUR-A54	44.4 ± 3.4 ab
CFFSUR-A68	39.2 ± 3.4 abc
CFFSUR-A67	37.6 ± 1.9 abc
CFFSUR-A66	36.0 ± 3.5 bc
CFFSUR-A63	32.0 ± 2.7 bc
CFFSUR-A60	31.2 ± 1.6 bc
CFFSUR-A65	28.8 ± 2.9 c
Control	—

*Values in the same column followed by the same letter are not significantly different (Tukey $P \geq 0.05$).
**Total mortality to 21 d post inoculation.

Discussion

Results from the pathogenicity bioassays confirmed that *P. lilacinum* isolates were pathogenic to insects (Fiedler & Sosnowska 2007; Rambadan et al. 2011). We found interspecific variation in both the pathogenicity and virulence bioassays. Intraspecific variation has been reported for other entomopathogenic fungi (Hajek & Leger 1994). Based on the LC_{50} values of the isolates evaluated in this study, strain CFFSUR-A53 seems to be less virulent than some other pathogen species. For example, isolates of *B. bassiana* (applied at 5.13×10^5 to 9.07×10^6 conidia per mL) and *M. anisopliae* (4.38×10^6 to 9.47×10^6 conidia per mL) kill more quickly (Campos-Carbajal 2000; De la Rosa et al.

Table 2. Mean Lethal Concentration of 2 *Purpureocillium lilacinum* isolates on *Anastrepha ludens* adults (N = 1,250 flies per strain).

Strain code	LC_{50} (conidia per mL)*	Fiducial limits 95% (conidia per mL)	Equation
CFFSUR-A53	7.619×10^6 a	$1.408 \times 10^6 - 1.317 \times 10^7$	$\gamma = 0.184\chi - 1.222$
CFFSUR-A60	5.203×10^9 b	$8.083 \times 10^8 - 9.518 \times 10^9$	$\gamma = 0.236\chi - 2.228$

*Values in the same column followed by the same letter are not significantly different according their fiducial limits at 95%.
χ = Log10 (doses).

Downloaded From: https://bioone.org/journals/Florida-Entomologist on 21 Sep 2019
Terms of Use: https://bioone.org/terms-of-use

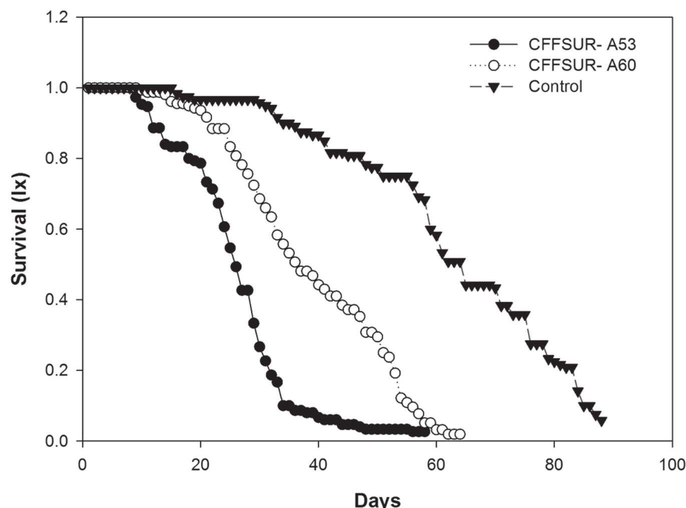

Fig. 1. Daily survival (l_x) of *Anastrepha ludens* adults infected by 2 *Purpureocillium lilacinum* strains (CFFSUR-A53 and CFFSUR-A60) and their non-infected control.

2002). The LT_{50} values obtained for CFFSUR-A53 (18–54 d) compared to *B. bassiana* (2.82–5.9 d) and *M. anisopliae* (4.07–4.95 d) confirm that the isolate is less virulent because it requires more time to kill. This is consistent with the concept of Steinhaus and Martignoni (1970) who defined virulence as the power or force with which the disease occurs in the host. The low virulence of *P. lilacinum* compared with other entomopathogenic fungi species could be related to the low production of toxins, proteases, and chitinases associated with the infection process of this fungus (Khan et al. 2004). Virulence is a characteristic regulated by the expression of genes that encode extracellular proteins or toxins (Quesada-Moraga & Vey 2003; Gao et al. 2011; Ortiz-Urquiza et al. 2010; Pedrini et al. 2013; Staats et al. 2014). The expression of genes can be modified by the density and viability of the inoculum (Herlinda 2010), the number of subcultures, and the nutritive quality from culture medium where the inoculum is multiplied, and finally the inoculation methods and environmental conditions in which the experiment is conducted (Quesada-Moraga & Vey 2003; Ortiz-Urquiza et al. 2010; Inglis et al. 2012).

It is possible that the differences in virulence mentioned above could be accentuated by differences on inoculation efficiency methods used in the tests. The immersion method is recommended for small insects (like aphids), but with larger insects a homogeneous distribution of the inoculum is not achieved. On the contrary, the spray method allows for adjusting the droplet size and distribution density of the inoculum deposited on insects (Inglis et al. 2012). Other authors suggest that the nutritional value of the medium on which the entomopathogenic fungus grows can promote the synthesis of enzymes and toxins closely related to the virulence and pathogenesis process. Ortiz-Urquiza et al. (2010) reported that nutritive media increased the pathogenicity expression of *B. bassiana* isolates. However, after 2 steps by Sabourad Dextrose Agar, the isolate virulence decreased. The major effect is obtained by inoculating the suspension on its host (Quesada-Moraga & Vey 2003).

Sublethal effects of entomopathogenic fungi on its host can affect the host behavior and reduce the rate of reproduction. Isolate CFFSUR-A53 reduced egg laying from 1,131 to 247 eggs per female, and the mechanism of this reduction in fecundity is unknown. Therefore, it will be important to investigate the cause of this observed reduced fecundity caused by *P. lilacinum* infection.

Purpureocillium lilacinum infection also caused a reduction in egg hatch, or fertility. As far as we know, this is the first report of reduced fertility in *A. ludens* caused by fungal infection, because other authors (Toledo et al. 2007) found no significant differences between the percentages of eggs hatching from *B. bassiana*-infected females and those hatching from uninfected females. According to Huang et al. (2010), *I. fumosorosea* reduced the net reproductive rate (Ro) and the intrinsic rate of population growth (r) of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). Several authors have noted that many types of entomopathogenic fungi decrease the survival of subsequent generations after infection of the adults. Torrado-Leon et al. (2006) reported that survival was decreased in 3 generations after the infection of *B. tabaci* by *B. bassiana*, and the most noticeable effect was on the F1 generation. We do not know whether infection with *P. lilacinum* can be transmitted vertically. Therefore, it will be important to study the effect of *P. lilacinum* infection on *A. ludens* offspring and further generations.

Adults infected with isolates of *P. lilacinum* had a greater survival rate compared to that reported by Toledo et al. (2007) for adult flies infected with *B. bassiana*. The pathogen strategy of slowly killing its host may be the result of an adaptation process that allows the pathogen to survive for long periods in the host before it infects a new host (Roy et al. 2006).

In our opinion, the *P. lilacinum* isolates are likely to be more epizootic in the field than are *B. bassiana* isolates because they kill slowly and allow cross-infection with other uninfected flies. The slow killing effect may be useful for the horizontal transmission of entomopathogenic fungi (Kaaya & Okech 1990; Maniania 1998; Klein & Lacey 1999; Dimbi et al. 2003; Quesada-Moraga et al. 2008). Toledo et al. (2007) proposed to inoculate sterile fruit flies with fungi for horizontal transmission of the pathogen during mating and interactions with wild flies. They noted that a limitation of this method was that *B. bassiana* isolates showed a relatively short lethal time (LT_{50} of 4.04–4.20 d), reducing the opportunity for transmission to healthy insects, and possibly reducing the sterile fly population. Toledo et al. (2007) suggested that the use of strains with longer lethal times could be more efficient from a practical viewpoint. Thus, the use of *P. lilacinum*, a slow killing agent, could be useful in the field because it could allow a higher rate of transmission and a larger number of infected wild flies compared to the use

Table 3. Demographic parameters of *Anastrepha ludens* adults infected with *Purpureocillium lilacinum* isolates (CFFSUR-A53 and CFFSUR-A60) and untreated adults*.

Strain	Mean life expectancy (± SE) in d**	Mean gross fecundity (± SE) sum of eggs per female**	Mean net fecundity (Ro) (± SE) (eggs per female)**
Control	62 ± 1.7 a	1180 ± 56.6 a	1131 ± 82.6 a
CFFSUR-A60	39 ± 2.8 b	923 ± 85.1 a	728 ± 75.8 b
CFFSUR-A53	22 ± 1.0 c	479 ± 67.3 b	247 ± 60.9 c

*Demographic parameters as defined by Carey (1993).
**Values in the same column followed by the same letter are not significantly different (Tukey $P \geq 0.05$).
$R_0 = \sum l_x m_x$

Downloaded From: https://bioone.org/journals/Florida-Entomologist on 21 Sep 2019
Terms of Use: https://bioone.org/terms-of-use

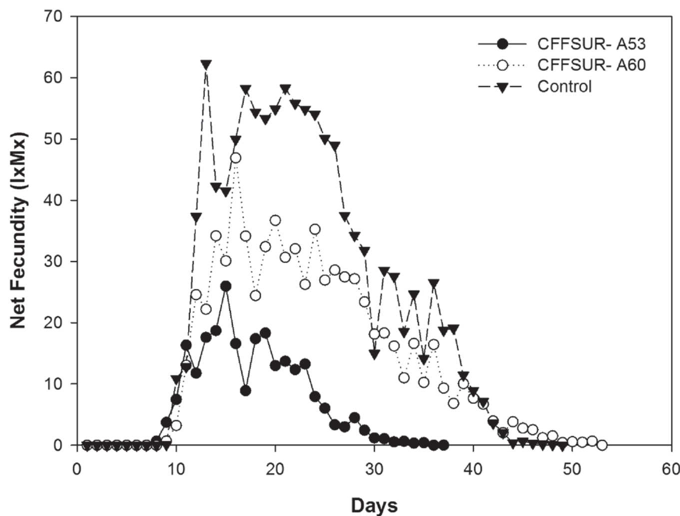

Fig. 2. Mean number of eggs laid per d (net fecundity $l_x m_x$) by *Anastrepha ludens* females infected by 2 strains of *Purpureocillium lilacinum* (CFFSUR-A53 and CFFSUR-A60) and their non-infected control.

of *B. bassiana*. The potential of this approach for pest control needs to be evaluated. In this study, we demonstrate that *P. lilacinum* isolates have the potential to infect and kill fruit flies. However, future investigations are required to evaluate its effect under field conditions, and to determine the most viable route for its application.

Acknowledgments

We thank Gustavo Rodas, Ezequiel de Leon, Sergio Campos, Emigdio Espinosa, Rachel Ferrera, Ingrid Quintero, and Angelica Hernandez for their technical support in the establishment of the bioassays. To the MOSCAFRUT facility (SAGARPA–SENASICA–IICA) for providing *A. ludens* fruit flies. To the Consejo Nacional de Ciencia y Tecnología (CONACYT) for the scholarship granted to RATH.

References Cited

Abbot W. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomologist 18: 265–267.

Agresti A. 2006. Logistic regression, pp. 99–136 *In* An Introduction to Categorical Data Analysis, 2nd edition. John Wiley & Sons, Inc., Hoboken, New Jersey, USA.

Aluja M. 1994. Bionomics and managements of *Anastrepha.* Annual Review of Entomology 39: 155–178.

Aluja M, Mangan RL. 2008. Fruit fly (Diptera: Tephritidae) host status determination: critical conceptual, methodological, and regulatory considerations. Annual Review of Entomology 53: 473–502.

Amala U, Jiji T, Naseema A. 2013. Laboratory evaluation of local isolate of entomopathogenic fungus, *Paecilomyces lilacinus* Thom Samson (ITCC 6064) against adults of melon fruit fly, *Bactrocera cucurbitae* Coquillett (Diptera; Tephritidae). Journal of Tropical Agriculture 51: 132–134.

Atkins SD, Clark IM, Pande S, Hirsch PR, Kerry BK. 2005. The use of real-time PCR and species-specific primers for the identification and monitoring of *Paecilomyces lilacinus.* FEMS Microbiology Ecological 51: 257–264.

Barnett HL, Hunter BB. 1998. Illustrated genera of imperfect fungi. 4th edition APS Press, St. Paul, Minnesota, USA.

Butt TM, Goettel MS. 2000. Bioassays of entomogenous fungi, pp. 141–195 *In* Ascher KRS, Navon A [eds.], Bioassays of Entomopathogenic Microbes and Nematodes. CAB International, Wallingford, United Kingdom.

Campos-Carbajal SE. 2000. Selección de cepas de *Metarhizium anisopliae* (Metch) Sorokin virulentas a la mosca mexicana de la fruta, *Anastrepha ludens* (Loew) en condiciones de laboratorio. BS Thesis. Universidad Autónoma de Chiapas, Huehuetán, Chiapas, México.

Carey JR. 1993. Applied demography for biologists with special emphasis on insects. Oxford University Press, New York, USA.

Castillo MA, Moya PM, Hernández E, Primo-Yúfera E. 2000. Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to entomopathogenic fungi and their extracts. Biological Control 19: 274–282.

Daniel C, Wyss E. 2009. Susceptibility of different life stages of the European cherry fruit fly, *Rhagoletis cerasi*, to entomopathogenic fungi. Journal of Applied Entomology 133: 473–483.

De la Rosa W, López FL, Liedo P. 2002. *Beauveria bassiana* as a pathogen of the Mexican fruit fly (Diptera:Tephritidae) under laboratory conditions. Journal of Economic Entomology 95: 36–43.

Dimbi S, Maniania NK, Ekesi S. 2009. Effect of *Metarhizium anisopliae* inoculation on the mating behavior of three species of African tephritid fruit flies, *Ceratitis capitata*, *Ceratitis cosyra*, and *Ceratitis fasciventris*. Biological Control 50: 111–116.

Dimbi S, Maniania NK, Lux SA, Ekesi S, Mueke JK. 2003. Pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin, to three adult fruit fly species: *Ceratitis capitata* (Weidemann), *C. rosa* var. *fasciventris* Karsch and *C. cosyra* (Walker) (Diptera: Tephritidae). Mycopathologia 156: 375–382.

FAO/IAEA/USDA (Food and Agriculture Organization of the United Nations/International Atomic Energy Agency/U.S. Department of Agriculture). 2003. Manual for product quality control and shipping procedures for sterile mass-reared Tephritid fruit flies, Version 5.0. International Atomic Energy Agency, Vienna, Austria.

Fernandes EG, Valério HM, Borges MAZ, Mascarin GM, Silva CE, Van Der Sand ST. 2013. Selection of fungi for the control of *Musca domestica* in aviaries. Biocontrol Science and Technology 23: 1256–1266.

Fiedler Z, Sosnowska D. 2007. Nematophagous fungus *Paecilomyces lilacinus* (Thom) Samson is also a biological agent for control of greenhouse insects and mite pests. BioControl 52: 547–558.

Gao Q, Jin K, Ying SH, Zhang Y, Xiao G, Shang Y, Wang C. 2011. Genome sequencing and comparative transcriptomics of the model entomopathogenic fungi *Metarhizium anisopliae* and *M. acridum*. PLOS Genetics 7: 1–18.

Goettel MS, Inglis GD. 1997. Fungi: Hyphomycetes, pp. 213–218 *In* Lacey LA [ed.], Manual of Techniques in Insect Pathology. Academic, San Diego, California, USA.

Goffré D, Folgarait PJ. 2015. *Purpureocillium lilacinum*, potential agent for biological control of the leaf-cutting ant *Acromyrmex lundii*. Journal of Invertebrate Pathology 130: 107–115.

Gökce A, Er MK. 2005. Pathogenicity of *Paecilomyces* spp to the glasshouse whitefly, *Trialeurodes vaporariorum*, with some observations on the fungal infection process. Turkish Journal of Agriculture and Forestry 29: 331–339.

Gutierrez JM. 2010. El programa moscas de la fruta en México, pp. 3–10 *In* Montoya P, Toledo J, Hernández E [eds.], Moscas de la Fruta: Fundamentos y Procedimientos para su Manejo. S y G Editores, México City, Mexico.

Hajek AE, Leger RJ. 1994. Interactions between fungal pathogens and insect hosts. Annual Review of Entomology 39: 293–322.

Hajek AE, Lund J, Smith M. 2008. Reduction in fitness of female Asian longhorned beetle (*Anoplophora glabripennis*) infected with *Metarhizium anisopliae*. Journal of Invertebrate Pathology 98: 198–205.

Herlinda S. 2010. Spore density and viability of entomopathogenic fungal isolates from Indonesia, and their virulence against *Aphis gossypii* Glover (Homoptera: Aphididae). Tropical Life Sciences Research 21: 11–19.

Huang Z, Shaukat A, Shun-Xiang E, Jian-Hui W. 2010. Effect of *Isaria fumosoroseus* on mortality and fecundity of *Bemisia tabaci* and *Plutella xylostella*. Insect Science 17: 140–148.

Inglis GD, Enkerli J, Goettel MS. 2012. Laboratory techniques used for entomopathogenic fungi: Hypocreales, pp. 189–253 *In* Lacey LA [ed.], Manual of Techniques in Invertebrate Pathology. 2nd edition. Academic Press, London, United Kingdom.

Kaaya GP, Okech MA. 1990. Horizontal transmission of mycotic infection in adult tsetse, *Glossina morsitans morsitans*. Entomophaga 35: 589–600.

Khan A, Williams K, Nevalainen H. 2004. Effects of *Paecilomyces lilacinus* protease and chitinase on the eggshell structures and hatching of *Meloidogyne javanica* juveniles. Biological Control 31: 346–352.

Klein GM, Lacey LA. 1999. An attractant trap for autodissemination of entomopathogenic fungi into populations of the Japanense beetle *Popillia japonica* (Coleoptera: Scarabaeidae). Biological Science and Technology 9: 151–158.

Lecuona RE, Papierok B, Riba G. 1996. Hongos entomopatógenos, pp. 35–60 *In* Lecuona RE [ed.], Microorganismos Patógenos Empleados en el Control Microbiano de Insectos Plaga. Talleres Gráficos Mariano Mas, Buenos Aires, Argentina.

Lezama-Gutierrez R, Trujillo-de la Luz A, Molina-Ochoa J, Rebolledo-Dominguez O, Pescador AR, Lopez-Edwards M, Aluja M. 2000. Virulence of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) on *Anastrepha ludens* (Dip-

Downloaded From: https://bioone.org/journals/Florida-Entomologist on 21 Sep 2019
Terms of Use: https://bioone.org/terms-of-use

tera: Tephritidae): laboratory and field trials. Journal of Economic Entomology 93: 1080–1084.

Luz C, Tai MHH, Santos AH, Rocha LFN, Albernaz DAS, Silva HHG. 2007. Ovicidal activity of entomopathogenic hyphomycetes on *Aedes aegypti* (Diptera: Culicidae) under laboratory conditions. Journal of Economic Entomology 44: 799–804.

Maniania NK. 1998. A device for infecting adult tsetse flies *Glossina* spp., with an entomopathogenic fungus in the field. Biological Control 11: 248–254.

Marti GA, López-Lastra CC, Pelizza SA, García JJ. 2007. Isolation of *Paecilomyces lilacinus* (Thom) Samson (Ascomycota: Hypocreales) from the Chagas disease vector, *Triatoma infestans* Klug (Hemiptera: Reduviidae) in an endemic area in Argentina. Mycopathologia 162: 369–372.

Ortiz-Urquiza A, Riveiro-Miranda L, Santiago-Álvarez C, Quesada-Moraga E. 2010. Insect-toxic secreted proteins and virulence of the entomopathogenic fungus *Beauveria bassiana*. Journal of Invertebrate Pathology 105: 270–278.

Panyasiri C, Attathom T, Poehling HM. 2007. Pathogenicity of entomopathogenic fungi-potential candidates to control insect pests on tomato under protected cultivation in Thailand. Journal of Plant Diseases and Protection 114: 278–287.

Pedrini N, Ortiz-Urquiza A, Huarte-Bonnet C, Zhang S, Keyhani NO. 2013. Targeting of insect epicuticular lipids by the entomopathogenic fungus *Beauveria bassiana*: hydrocarbon oxidation within the context of a host-pathogen interaction. Frontiers in Microbiology 4: 1–18.

Posada FJ, Marin P, Perez M. 1998. *Paecilomyces lilacinus*, enemigo natural de adultos de *Hypothenemus hampei*. Cenicafé (Colombia) 49: 72–77.

Quesada-Moraga E, Vey A. 2003. Intra-specific variation in virulence and in vitro production of macromolecular toxins active against locust among *Beauveria bassiana* strains and effects of *in vivo* and *in vitro* passage on these factors. Biocontrol Science and Technology 13: 323–340.

Quesada-Moraga E, Martin-Carballo I, Garrido-Jurado I, Santiago-Alvarez C. 2008. Horizontal transmission of *Metarhizium anisopliae* among laboratory populations of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Biological Control 47: 115–124.

Quesada-Moraga E, Ruiz-Garcia E, Santiago-Alvarez C. 2006. Laboratory evaluation of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against puparia and adults of *Ceratitis capitata* (Diptera: Tephritidae). Journal of Economic Entomology 99: 1955–1966.

R Development Core Team. 2010. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rambadan S, Jugmohan H, Khan A. 2011. Pathogenicity and haemolymph protein changes in *Edessa meditabunda* F. (Hemiptera: Pentatomidae) infected by *Paecilomyces lilacinus*. Journal of Biopesticides 4: 169–175.

Roy HE, Steinkraus DC, Eilenberg J, Hajek AE, Pell JK. 2006. Bizarre interactions and endgames: entomopathogenic fungi and their arthropod hosts. Annual Review of Entomology 51: 331–357.

Santiago-Martínez G. 2010. Aplicación del concepto áreas libres de plagas, pp. 229-242 *In* Montoya P, Toledo J, Hernández E [eds.], Moscas de la Fruta: Fundamentos y Procedimientos para su Manejo. S y G Editores, México City, Mexico.

Sookar P, Bhagwant S, Awuor-Ouna E. 2008. Isolation of entomopathogenic fungi from the soil and their pathogenicity to two fruit fly species (Diptera: Tephritidae). Journal of Applied Entomology 132: 778–788.

Staats CC, Junges Â, Guedes RLM, Thompson CE, de Morais GL, Boldo JT, Schrank A. 2014. Comparative genome analysis of entomopathogenic fungi reveals a complex set of secreted proteins. BMC Genomics 15: 822. doi: 10.1186/1471-2164-15-822

Steinhaus EA, Martignoni ME. 1970. An abridged glossary of terms used in invertebrate pathology. 2nd edition. USDA Forest Service, Pacific Northwest Forest and Range Experiment Station, Corvallis, Oregon, USA.

Tefera T, Pringle KL. 2003. Food consumption by *Chilo partellus* (Lepidoptera: Pyralidae) larvae infected with *Beauveria bassiana* and *Metarhizium anisopliae* and effects of feeding natural versus artificial diets on mortality and mycosis. Journal of Invertebrate Pathology 84: 220–225.

Tigano-Milani MS, Carneiro GR, De Faria RM, Frazao SH, McCoy WC. 1995. Isozyme characterization and pathogenicity of *Paecilomyces fumosoroseus* and *P. lilacinus* to *Diabrotica speciosa* (Coleoptera: Chrysomelidae) and *Meloidogyne javanica* (Nematoda: Tylenchidae). Biological Control 5: 378–382.

Toledo J, Campos SE, Flores S, Liedo P, Barrera JF, Villaseñor A, Montoya P. 2007. Horizontal transmission of *Beauveria bassiana* in *Anastrepha ludens* (Diptera: Tephritidae) under laboratory and field cage conditions. Journal of Economic Entomology 100: 291–297.

Torrado-Leon E, Montoya-Lerma J, Valencia-Pizo E. 2006. Sublethal effects of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) on the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) under laboratory conditions. Mycopathologia 162: 411–419.

Wraight SP, Inglis GD, Goettel MS. 2007. Fungi, pp. 223–248 *In* Lacey LA, Kaya HK [eds.], Field Manual of Techniques in Invertebrate Pathology. Springer, Amsterdam, The Netherlands.

Downloaded From: https://bioone.org/journals/Florida-Entomologist on 21 Sep 2019
Terms of Use: https://bioone.org/terms-of-use