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Entomopathogenic fungi as biological control agents of *Phyllocoptruta oleivora* (Prostigmata: Eriophyidae) under greenhouse conditions

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Abstract

The citrus rust mite, *Phyllocoptruta oleivora* Ashmead (Acari: Eriophyidae), is a major pest of citrus, affecting both quality and yield. Although entomopathogenic fungi such as *Beauveria bassiana* (Bals.-Criv.) Vuill. (Cordycipitaceae), *Metarhizium anisopliae* (Metschn.) Sorokīn (Clavicipitaceae), and *Isaria fumosorosea* Wize (Cordycipitaceae) have been used for insect and mite control, the last 2 have never been applied to control citrus rust mite. The aim of this study was to evaluate the effect of 3 concentrations of *B. bassiana*, *M. anisopliae*, and *I. fumosorosea* on mortality and growth rate (*r*) of *P. oleivora* populations under greenhouse conditions. The fungi were isolated from agricultural soil samples. Mobile stages of *P. oleivora* were collected from leaves of commercial Valencia orange groves. At a concentration of 1×10^8 conidia mL^{-1}, *B. bassiana* and *M. anisopliae* caused > 60% mortality in *P. oleivora* populations, whereas mortality due to *I. fumosorosea* never exceeded 50%. There was a significant inverse relationship between the percent mortality and the LT_{50}. Application of *B. bassiana* resulted in the slowest growth rate of *P. oleivora* populations, which was 10.5 × slower than the control population receiving no fungus application. Our results demonstrate that *B. bassiana* and *M. anisopliae* are efficient in control of *P. oleivora* populations under greenhouse conditions, whereas *I. fumosorosea* produces moderate control.

Key Words: citrus rust mite; biocontrol; *Beauveria bassiana*; *Metarhizium anisopliae*; *Isaria fumosorosea*; mean lethal time

Resumen

El ácaro tostador de los cítricos, *Phyllocoptruta oleivora* Ashmead (Acari: Eriophyidae), es una plaga importante, que afecta tanto la calidad como el rendimiento. Hongos entomopatógenos como *Beauveria bassiana* (Bals.-Criv.) Vuill. (Cordycipitaceae), *Metarhizium anisopliae* (Metschn.) Sorokīn (Clavicipitaceae), e *Isaria fumosorosea* Wize (Cordycipitaceae), se han utilizado para el control de insectos y ácaros, los 2 últimos nunca se han aplicado contra el ácaro tostador de los cítricos. El objetivo de este estudio fue evaluar el efecto de 3 concentraciones de *B. bassiana*, *M. anisopliae*, e *I. fumosorosea* sobre la mortalidad y la tasa de crecimiento (*r*) de poblaciones de *P. oleivora* en condiciones de invernadero. Los hongos fueron aislados de muestras de suelo agrícola. Se colectaron estados móviles de *P. oleivora* de hojas de naranjos comerciales variedad Valencia. Las concentración de 1×10^8 conidias mL^{-1}, *B. bassiana* y *M. anisopliae* causaron > 60% de mortalidad en las poblaciones de *P. oleivora*, mientras que la mortalidad debida a *I. fumosorosea* no excedió el 50%. Hubo una relación inversa significativa entre el porcentaje de mortalidad y el LT_{50}. La aplicación de *B. bassiana* dio como resultado una tasa de crecimiento más lenta en las poblaciones de *P. oleivora*: 10.5 × más lenta que la población control que no recibió aplicación de hongos. Nuestros resultados demuestran que *B. bassiana* y *M. anisopliae* son eficientes en el control de poblaciones de *P. oleivora* en condiciones de invernadero, mientras que *I. fumosorosea* produce un control moderado.

Palabras Clave: *á*caro tostador de los cítricos; biocontrol; *Beauveria bassiana*; *Metarhizium anisopliae*; *Isaria fumosorosea*; tiempo letal medio

The citrus rust mite, *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae), is a pest of citrus in tropical regions, where environmental conditions are optimal for population growth (Childers et al. 1996; Aghajanzadeh & Mallik 2007; Rogers & Stansly 2016). Together with the Mexican fruit fly, *Anastrepha ludens* Loew (Diptera: Tephritidae), *P. oleivora* contributes to severe phytosanitary problems for citrus production in the state of Tamaulipas in northeastern Mexico (Varela-Fuentes et al. 2005; Varela-Fuentes et al. 2013; Vanoye-Eligio et al. 2017). *Phyllocoptruta oleivora* can infest leaves and green branches of citrus.

Damage occurs when *P. oleivora* feed on the developing fruit; penetration of epidermal cells by the mite's stylet causes dark brown areas to develop on the fruit surface (McCoy & Albrigo 1975). Citrus rust mites are present throughout the year, selecting fruits that are

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exposed to sunlight, but they avoid the heat in the outer portions of the fruit that get very hot from exposure to sunlight (Varela et al. 2005; Varela-Fuentes et al. 2013). Heavy infestation with *P. oleivora* reduces the size and weight of fruit; this reduces juice volume, increases acidity and soluble solids, and reduces the aesthetic appearance of the fruit, limiting their marketability (Varela et al. 2005; Imbachi et al. 2012; Mesa & Rodríguez 2012; Varela-Fuentes et al. 2013).

Throughout Mexico, including northeastern Mexico, the principal control method for *P. oleivora* is the use of broad spectrum insecticides (Alves et al. 2005). However, this practice increases the risk of insecticide resistance and environmental degradation of agroecosystems (Varela-Fuentes et al. 2013). The use of entomopathogenic fungi for pest control is an alternative strategy, particularly for the problems of resistance (Omoto et al. 1994) and environmental pollution, thus improving the economic and biological sustainability of agroecosystems (Alves et al. 2004).

The majority of fungi that contribute to natural regulation of insect and mite populations are from the orders Hypocreales and Entomophthorales (Dolinski & Lacey 2007). The latter are difficult or impossible to mass-produce, and therefore have not been produced commercially or applied on a large scale. In contrast, several species of Hypocreales are commercially available for control of insect and mite pests; some of these belong to the genera *Beauveria*, *Metarhizium*, *Isaria*, *Aschersonia*, *Hirsutella*, and *Lecanicillium* (Alves 1998; Inglis et al. 2001; Goettel et al. 2005).

The fungi *Beauveria bassiana* (Bals.), *Metarhizium anisopliae* (Metschinkof), and *Isaria fumosorosea* (Wize) have been studied widely as biological control agents, including as mycoacaricides (Tamai et al. 1999, 2002; Amjad et al. 2012; El-Sharabasy 2015). There have been a few studies on the efficacy of *B. bassiana* to control *P. oleivora* under laboratory conditions (Alves et al. 2005); despite this, there have been no studies under greenhouse and field conditions. Similarly, no studies have been conducted on *M. anisopliae* to control *P. oleivora*. *Paecilomyces fumosoroseus* (= *Isaria fomosorosea*) (Wize) A. H. S. Br. & G. Sm. (Cordycipitaceae) has been evaluated to control 2 other mites, the citrus brown mite, *Eutetranychus orientalis* (Klein) (Prostigmata: Tetranychidae), and the 2-spotted spider mite, *Tetranychus urticae* Koch (Prostigmata: Tetranychidae) under laboratory and greenhouse conditions, respectively (Luangsa-Ard et al. 2005; Kim et al. 2008; El-Sharabasy 2015).

Alternative control strategies for *P. oleivora* that contribute to economic gain through effective pest control and reducing resistence, and are environmentally friendly, are urgently required. In the present study we evaluated mortality and population growth rate of mobile stages of *P. oleivora* following application of different concentrations of 3 entomopathogenic fungi: *B. bassiana*, *M. anisopliae*, and *I. fumosorosea*. We also determined lethal times for these entomopathogens to kill 50% of citrus rust mite.

Materials and Methods

This greenhouse study was done at the Institute of Applied Ecology, Autonomous University of Tamaulipas, Victoria, Tamaulipas, Mexico. Eighty *Citrus sinensis* (L.) Osbeck (Rutaceae) variety 'Valencia' plants that were certified free of pests and diseases were obtained from the Francisco Villa Experimental Facility (23.897222°N, 99.131944°W), Güemez, Tamaulipas, Mexico. Plants were 65 ± 5 (average ± SD) cm in height and were placed in the greenhouse in pots at a distance of 30 cm apart. Environmental conditions in the greenhouse during the experiment were 35 ± 5 °C, 80 ± 10% RH, and a 11:13 (L:D) photoperiod. Plants were irrigated every 2 d with 200 mL of water, and fertilized once using HUMIMAX (Humic substances derived from leonardite 12%, soluble potassium 2%, amino acids 0.50%, manganese 3%, iron 3%, zinc 1%, boron 0.50%, moisturizers, dispersing and penetrating 78%) at a dose of 2.5 mL per L of water. Fertilization was performed 20 d before the infestation to avoid any effect on the mites.

COLLECTION OF MOBILE STAGES OF *PHYLLOCOPTRUTA OLEIVORA* AND ESTABLISHMENT ON EXPERIMENTAL TREES IN THE GREENHOUSE

Mobile mite stages were collected from leaves of commercial Valencia orange groves from 3 localities: Mártires Chinameca (24.096111°N, 99.140555°W), Úrsulo Galván (24.093611°N, 99.136666°W), and La Misión Victoria (23.812500°N, 99.159444°W) in Tamaulipas State, Mexico. Collections were made in Mar and Apr 2017 when we predicted that the mite populations were likely to be high (Landeros et al. 2003). We selected mature and immature leaves and fruits that were showing symptoms of damage by *P. oleivora*. Samples were collected into brown paper bags, placed inside iceboxes at 5 ± 1 °C, and transported to the laboratory at Institute of Applied Ecology, Autonomous University of Tamaulipas. The presence of mites on samples was confirmed using a stereoscopic microscope (UNICO Stereo & Zoom Microscopes ZM180, Dayton, New Jersey, USA).

Colonies of mites were established on all trees in the greenhouse over a period of 18 d; every 2 d a leaf disc containing 10 to 12 mites was placed at the top of each tree (9 infested leaf discs added in total to every tree). Branches with infested leaves were labelled for subsequent monitoring. Two d before the fungal applications, 1 leaf was taken from each tree and from trees being allocated to different treatments, and the numbers counted under a microscope to confirm that mite colonization had been achieved, and the count was taken to be the initial population of mites (X_0); after observing the infested leaves, they were returned to the trees from which they had been sampled.

SOURCE AND PRODUCTION OF ENTOMOPATHOGENIC FUNGI

Beauveria bassiana, *M. anisopliae*, and *I. fumosorosea* were isolated from agricultural soil samples according to Sánchez-Peña et al. (2011) and grown on Potato Dextrose Agar (PDA, DIBICO, Mexico City, México) in 90 × 15 mm diam Petri dishes for 21 d at 25 ± 2 °C, 70% RH, 12:12 h (L:D) photoperiod. Isolates were identified using a light microscope (Standard 25 ICS, Carl Zeiss AG, Oberkochen, Germany) (Humber 1997). Under sterile conditions, conidia from each isolate were scraped into sterile tubes of 50 mL 0.02% Tween® 20 aqueous solution to produce a suspension; the conidial concentration was estimated using a Neubauer hemocytometer (Fisher Scientific, Pittsburgh, Pennsylvania, USA), adjusted to 1×10^8 conidia mL^{-1}, and used to inoculate 1 of the 250 mL flasks (Pyrex, New York, USA), each containing 100 mL of Sabouraud Dextrose Broth (Bioxon, New Jersey, USA); 10 mL of suspension was added to each flask. The flask cultures were incubated at 28 °C and shaken manually every third d. After 5 d, 30 mL from the flask cultures was inoculated onto sterile rice (*Oryza sativa* L.) (Poaceae) in polyethylene bags: 200 g of rice sterilized in 100 mL water per bag (capacity = 1 kg) inoculated with 30 mL aliquots of suspension. The bags of rice were incubated for 14 d under ambient conditions (25–28 °C, 12:12 h [L:D] photoperiod). After 15 d of incubation, the conidia were harvested by adding 100 mL of 0.02% Tween® into each bag of rice, which was then shaken. The solution was filtered through a sieve (500 µm, Gilson®) to recover the liquid. Conidial concentration was estimated using a Neubauer hemocytometer and adjusted to provide 1×10^8, 5×10^7, and 1×10^7 conidia per mL^{-1} suspensions of each isolate for the greenhouse experiment.

GREENHOUSE EXPERIMENT

The experiment was carried out in the following manner: 10 to 12 mites were released 2 days before the application of the fungus. Three leaves of 6 randomly sampled trees were taken for treatment, and the number of mites counted from those leaves were called the initial population (X_0). Further, initial mite count determined the homogeneous distribution and establishment of the *P. oleivora* population within the greenhouse.

After the application of fungus, the study was carried out using 1-way designs per counting date. The structure of this design is a set of t treatments, also called a unifactorial treatment structure. Each way was designed to analyze the effects of concentrations of each fungus per count. Three replicates (leaves) were counted for each treatment, analyzing 36 replicates per count date. The sample unit was the number of mites on the leaves. For each concentration, 6 trees were randomly used. Each treatment was separated by 2 m to avoid cross contamination. Each treatment tree was sprayed with 0.5 L of the relevant fungal species and concentration using a Truper® manual sprayer (Model 14687; Truper®, Victoria City, Tamaulipas, Mexico). The control treatment (n = 18 trees total) were sprayed with only the carrier Tween® without conidia. The counts were made at 3, 5, 7, and 10 d after the application of each treatment. The mortality criterion was determined by mites with evidence of mycelial growth, which was determined by observation under the stereoscope. Also, live mites were counted to obtain their growth rate. After each count, the leaves were destroyed (total of 36 leaves per treatment per concentration combination and control for each sampling occasion). Environmental conditions in the greenhouse during the experiment were 35 ± 5 °C, 80 ± 10% RH, and 11:13 h (L:D) photoperiod.

STATISTICAL ANALYSIS

The Henderson and Tilton (1955) formula was used to correct the data for control population size, and to establish the percentage of mortality for each treatment compared with the control. This was necessary because in the absence of treatment (the control) mite populations may still fluctuate (increase or decrease) due to the biology of the mite or the quality of food.

Arccosine transformation was used to stabilize variance in population counts. Data on mortality counts were then analyzed by ANOVA and the mean comparisons using Tukey's hsd test ($P < 0.05$). The first count of live individuals was analyzed by 1-way ANOVA, and thereafter counts were analyzed by 2-way ANOVA. The mean lethal time values (LT_{50}) were calculated using Probit analysis (Finney 1971). SAS software was used for all analyses (SAS 2002).

Population growth rates in different treatments were estimated using the equation proposed by Odum (1971), $r = (1/t)(X_t/X_0)$, and used by Soler-Salcedo et al. (2006) and Chacón-Hernández et al. (2017), where t is the time in d, X_0 and X_t the number of mites at time 0 (or initial number of individuals), and the number of mites at time t at the end of the next sampling period, respectively. The units of this parameter represent the number of offspring that each individual produced per d throughout the study period and is expressed as d^{-1} (Soler-Salcedo et al. 2006).

Results

We evaluated the effect of applications of *B. bassiana*, *M. anisopliae*, and *I. fumosorosea* at 3 concentrations on populations of the mobile stages of *P. oleivora* in the greenhouse (Table 1). On d 3 after application there was a significant effect of concentration on the number of mobile stages of *P. oleivora* ($F_{9, 20} = 27.73$; $P < 0.0001$). At this time, mortality ranged between 21.01% (*I. fumosorosea* 5×10^7) and 27.71% (*B. bassiana* 1×10^8). On d 5, significantly higher mortality was achieved in the *B. bassiana* 1×10^8 conidia mL^{-1} treatment compared with all the other treatments ($F_{9, 20} = 180.08$; $P < 0.0001$). By d 7, the greatest mortalities were seen in the *B. bassiana* 1×10^8 conidia mL^{-1} and *M. anisopliae* 1×10^8 conidia mL^{-1} treatments ($F_{9, 20} = 9.54$; $P < 0.0001$). By d 10, the greatest mortalities also were found in the *B. bassiana* 1×10^8 conidia mL^{-1} and *M. anisopliae* 1×10^8 conidia mL^{-1} treatments ($F_{9, 20} = 125.87$; $P < 0.0001$). All concentrations of *I. fumosorosea* caused moderate mortality in *P. oleivora* (Table 1).

The time for 50% mortality (LT_{50}) values ranged from 5 to 23 d; *B. bassiana* had shorter LT_{50}s than *M. anisopliae* and *I. fumosorosea*, but asymptotically, concentrations were significantly different according to 95% CI. The trend for LT_{50} values indicate a significant negative relationship with concentration, i.e., the higher the concentration of conidia mL^{-1}, the shorter the mean lethal time (Table 1).

The initial densities of *P. oleivora* before application of entomopathogenic fungi were not significantly different among treatments ($F_{3, 8} = 0.39$; $P > 0.05$) (Fig. 1), confirming that populations had established homogenously inside the greenhouse. After application of entomopathogenic fungi, there were significant differences in population sizes of live mites among treatments ($F_{9, 80} = 31.75$; $P < 0.0001$), on fluctuating population of *P. oleivora* ($F_{3, 80} = 99.68$; $P < 0.0001$), and there was a significant interaction ($F_{27, 80} = 6.69$; $P < 0.0001$). Thus, populations of live mobile stages of *P. oleivora* in the control increased in number more than in all the fungal treatments. Populations rapidly increased on control trees in the first 5 d and remained steady thereafter. Indi-

Table 1. Virulence and lethal mean time (LT_{50}) of entomopathogenic fungi to control mobile stages of *Phyllocoptruta oleivora*.

Fungus	Conidia mL^{-1}	Mortality ± SE (%)*				Lethal mean time LT_{50} (d)
		D 3	D 5	D 7	D 10	
Beauveria bassiana	1×10^8	27.71 a** ± 1.98	43.94 a ± 1.84	56.64 a ± 3.98	67.66 a ± 1.70	5.05 (4.58–5.51) a
	5×10^7	26.26 ab ± 3.91	39.85 ba ± 1.81	48.29 ab ± 2.97	59.39 ab ± 4.38	6.04 (5.43–6.76) ab
	1×10^7	22.34 ab ± 1.70	37.91 ± 2.29	47.74 b ± 2.37	57.47 b ± 2.64	6.47 (5.86–7.21) ab
Metarhizium anisopliae	1×10^8	27.41 ab ± 3.02	38.74 bc ± 0.85	53.90 ab ± 1.44	61.20 ab ± 4.24	5.64 (5.09–6.24) ab
	5×10^7	22.34 ab ± 1.70	34.28 cd ± 1.70	51.54 ab ± 2.89	55.72 b ± 1.70	6.54 (5.94–7.28) bc
	1×10^7	20.04 b ± 1.95	33.30 ed ± 1.70	50.21 ab ± 0.96	52.86 b ± 3.25	6.98 (4.74–17.12) bc
Isaria fumosorosea	1×10^8	24.72 ab ± 4.61	30.77 ef ± 1.33	38.46 c ± 2.89	43.08 c ± 3.33	11.07 (8.72–18.21) d
	5×10^7	21.01 ab ± 2.67	28.23 f ± 1.72	29.57 d ± 4.74	41.15 c ± 3.33	13.99 (10.50–26.48) e
	1×10^7	21.30 ab ± 1.58	20.04 g ± 1.95	27.66 d ± 4.22	35.11 c ± 1.70	23.34 (14.14–111.4) f
Control		0 c	0 h	0 e	0 d	

*Standard error in percent. **Averages followed by the same letter in the same column are not significantly different ($P > 0.05$; ANOVA and Tukey test).

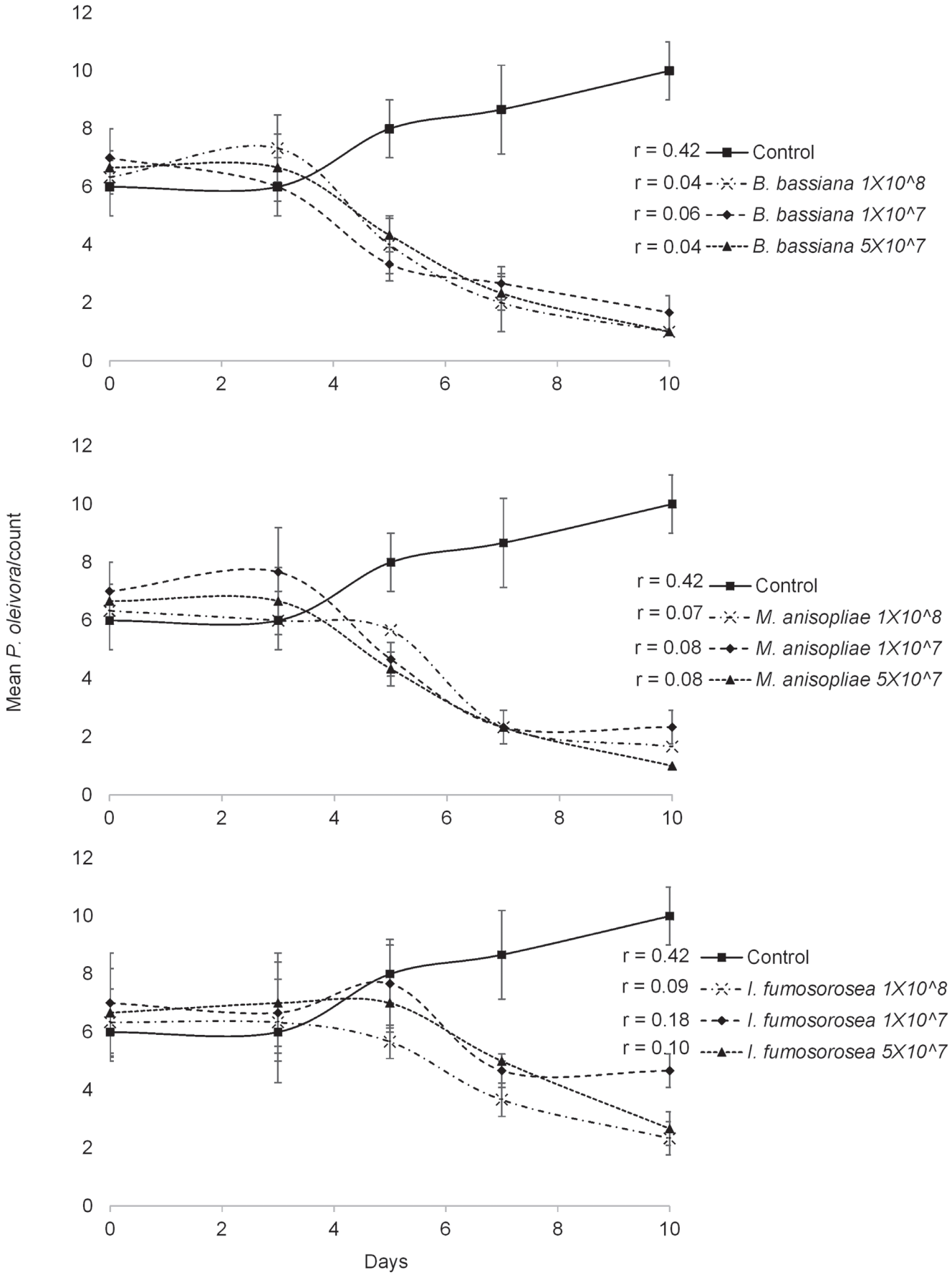

Fig. 1. Average ± SD and growth rate "*r*" per individual (d^{-1}) of mobile stages of *Phyllocoptruta oleivora*.

viduals in control populations had a mean offspring production rate of 0.42 d^{-1}; in the *B. bassiana* 1×10^8, 5×10^7, and 1×10^7 conidia mL^{-1} treatments, it was 0.04, 0.04, and 0.06 d^{-1}, respectively; in the *M. anisopliae* 1×10^8, 5×10^7 and 1×10^7 conidia mL^{-1} treatments, it was 0.07, 0.08, and 0.08 d^{-1}, respectively; in the *I. fumosorosea* 1×10^8, 5×10^7, and 1×10^7 conidia mL^{-1} treatments, it was 0.09, 0.10, and 0.17 d^{-1}, respectively (Fig. 1). The population fluctuations observed over the first 3 d suggest that mortality was occurring most rapidly in the *B. bassiana* treatments, presumably due to infection.

The overall population growth rate was lowest in the 3 *B. bassiana* treatments (all 3 concentrations) due to higher fungus-related mortality. The opposite was apparent in the *I. fumosorosea* treatments, presumably because it was less pathogenic to *P. oleivora*. *Metarhizium anisopliae* was intermediate in its effect on population growth (Fig. 1, Table 1). There was a dose-related effect of different concentrations of all 3 fungi on *P. oleivor* population growth rates; at 1×10^8, 5×10^7, 1×10^7 conidia mL^{-1} of *B. bassiana*, growth rates were 10.5, 10.5, and 7.0, respectively, slower than the control; for *M. anisopliae* they were 6.4, 5.6, and 5.0 slower, respectively; and for *I. fumosorosea* they were 4.6, 4.2, and 2.5, respectively, slower. This indicates that *B. bassiana* is more effective in controlling populations of citrus rust mite under greenhouse conditions than the other 2 fungal species.

Discussion

Mortality in the mobile stages of the citrus rust mite caused by entomopathogenic fungi increased with time after application. *Beauveria bassiana* caused the greatest mortality in populations of *P. oleivora* between d 3 and the end of the experiment. This fits with the fact that *B. bassiana* requires only 24 h to colonize its host (Alves et al. 2005). Lower concentrations of each fungus would reduce the numbers of hosts killed, and accounts for the dose response we observed.

The mortalities observed were less than in studies of *P. oleivora* by Alves et al. (2005), who evaluated *B. bassiana* on citrus rust mite, whereas Barreto et al. (2004) and Bugeme et al. (2008) evaluated *B. bassiana* and *M. anisopliae* on *Mononychellus tanajoa* Bondar and *Tetranychus evansi* Baker and Pritchard (both Prostigmata: Tetranychidae), respectively, and Zemek et al. (2016) evaluated *I. fumosorosea* to control *Tetranychus urticae* Koch (Acari: Tetranychidae), where they found mortality above 90%, at 5, 8, 10, and 15 d after application. This may be due to differences in environmental conditions in the studies. In our study, the temperature was 35 ± 5 °C and RH was 80 ± 10%, whereas the other studies were done under laboratory conditions of 25 ± 2 °C, and 70 to 98% RH. Bugeme et al. (2008) state that mortality varies with species of entomopathogenic fungus, isolates, and temperature. The same authors found that *B. bassiana* and *M. anisopliae* also provided good control of the red spider mite, *T. evansi*, at temperatures of 30 ± 5 °C. Furthermore, it has been reported that several isolates of fungi are pathogenic to arthropod pests at temperatures ranging from 25 to 35 °C (Hsiao et al. 1992; Fargues & Remaudiere 1977; Thomas & Jenkins 1997; Ekesi et al. 1999; Milner et al. 2003; Dimbi et al. 2004; Cuthbertson et al. 2005; Bugeme et al. 2008; Mishra et al. 2015).

In our study, all concentrations of *I. fumosorosea* achieved mortalities of less than 50% in 10 d, which was lower than figures reported by Zemek et al. (2016), who found that at a concentration of 4×10^7 conidia mL^{-1}, *I. fumosorosea* caused 92.1% of mortality of *T. urticae* females in laboratory experiments, whereas Chandler et al. (2005) reported mortalities of 21 to 22% and 97% (concentrations 1×10^7 and 1×10^8 conidia mL^{-1}, respectively) of mobile stages of *T. urticae* on tomato *Lycopersicon esculentum* Miller (Solanaceae) under laboratory and greenhouse conditions, respectively.

Environmental factors, such as temperature and humidity, affect not only the survival and virulence of entomopathogenic fungi, but also influence host-pathogen interactions (Kiewnick 2006). Optimal temperature and humidity requirements for survival and virulence vary among different fungal isolates (Fargues & Remaudiere 1977). *Beauveria bassiana* is effective over a wide temperature range (25–35 °C) under favorable humidity conditions (90% RH) (Bugeme et al. 2008; Mishra et al. 2015). The efficiency of entomopathogenic fungi is influenced by temperature and relative humidity (Mishra et al. 2015), and thus was reflected in the LT_{50}, since these environmental factors in this study ranged between 35 to 40 °C, and 70 to 90% R.H.

In concentrations of 1×10^8, 5×10^7, 1×10^7 conidia mL^{-1} of *B. bassiana*, the LT_{50}s achieved were 5.05, 6.47, and 6.04 d, respectively, and for *M. anisopliae* the values achieved were 5.64, 6.98, and 6.54 d, respectively. This is comparable with reports of Bugeme et al. (2008), who found that at a concentration of 1×10^7 conidia.mL^{-1} of *B. bassiana* isolates ICIPE278 and ICIPE279, LT_{50}s of 1.9 and 2.0, respectively, were achieved at 35 °C to control adult female populations of *T. evansi*. In contrast with a concentration of 1×10^7 conidia per mL, isolates ICIPE55, ICIPE59, and CIPE78 of *M. anisopliae* achieved LT_{50}s of 2.6, 3.4, and 2.7 d, respectively, to control *T. evansi* at 35 °C, which was faster. In our study, LT_{50} can be correlated with mortality rate ($r = -0.8976$; $P < 0.001$), which implies that high mortality and short LT_{50}s are linked, and that mortality is related to concentration, i.e., the infection rates are dependent on conidial concentrations. The LT_{50}s obtained showed that with increasing concentrations (1×10^7, 5×10^7, and 1×10^8), the decrease in population size of citrus rust mite was more evident; however, the population growth rate (r) is reduced, and the reproductive potential of the females is possibly reduced, decreasing the fertile period and the oviposition time.

The present study revealed that *B. bassiana* and *M. anisopliae* are efficient in controlling *P. oleivora* populations under greenhouse conditions, whereas *I. fumosorosea* produces moderate control. The mortality of the population of mobile stages of *P. oleivora* depends on the isolate concentration and fungus. Also, the higher the concentration, the higher the mortality in the citrus rust mite population. Our results demonstrate that entomopathogenic fungi can be used as a potential microbial control on *P. oleivora* in a program of integrated pest management (IPM) to control citrus rust mite in the orange crop under greenhouse conditions.

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