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Effect of photoperiod and host distribution on the horizontal transmission of *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) in greenhouse whitefly using a novel model bioassay

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Abstract
A model bioassay was used to evaluate the epizootic potential and determine the horizontal transmission efficiency of *Isaria fumosorosea* Trinidadian strains against *Trialeurodes vaporariorum* pharate adults under optimum conditions (25 ± 0.5 °C, ~100% RH) at two different photoperiods. Untreated pharate adults were arranged on laminated graph paper at different distributions to simulate varying infestation levels on a leaf surface. Four potential hosts were located 7, 14, and 21 mm away from a central sporulating cadaver simulating high, medium and low infestation levels, respectively. Percent hosts colonized were recorded 7, 12, 14 and 21 days post-treatment during a 16 and 24 hour photophase. After 21 days, mean percent hosts colonized at the highest, middle and lowest infestation levels were 93 and 100%, 22 and 58%, 25 and 39% under a 16 and 24 hour photophase, respectively. From the results, it was concluded that the longer the photophase, the greater the percentage of hosts colonized, and as host distance increased from the central sporulating cadaver, colonization deceased. The use of this novel model bioassay technique is the first attempt to evaluate the epizootic potential and determine the horizontal transmission efficiency of *I. fumosorosea* Trinidadian strains under optimal environmental conditions at different photoperiods. This bioassay can be used to assess transmission efficiency for the selection of fungi being considered for commercial biopesticide development.

Keywords: blastospore, epizootic potential, whitefly, leaf model bioassay, secondary cycling

Introduction

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) is a pest of agricultural crops worldwide that necessitates frequent control using insecticides (Castañé & Albajes 1992; Antonious & Snyder 1995; Wang et al. 2003). Due to the inappropriate use of insecticides, environmental concerns and evolution of resistance to important insecticides, there is need for alternative methods to control whiteflies (Omer et al. 1992; Sanderson & Rousch 1992; Bi & Toscano 2007).

Entomopathogenic fungi are being developed as major components of integrated pest management programs for controlling *T. vaporariorum* and other whiteflies (Fransen 1993; Poprawski & Jones 2000; Meekes et al. 2002; Vidal et al. 2003; Cuthbertson & Walters 2005; Cuthbertson et al. 2008). Most research using fungi for controlling aleyrodids has focused primarily on using *Beauveria bassiana* (Balsamo) Vuillemin, *Lecanicillium muscarium* (Petch) Zare & Gams and *Aschersonia* species; however, *Isaria fumosorosea* (=*Paecilomyces fumosoroseus*) Wize (Hypocreales: Cordycipitaceae), has also been shown to cause epizootics in whitefly populations on various host plants (Lacey et al. 1995; Wraight et al. 2000; Meekes et al. 2000; Gökçe & Er 2005; Saito 2005; Alma et al. 2007).

To determine the efficacy of an entomopathogen used in any control program, both biotic (host density, developmental stage, etc.) and abiotic (humidity, temperature, photoperiod, etc.) factors that regulate epizootics in insect pests need to be evaluated (Shah & Pell 2003; Vidal et al. 2003; Pell 2008; Vidal & Fargues 2008). One of many factors important in epizootics, such as epizootic potential, depends not only on their ability to sporulate on cadavers, but also their ability to spread to healthy insects, a process known as secondary cycling (Thomas et al. 1995, Luz & Fargues 1997, 1999; Luz et al. 1999; de la Rosa et al. 2000; Long et al. 2000a; Arthurs & Thomas 2001a, b). Factors that promote secondary cycling are important for achieving long term control and can increase the horizontal transmission efficiency of the entomopathogen. Horizontal transmission efficiency is dependent upon several parameters including the number and distribution of individuals in the infected population. The importance of density dependent transmission efficiency has been confirmed in the laboratory (Galani & Almasan 1984; Carruthers & Soper 1987) and field (Brown & Hasibuan 1995; Keller et al. 1997; de la Rosa et al. 2000; Long et al. 2000b; Kreutz et al. 2004).

Epizootic parameters of entomopathogenic fungi against various arthropods have been studied under laboratory conditions using different model systems. Hall (1984) used leaf disc bioassays to study the epizootic potential of *L. muscarium* isolates against aphids, whereas Mier et al. (1991) used glass slides in moisture chambers to conduct *in vitro* pathogenicity tests on whitefly nymphs with Mexican isolates of *L. muscarium.* Landa et al. (1994) used a glass slide bioassay to compare the pathogenicity of conidia from different isolates of *I. fumosorosea, L. muscarium* and *B. bassiana* against early fourth-instar whitefly nymphs. Later, Landa and Bohatá (1999) used glass slides to determine the compatibility of *I. fumosorosea* with natural insecticides against whitefly nymphs. Most recently, Avery et al. (2004) used a glass slide bioassay to compare the pathogenicity of blastospores and conidia of *I. fumosorosea* Trinidadian strains against pharate adults of *T. vaporariorum* at two different photoperiods. Using this model, horizontal transmission efficiency of *I. fumosorosea* strains after colonization could not be assessed, therefore; a novel model bioassay simulating a leaf surface was designed.

The purpose of this study was to determine the horizontal transmission efficiency of *I. fumosorosea* Trinidadian fungal strains in colonizing susceptible pharate adults of the greenhouse whitefly at different distances from a sporulating cadaver under different photoperiod regimes. Two photoperiods were chosen to compare the horizontal transmission of the fungal strains under different growing conditions in a greenhouse. This study reports the use of a simulated model bioassay to determine the epizootic potential and horizontal transmission of *I. fumosorosea* Trinidadian strains at two different photophases.

Materials and methods

Test insects

Greenhouse whitefly pharate adults (17-18 d old; = 5^{th} instar) were supplied by British Crop Protection Ltd., England. Prior to the assay, detached leaves (*Nicotiana tabacum* L.) were washed with sterile distilled water to remove other potential fungi contaminating the hosts and allowed to dry in a fume hood. Pharate adults were carefully removed at random from the semi-desiccated leaf surface with a probe made from a flattened hypodermic needle (Landa et al. 1994) for use in the bioassays.

Fungal strains

Isaria fumosorosea (*Ifr*) strains T, T10 and T11 used in this study were originally collected in Trinidad and maintained at CABI BioScience (Egham, UK) on potato dextrose agar (PDA; Oxoid™) slants. Strains T and T11 were both originally isolated from *Bemisia tabaci* (Gennadius) in 1990 and 1991, respectively, and strain T10 from an unknown aphid species in 1990. *Ifr* Trinidadian strains were maintained on PDA slants at Birkbeck, University of London and later cultured at 25 ± 0.5 °C on PDA prior to use in the experiments.

Liquid culture media for blastospores

Liquid culture medium modified from Jackson et al. (1997) was used to produce the blastospores of *Ifr* strains. The liquid culture media contained the following ingredients per liter: KH_2PO_4, 2 g; $CaCl_2.2H_2O$, 0.4 g; $MgSO_4.7H_2O$, 0.3 g; $Fe_2SO_4.7H_2O$, 50 mg; $CoCl_2.6H_2O$, 37 mg; $MnCl_2.4H_2O$, 16 mg; $ZnSO_4.7H_2O$, 14 mg; glucose, 80 g and Casamino acids, 13.2 g. To supply the required vitamins for the blastospore growth (Jackson et al. 1997), a 1 mL aliquot of *Aspergillus* vitamin solution was added to the medium which contained the following: biotin stock, 15 mL, nictinomide, 100 mg, pyridoxin, 250 mg, p-aminobenzoic acid, 200 mg, riboflavin, 100 mg, aneuren HCl, 0.5 mg, and pantothenic acid, 200 mg per liter of solution. Lastly, glucose was added to the liquid culture media stock, adjusted to a pH of 5.5 by the addition of HCl, autoclaved and then combined with the sterile *Aspergillus* vitamin stock solution.

Ifr conidial inoculum preparation and blastospore production

A circular plug (0.7 cm diameter) was removed from a conidial culture grown on PDA for 14 d at 25 ± 0.5 °C under constant fluorescent light for each strain, i.e. conditions previously determined to be optimum for conidial production for *Ifr* on PDA solid media (Avery 2002). Each plug was transferred to a PDA slant in a glass tube and incubated at 25 ± 0.5 °C light for 14 d under constant fluorescent light. These new PDA slant conidial cultures served as the inoculum for the liquid-culture media for producing blastospores. Conidial suspensions for

inoculating the submerged cultures were prepared by flooding each PDA slant with 10 mL of sterile, distilled water. Sterile glass beads (20-25 beads; 1 mm in diameter) were then added, and the tube suspension was agitated using a vortex mixer. The concentration of the inoculum for submerged cultures per strain was standardized using a hemocytometer to $2.2 \pm 0.2 \times 10^6$ conidia mL^{-1}.

Ifr inoculum suspensions (10 mL) were then poured into three separate flasks containing the liquid-culture medium. Liquid-cultures (100 mL in 250 ml Erlenmeyer flasks) were incubated at 25 °C on an orbital shaker (Gallenkamp™) at 140 rpm for 4 d and the pH was not buffered during culture growth. After incubation, each liquid culture broth was filtered through a double layer of sterile muslin to separate any fungal hyphae from the blastospores.

From each replicate filtered culture, a 30 µL pipette sample was spread with a bent sterile glass rod onto two separate PDA plates to determine the purity of each culture. All PDA plates were cultured at 25 ± 0.5 °C under constant fluorescent light for 2-3 d and observed to determine if contaminated with other fungi. Pure blastospore suspension per *Ifr* strain (based on observed plate culture) was chosen as the source of inoculum for producing the cadavers at both photoperiod regimes. The mean number ± SEM of viable blastospores mL^{-1} used to inoculate the whitefly pharate adult hosts for all *Ifr* strains produced in each flask was $1.8 \pm 0.13 \times 10^7$ (Avery et al. 2004).

Cadaver conidial production

Filtered blastospore droplets (~2 µL per drop) of each *Ifr* strain suspended in 0.01% Triton X-100 were placed on four sterile glass slides using an inoculating loop as described by Avery et al. (2004). Pharate adults of similar size were randomly removed from one of the two semi-desiccated tobacco leaves, placed in the middle of each suspension drop on the slide and allowed to dry in the fume hood. After the drops had dried, each slide containing 10 pharate adults were placed inside a sterile plastic Petri dish (100 x 15 mm) containing PDA and sealed using Parafilm®. The PDA in the Petri dishes maintained a high relative humidity (RH ~100%) for the duration of the assay. Each Petri dish was then placed in a growth chamber, and the assays were incubated at 25 ± 0.5 °C under a 24 h photophase. After 8 d of incubation, 5 sporulating cadavers for each strain were selected and scraped from a slide with a sterile razor blade and vortexed in 1 mL of 0.01% Triton X-100 (v/v) for 60 s. Ten individual aliquots (10 µL each) were taken from each suspension and the average conidial concentration per cadaver per *Ifr* strain was determined using a hemocytometer. The mean number ± SEM of conidia mL^{-1} produced per cadaver for strain T, T10 and T11 was 11.6 ± 3.2, 10.3 ± 1.4 and $3.3 \pm 0.5 \times 10^3$, respectively.

Model bioassay protocol

Ifr-infected pharate adult cadavers used for these studies were produced as described above. The dried inoculated pharate adults were then carefully transferred to the center of each sterile (alcohol swabbed) laminated graph paper square (5 x 5 mm) using the flattened hypodermic needle and adhered to the surface by a drop of water. Four healthy un-treated pharate adults were gently removed from a semi-desiccated leaf and placed surrounding the central inoculated pharate adult (cadaver) according to the specific grid arrangement (Figure 1). Grid arrangement A, B and C, simulated high, medium and low infestation levels on a leaf surface, respectively.

Each complete square (5 total insect hosts including the central cadaver) was carefully laid onto a sterile V-shaped glass rod previously placed on PDA agar in a plastic Petri dish (100 x 15 mm) and then sealed with Parafilm®. The glass rod was used to elevate the plastic square above the PDA so as to minimize contamination of other possible fungal pathogens present on the susceptible insect hosts. The sealed dishes were incubated at 25 ± 0.5 °C in growth chambers under a 16 and 24 h photophase. Each dish which contained the completed grid with insect hosts surrounding a central cadaver resting on the glass rod represented a model bioassay.

At assessment of each bioassay, Petri dish lids were carefully removed and the total number of hosts (including exuviae or eclosed adults) colonized by the fungus was recorded 7, 12, 14 and 21 d post-treatment. Colonized hosts were defined as producing conidia. To enhance observation of the transparent hyphal growth across the laminated surface, a colored plastic Petri dish was placed under the bioassay plate. The number of hosts colonized at both photoperiods was observed and recorded during the same period. All experiments were conducted using random samples of pharate adults from a single batch of whiteflies on two different leaves and tests under different photoperiods were conducted simultaneously. The different densities in the bioassay per *Ifr* strain tested were replicated 3 times and the experiment was repeated under a 16 and 24 h photophase.

Statistical analysis

Results were subjected to a three-way RMANOVA ($\alpha = 0.05$) factorial analysis to determine the effect of time, fungal strain, host distribution and photoperiod on host colonization. If no interaction was determined, than the mean percent hosts colonized between strains and infestation levels were separated using a one-way ANOVA + Tukey HSD test ($\alpha = 0.05$) after being arcsine transformed. Where no significant differences in percent colonization were found between the strains at the different densities over time, the data were pooled and re-analyzed. Percent of non-treated hosts colonized at different infestation levels per bioassay over time were arcsine transformed prior to being analyzed using a repeated measures (RM) ANOVA ($\alpha = 0.05$). To determine the relationships between host distance from the central cadaver and percent colonization, data were submitted to regression analyses and compared at both photoperiods. These statistical tests were conducted using PROC GLM procedures of SAS WIN_PRO platform (SAS Institute Inc., Cary, NC, 1999-2001).

Using Least Squares Dummy Regression Analysis (LSDR) we tested the null hypothesis: Ho: $B_{11} = B_{12} = 0$. That is, the regression coefficients for distance predicting colonization would be the same across the two photoperiods. For this analysis, distance was coded 1 for the 16:8 LD photoperiod and 2 for the 24:0 LD photoperiod. The two conceptual regression models were as follows:

Model 1: $y_1 = \beta_{01} + \beta_{11} x$ for $x \leq$ 24:0 LD .

Model 2: $y_2 = \beta_{02} + \beta_{12} x$ for 16:8 LD $x \geq$ 24:0 LD .

Where

y_1 = photoperiod 1 (16:8 LD)

y_2 = photoperiod 2 (24:0 LD)

x = Distance

These statistical tests were conducted using the PROC REG and 'TEST' statements in SAS for Windows version 9.1 (SAS Institute Inc., Cary, NC, 1999-2001).

Results

Effect of photoperiod regimes and fungal strains on colonization of insect hosts

Percent of hosts colonized at each infestation level for both photoperiod regimes increased significantly over time ($F = 3.53$; df = 3, 215; $P = 0.0170$). A significant interaction effect of host distribution on the percent colonization was observed amongst all the levels ($F = 4.74$; df = 2, 215; $P < 0.0001$) and, when comparing infestation levels, host colonization was significantly greater at the highest level ($F = 34.9$; df = 2, 215; $P < 0.0001$) compared to the medium or the lowest level after 7 days (Table 1). No differences ($F = 0.27$; df = 2, 53; $P = 0.767$) were observed between the performance of the fungal strains T, T10 and T11 on the percent colonization at either photoperiod regime for 7, 12, 14 and 21 days post treatment.

The percent of hosts colonized 21 d post-treatment under constant light for the highest infestation level (mean ± SEM: 100 ± 0.0) was 42% and 61% higher ($F = 6.21$; df = 2, 26; $P = 0.0017$) compared to the medium (58 ± 12.5) and lowest (39 ± 11.1) level which were similar, respectively (Table 2). Under a 16 h photophase, the percent hosts colonized 21 d post treatment for the highest infestation level (mean ± SEM: 92 ± 4.2) was 70% and 67% higher compared to the medium (22 ± 5.7) and lowest (25 ± 8.3) level which were similar, respectively.

Effect of time, infestation level and photoperiod regimes on colonization of hosts

Colonization of the whitefly hosts by all fungal treatments was significantly affected by time (RMANOVA: $F = 21.78$; df = 3, 211; $P < 0.0001$), infestation level ($F = 84.18$; df = 2, 211; $P < 0.0001$) and photoperiod ($F = 134.34$; df = 1, 211; $P < 0.0001$). At all infestation levels, colonization increased over time ($F = 4.74$; df = 6, 211; $P = 0.0002$) and the longer photoperiod (24 h) had a significant positive effect ($F = 3.53$; df = 1, 211; $P = 0.0170$) compared to the shorter photoperiod (16 h) over the 21 d observation period.

Linear regression slopes of percent colonization of the susceptible pharate hosts were significant at 16 h ($F = 26.6$; df = 1, 26; $P < 0.0001$) and 24 h ($F = 20.1$; df = 1, 26; $P < 0.0001$) 21 d post treatment (Figure 2). As the distance from the sporulating cadaver and the surrounding susceptible hosts increased, the percentage of hosts colonized significantly decreased at both 16 h ($R^2 = - 0.6297$; $P < 0.001$) and 24 h ($R^2 = - 0.8326$; $P < 0.001$) photoperiod regimes. Regression estimates suggest that distance is a stronger predictor of colonization for the 24:0 photoperiod (slope = -4.32046) than for the 16:8 LD photoperiod (slope = -4.71409). Results from the F test confirmed a significant difference between the slopes (F=18.50, p = 0.0007) with the 24 h photophase having a higher percent of susceptible hosts colonized at all infestation levels compared to the 16 h photoperiod. The null hypothesis that the two slopes were identical was rejected, confirming that the slopes do indeed differ significantly across the 2 photoperiods.

Discussion

Epizootic potential of fungal strains against whitefly hosts

In this study, potential hosts surrounding the central sporulating cadaver were colonized either as pharate adults or exuviae with eclosed adults. Sometimes *Ifr* hyphae were observed to entangle the eclosing adults by surrounding the nymphal casing (exuviae) with a netted mycelial mass and then sporulate. Wraight et al. (1998) observed postmortem hyphal growth and sporulation of *Ifr*

(20°C and 100% RH) rapidly covered the dead whitefly host and extended several millimeters onto the surrounding leaf surface.

In the distribution studies, with a single pharate adult being the only major nutrient resource for the fungus, the *Ifr* hyphae were able to colonize some of the surrounding hosts or host exuviae with eclosed adults located 21 mm away. However, honeydew transferred with the initially infected nymphs may also have provided a secondary carbon resource for the fungal hyphae. Fokkema et al. (1983) noted that aphid honeydew stimulated the growth of various saprophytic fungi on the wheat leaf phylloplane.

Colonization of healthy whitefly hosts may have been influenced by possible auto-dissemination of spores by the eclosed adults (range of eclosed adults per density: 0-50%) while inside each bioassay chamber. Auto-dissemination of *Ifr* PFR 97 spores by whitefly adults was recorded in greenhouse conditions on cucumber plants (Bohatá & Landa 2004). However, in this study, the auto-dissemination effect would be similar because the percent eclosion of the pharate adults after 21 d exposure to 16 h (10.9 ± 2.90) or 24 h (13.9 ± 7.35) photophases were similar for the different infestation levels. Once whiteflies eclosed, regardless of the photophase, they were observed to be impeded from flying freely and immediately became entangled in the infective fungal mycelium extending away from the central cadaver. Also, after analyzing the percent colonization over time, a consistent increase was found for all infestation levels at both photoperiod regimes.

The greatest distance infective hyphae spread from the central cadaver to susceptible hosts across the artificial surface was 21 mm. Wraight et al. (1998) indicated that the ability of *Ifr* hyphae to grow extensively over a leaf surface under humid conditions is a characteristic that certainly enhances its rapid spread through whitefly populations and its ability to cause epizootics. The increase in photophase from 16 to 24 h resulted in greater colonization of the whitefly hosts with *Ifr* conidia regardless of fungal strain. In contrast, *Ifr* Trinidadian conidial treatments cultured on PDA and applied against pharate whitefly adults on glass slides, indicated that the infection and colonization rates were similar under either photophase (Avery et al. 2004). The increased virulence may have resulted from the fungal strains being intially passaged through the central cadaver; however, this hypothesis was not verified in the present study. In addition to fungal virulence, an increased photophase has also been demonstrated to have a positive effect on the infection rate, conidiation and development of *Ifr* (Sakamoto et al. 1985; de la Torre & Cárdenas-Cota 1996; Avery et al. 2004).

In this model system, by varying distances healthy whitefly hosts were located from the central sporulating cadaver, it was possible to simulate high, medium and low infestation levels found on a leaf surface. However, this model system is limited and cannot account for behavioral parameters of the whitefly or plant characteristics that could affect the horizontal transmission of the fungus. For instance, in this study, whiteflies were held under optimum conditions for fungal development (Avery 2002), which may not reflect greenhouse or field environmental conditions due to the microclimatic conditions present on the leaf surface. In tritrophic interactions, parameters not included were the possible effects trichome type and density or allelochemicals found on the leaf surface on the growth of *Ifr*. In a similar study with greenhouse whitefly hosts, *Ifr* strains were not inhibited by the presence of exudates from the trichomes of *Pelargonium* cultivars (Avery 2002). However, Vega et al. (1997), and Lacey and Mercadier (1998) found that *Ifr* growth was inhibited by selected allelochemicals or secondary compounds commonly found

on the surface of leaves. In addition, Poprawski et al (2000) tested similar allelochemicals against conidial suspensions of both *Ifr* and *B. bassiana*. Perhaps these allelochemicals could be spread on the laminated surface of this model bioassay to determine what effect they may have on the potential infectivity, virulence and horizontal transmission of *Ifr* or other entomopathogenic fungi. Lastly, whitefly nymphs usually feed on the underside of the leaf surface, therefore the honeydew could be removed naturally from the phylloplane which may affect the colonization rate of *Ifr*.. The leaf model bioassay was designed simply to determine the effect of photoperiod on *Ifr* strains and whether the hyphae from one infected insect (cadaver) could colonize surrounding hosts at different spatial arrangements simulating different infestation levels under optimum conditions of temperature and humidity.

The choice of a 24 h photophase used in this model bioassay was based on previous data showing that *Ifr* Trinidadian strains grew faster under constant light compared to a shorter photophase (Avery 2002, Avery et al. 2004). In addition, certain plants such as pepper plants grown under continuous lighting (24 h) have resulted in earlier flowering and fruiting giving better yields under greenhouse conditions (Demers and Gosselin 1999). Therefore in this model, the choice of comparing the virulence and horizontal transmission of *Ifr* during either a 24 or 16 h photoperiod may have some practical application. For instance, although the colonization was faster under constant light, the end result after 21 days was not significantly different. Therefore, in greenhouse production, the added expense incurred for increasing the photoperiod to supplement the daylight hours is not warranted according to this model. The same result could be obtained at a shorter photophase over time and theoretically save the grower the added expense of using more electricity for lights in the greenhouse. However, this model is based on laboratory data and needs to be confirmed under greenhouse conditions.

Effect of environmental conditions and host distribution on transmission efficiency

Many authors have indicated that epizootics are dependent upon various abiotic and biotic factors which include environmental conditions and population distribution. For example, in a laboratory experiment using hypocrealean under optimum growing conditions, conidia transfer between inoculated *B. bassiana* and untreated spruce bark beetles at different ratios resulted in 96% mortality at a ratio of 1:1 and 90% mortality at a ratio of 1:2 (Kreutz et al. 2004). In addition at ratios of 1:5, 1:10 and 1:20 the mortalities after 7 days were 83, 77 and 75%, respectively. In soil experiments, horizontal transmission efficiency of infection by Hypocreales was correlated with the distribution of the pest population (Keller et al. 1997; Long et al. 2000a, b).

In this study, the colonization of whitefly hosts was positively correlated to the distribution of the population under optimum environmental conditions. The number of susceptible hosts colonized after 21 days was greatest when nearest to the sporulating cadaver. It was observed that host colonization occurred 61 and 67 % faster at the high infestation level than with the low level under a 24 and 16 hour photoperiod regime, respectively. By decreasing the distance susceptible hosts are located from the cadaver, the contact time by hyphae from the infected host is potentially shortened and subsequently the transmission efficiency should increase. Transmission efficiencies of *Ifr* thus appear to be dependent upon host distribution under high humidity. However, this was a model system and it does not take into account all the parameters involved in epizootics. In closed production systems, e.g. greenhouses, where optimum growing conditions for *Ifr* can be provided by short periods of misting, secondary cycling of the fungus

may occur as well. Vega et al. (1999) found that the *Ifr* blastospore formulation can germinate in 6-8 h under high humidity conditions. *Ifr* blastospore products, PFR 97™ 20% WDG (Certis, USA) and PreFeRal® WG, (BioBest, Belgium) are both recommended to be most effective when application is initiated before or at the first sign of insect presence. Also, these products are recommended to be applied when populations of whitefly are low in 'hot spots" before high infestations occur. Therefore, based on this model system, with only one cadaver infected with *Ifr*, at the lowest infestation level, the fungal hyphae were still able to infect the other whitefly hosts 21 mm away. This implies that transmission efficiency and secondary cycling can occur at very low infestation levels with *Ifr* under high humidity conditions readily available in a greenhouse. Commercial growers could remediate with *Ifr* at this low infestation level with potential infection and secondary cycling occurring for the next whitefly generation.

Model systems as tools for predicting fungal epizootics

Most modeling on transmission efficiency has dealt with entomopthoralean fungi (Brown and Hasibaun 1995; Oduor et al. 1997; Verghese and Sreedevi 2006); however, similar work for hypocrealean-host relationships is both lacking and needed. By using model systems, such as those used in this study, the likelihood of epizootics developing may be more accurately predicted for a specific fungus, given certain environmental and population parameters. In addition, this model bioassay can be used for simulating and predicting the virulence and horizontal transmission of *Ifr* or other entomopathogenic fungi against aleyrodid species or insects with a sessile developmental stage prior to application in the field.

This novel model bioassay technique is the first to evaluate the epizootic potential and determine the horizontal transmission efficiency of blastospores of *I. fumosorosea* Trinidadian strains under optimal environmental conditions at different photoperiods.

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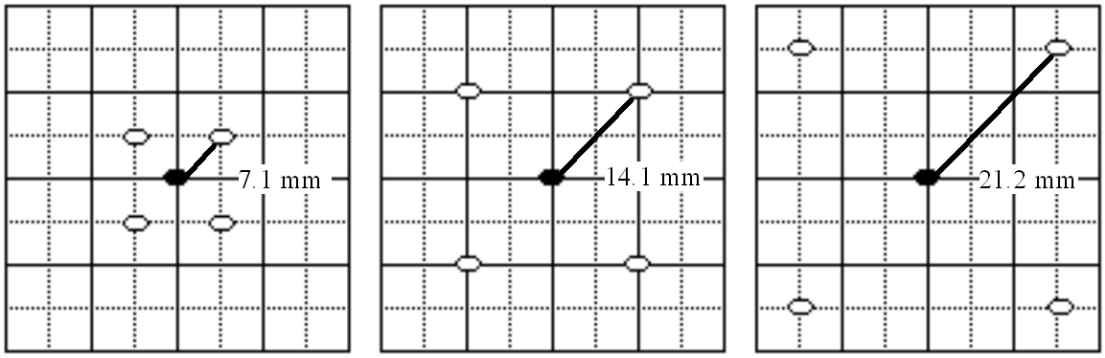
Figures

Figure 1. Distribution of infected (solid circles) and susceptible (open circles) *Trialeurodes vaporariorum* hosts and characteristics for the various grid arrangements A, B and C observed under 16:8 and 24:0 hour LD photoperiod regimes. Each unit square measures 5 x 5 mm.

Figure 2. Correlation between the distance of surrounding susceptible *Trialeurodes vaporariorum* hosts from the sporulating cadaver and the percentage colonized at different photoperiods when incubated at 25 ± 0.5 ºC with ~100 RH. There are 3 data points (1 per strain) for the varying distances from the cadaver represented, although some are obscured due to similar values.



Grid arrangement A Grid arrangement B Grid arrangement C

Grid arrangement	A	B	C
Infestation level	High	Medium	Low
Distance from cadaver (mm)	7.1	14.1	21.2

~~**Figure 1**~~

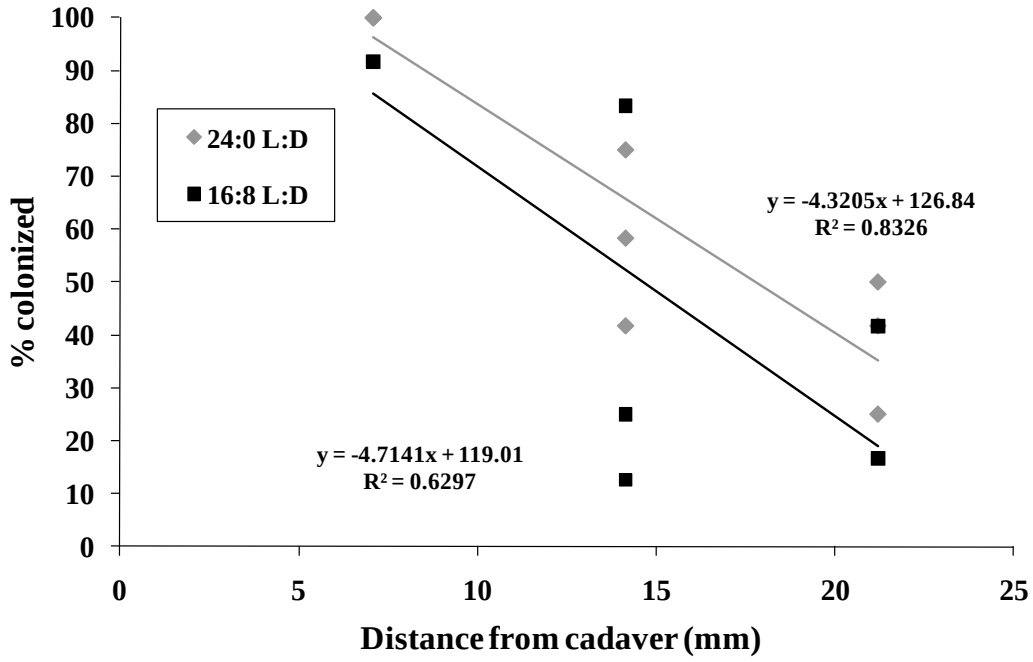

100
90
80
70
60
50
40
30
20
10
0
% colonized
24:0 L:D
16:8 L:D
y = -4.3205x + 126.84
R² = 0.8326
y = -4.7141x + 119.01
R² = 0.6297
0
5
10
15
20
25
Distance from cadaver (mm)

Figure 2

Table 1. Effect of photophase (hr), distance from the cadaver and Trinidadian fungal strains on the percent colonization of susceptible *Trialeurodes vaporariorum* (GW) hosts over time under laboratory conditions.

		Mean % GW hosts colonized[a] /days post-treatment			
Main Effect	n	7	12	14	21
Photophase (hr)					
16	27	0.0a	17.6a	19.4a	44.0a
24	27	45.4b	51.9b	57.4b	65.7b
distance from the cadaver[b]					
7	18	30.6a	65.3b	72.2b	95.8b
14	18	20.8a	22.2a	25.0a	36.8a
21	18	16.6a	16.7a	18.1a	31.9a
Fungal strain					
T	18	30.6a	40.3a	44.4a	62.5a
T10	18	19.4a	33.3a	36.1a	54.9a
T11	18	18.1a	30.6a	34.7a	47.2a

[a]Means followed by the same letter are not significantly different (Duncan multiple range test, $P < 0.05$)

[b]Four potential hosts were located 7.1mm (high), 14.1mm (medium) and 21.2 mm (low) from a central sporulating *T. vaporariorum* cadaver.(Infestation level)

Table 2. Main effect[a] of host distribution on the colonization of *Trialeurodes vaporariorum* (GW) with *Isaria fumosorosea* Trinidadian strains at different photoperiods (h L:D) incubated at 25 ± 0.5 °C with ~100% RH.

	Mean percentage ± SEM of GW colonized / day[bc]			
Host distribution (h L:D)	7	12	14	21
High (16:8)	0 ± 0.0c	53 ± 2.8b	58 ± 4.2b	92 ± 4.2a
High (24:0)	61 ± 11.1a	78 ± 7.7a	83 ± 7.2a	100 ± 0.0a
Medium (16:8)	0 ± 0.0c	0 ± 0.0c	6 ± 6.0c	22 ± 5.7c
Medium (24:0)	42 ± 11.7ab	44 ± 12.3b	50 ± 12.5b	58 ± 12.5b
Low (16:8)	0 ± 0.0c	0 ± 0.0c	0 ± 0.0c	25 ± 8.3c
Low (24:0)	33 ± 10.2b	33 ± 10.2b	36 ± 9.4b	39 ± 11.1cb

[a]The performance data for all *I. fumosorosea* strains for both photoperiods and distances from the cadaver were found similar (MANOVA, DMRT, $P > 0.05$) and were therefore combined prior to determining the mean percentage of GW colonized per day.

[b]Four potential hosts were located 7.1, 14.1 and 21.2 mm from a central sporulating GW cadaver for the highest, medium and lowest densities, respectively. (n = 9 replicates for all densities).

[c]Actual untransformed mean values in a column followed by different letters among the densities per hour (h) LD photoperiod are significantly different (Tukey HSD test, $P < 0.05$). Mean values were arcsine transformed prior to analysis.