Avery PB, Faull J, Simmonds MSJ. 2004. Effect of different photoperiods on the growth, infectivity and colonization of Trinidadian strains of *Paecilomyces fumosoroseus* on the greenhouse whitefly, *Trialeurodes vaporariorum*, using a glass slide bioassay. 10pp. *Journal of Insect Science*, 4:38, Available online: insectscience.org/4.38

Journal of Insect Science

insectscience.org

Effect of different photoperiods on the growth, infectivity and colonization of Trinidadian strains of *Paecilomyces fumosoroseus* on the greenhouse whitefly, *Trialeurodes vaporariorum*, using a glass slide bioassay

Pasco B. Avery[1, 2], Jane Faull[2], and Monique S. J. Simmonds[3]

[1]*Lee Academy, 4 Winn Road, Lee, Maine 04455, USA*
[2]*School of Biological and Chemical Sciences, Birkbeck College, Malet Street, London, WC1E 7HX, UK*
[3]*Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK*
pavery@leeacademy.lee.me.us

Received 23 January 2004, Accepted 22 August 2004, Published 3 December 2004

Abstract

Growth, infectivity and colonization rates for blastospores and conidia of Trinidadian strains T, T10, and T11 of *Paecilomyces fumosoroseus* (Wize) Brown and Smith were assessed for activity against late fourth-instar nymphs of *Trialeurodes vaporariorum* (Westwood) (Homoptera:Aleyrodidae) under two different photoperiods (24 and 16 hour photophase). A glass-slide bioassay and a fungal development index, modified for both blastospores and conidia, were used to compare the development rates of the fungal strains on the insect hosts. Fewer adult whiteflies emerged from nymphs treated with blastospores and reared under a 16:8 hour light:dark photoperiod than a 24:0 hour photoperiod. Eclosion times of whitefly adults that emerged from nymphs treated with the different strains of conidia were similar over the 8 day experimental period at both light regimes. The percent eclosion of adult whiteflies seems to be directly correlated with the speed of infection of the blastospore or conidial treatment and the photoperiod regime. The longer photophase had a significant positive effect on development index for blastospores; however, a lesser effect was observed for the conidia at either light regime. Blastospore strain T11 offered the most potential of the three Trinidadian strains against *T. vaporariorum* fourth-instar nymphs, especially under constant light. The glass-slide bioassay was successfully used to compare both blastospores and conidia of *P. fumosoroseus*. It can be used to determine the pathogenicity and the efficacy of various fungal preparations against aleyrodid pests.

Keywords: fungal development index; blastospore; conidia; eclosion rate, pathogenicity, entomopathogenic, virulent

Abbreviation:
FDI fungal development index

Introduction

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) is a major pest of agricultural crops and ornamental plants worldwide (Webb *et al*. 1974; Omer *et al*. 1992; Antonious and Snyder 1995; Orozco *et al*. 1995; Manzano *et al*. 2000; EWSN 2003). Overall, crops in the families Cruciferae, Leguminosae, Malvaceae and Solanaceae are mainly attacked by *T. vaporariorum* (Byrne *et al*. 1990). Geraniums, the most widely cultivated ornamental potted plants in the Spanish Mediterranean area, are also seriously affected by the greenhouse whitefly (Castañé and Albajes 1992, 1994).

T. vaporariorum has been controlled primarily using various insecticides. However, according to the European Whitefly Studies Network, the greenhouse whitefly continues to be a major problem on protected crops where populations have developed high levels of insecticide resistance (EWSN 2003).

Various studies have demonstrated the effectiveness of fungal pathogens for the control of *T. vaporariorum* and other whiteflies (Hall 1982, 1985; Landa 1984; Ramakers and Samson 1984; Samson and Rombach 1985; Fang *et al*. 1986; Fransen 1990, 1993; Ravensberg *et al*. 1990; van der Schaaf *et al*. 1991). Most of this research has been undertaken with *Verticillium lecanii* and species of *Aschersonia* (Lacey *et al*. 1995).

In 1989, an isolate of *Paecilomyces fumosoroseus* (Wize) Brown and Smith was isolated from a mealybug in Apopka, Florida (Osborne and Landa 1992). Smith (1996) tested and demonstrated that three Trinidadian strains, T, T10 and T11, of *P. fumosoroseus* conidia were highly virulent against nymphal instars of *Bemisia tabaci*. However, the efficacy of these strains against *T. vaporariorum* was not assessed.

A thorough knowledge of the growth, infectivity and colonization rates of entomopathogens is vital in predicting efficacy for any biocontrol spray program. In preliminary tests, blastospores of strain T11 of *P. fumosoroseus* exhibited faster radial growth than the Trinidadian strains T and T10 when grown in vitro (Avery 2002).

However, this fact gives no indication of the entomopathogenic activity once the fungal blastospores, conidia or hypha come in contact with the cuticle of the target host.

Both the speed of germination and initial infection rate of entomopathogens varies among strains and is dependent on the type of spores applied in a spray program. Vega *et al*. (1999) observed that blastospores of *P. fumosoroseus* germinated faster than conidia of the same strain on both the silverleaf whitefly cuticle and solid media. Other studies have indicated that blastospores were as effective as conidia (Kanagaratnam *et al*. 1982; Vandenburg *et al*. 1998) or less virulent (Bell 1975; Lane *et al*. 1991).

The development, virulence, and subsequent colonization of entomopathogens on various insect hosts may be affected by differences in photophases (Hoffman and Byrne 1986; Landa *et al*. 1994; Feng 1998). In contrast, Feng *et al*. (1999) studied the infectivity of *Pandora neoaphidis* to the pea aphid and found that changes in the photoperiod seemed less important than changes in temperature.

Successful development of entomopathogenic fungi as potential microbial insecticide agents requires careful assessment and selection of the most efficacious species and isolates (Wraight and Carruthers 1999; Hajek *et al*. 2001). Laboratory bioassay procedures have played a crucial role in screening different isolates for determining the pathogenicity and efficacy of various fungal preparations against target pests prior to being tested in field trials. Mier *et al*. (1991) conducted *in vitro* pathogenicity tests on whitefly nymphs with Mexican isolates of *V. lecanii* using glass slides in moisture chambers. Later, Landa *et al*. (1994) used a glass-slide bioassay for comparing pathogenicity of conidia of different isolates of *P. fumosoroseus* (isolate PFR 97), *V. lecanii*, and *Beauveria bassiana* against early fourth-instar nymphs of greenhouse and silverleaf whitefly. They used a fungal growth development index to determine the pathogenicity and colonization of conidia on the whitefly hosts.

The present study uses a glass-slide bioassay and a fungal development index (FDI) to determine the infectivity rate for blastospores and conidia of three Trinidadian strains (T, T10 and T11) of *P. fumosoroseus* against late fourth-instar nymphs (sub-stage 2 or 3) of *T. vaporariorum* under two different photoperiods. The FDI was based on the different developmental stages of blastospores of *B. bassiana* (Bidochka *et al*. 1987).

Materials and Methods

Test insects and fungi

Greenhouse whitefly fourth-instar nymphs in sub-stage 2 or 3 infesting tobacco leaves were supplied by British Crop Protection Ltd., England. The Trinidadian strains of *Paecilomyces fumosoroseus* were obtained from CABI BioScience, Egham, UK.

Laboratory glass-slide bioassay protocol

A glass-slide bioassay was used to determine the FDI for each fungal strain. Upon arrival (< 24 hours), the whitefly-infested leaves were washed with sterile distilled water and allowed to dry in a fume hood. Nymphs were then carefully removed from the leaf surface with a probe made from a flattened hypodermic needle. Each nymph was placed in either a droplet of distilled water, a Triton X-100 solution of *P. fumosoroseus* blastospore or a conidial suspension on a sterile microscope slide. Similar size drops ~2 ml per drop) of fungal treatments were placed on the slide using an inoculating loop.

The filtered blastospore and conidial suspension of the three strains of *P. fumosoroseus* were made up in distilled water as described by Avery (2002). Drops of distilled water or Triton X-100 (0.01% v/v) in solution served as the controls. The number of viable conidia for each suspension was 1.0 ± 0.06, 1.3 ± 0.08, and 1.2 ± 0.02 × 10^6 ml^{-1} and the number of blastospores was 2.1 ± 0.19, 2.1 ± 0.07, and 1.2 ± 0.03 × 10^7 ml^{-1} for *P. fumosoroseus* strain T, T10, and T11, respectively. These concentrations were not standardized as they reflect the number of viable blastospores and conidia collected from the three strains of *P. fumosoroseus* using a set procedure (Avery 2002). Within an experiment each nymph was considered as an individual replicate, and each glass slide with 7, 8, or 10 nymphs placed on it was considered as a block.

To determine the effect of a surfactant on the growth of blastospores of *P. fumosoroseus*, Triton X-100 (0.01% v/v) was added to the suspension and results were compared with those from testing the blastospore suspension containing no surfactant. A nymph was placed in the middle of each drop on the slide and allowed to dry in the fume hood. Nymphs placed in drops of distilled water or in Triton X-100 served as the controls. After the drops had dried, each slide was placed inside a sterile plastic Petri dish (100 × 15 mm) directly on top of potato dextrose agar (Difco, www.voigtglobal.com/DIFCO.htm). The agar in the Petri dishes maintained a high relative humidity (RH) at ~100% for the duration of the assay as evidenced by condensation on the dish, however, the tops were not sealed and the RH was not measured. Each Petri dish was then placed in a growth chamber, and the assays were maintained at 25 ± 0.5 °C under either 24:0 or 16:8 hour light:dark (LD) photoperiod regime.

The fungal blastospore assay at a 24:0 hour LD regime consisted of 10 nymphs per slide (2 drops at the edge for control, 8 drops for each replicate treatment) and was repeated twice. Thus the total number of nymphs was 16 nymphs for treatment and 4 for control per experiment. The fungal blastospore assay at a 16:8 hour LD regime consisted of 8 nymphs per slide (8 drops per treatment and control) and this was repeated three times. The total number of nymphs per experiment was 24 for each treatment and control.

The conidial assay at a 24:0 hour LD photoperiod regime consisted of 24 nymphs (8 drops per treatment) repeated three times, giving a total of 24 nymphs per experiment. Control treatments for the conidial assay consisted of 23 nymphs per experiment (8, 8, and 7 nymphs per slide). The conidial assay at a 16:8 hour LD photoperiod regime consisted of 5 nymphs per slide (5 drops per treatment) repeated three times, resulting in a total of 15 nymphs per experiment. Control treatments consisted of 16 nymphs per experiment (8 nymphs per slide).

Nymphs placed on the slides were monitored for 8 days for both photoperiod regimes. Petri dishes containing the nymphs on the glass slide were placed on the stage of a light microscope (400×) and the dish cover was carefully removed to allow determination of the stage of fungal development per nymph.

Experiments with blastospore and conidial treatments at the two photoperiods were conducted independently on different occasions with different cohorts of insects.

In these studies, nymphs covered with the fungus were assumed to have died from the infection due to exposure to either blastospore or conidial inoculations of *P. fumosoroseus*, although the mode of action for the death of insects was not addressed. Increases in mortality between treatments and controls was assumed to be due to infection by *P. fumosoroseus*.

Fungal development index (FDI)

The degree of fungal development for all *P. fumosoroseus* strains on the whitefly nymphs was assessed using the FDI (Figures 1a–f). All assays were rated daily until sporulation was completed (Figure 1f) on the insect host or until eclosion of the adult had occurred. Controls were used to assess the number of adult whitefly eclosed in these assays. When being evaluated, each nymph was assessed individually under a compound light microscope (400×), and the stage of fungal development on the nymph was recorded using the FDI.

FDI values of 0.0 = no fungal growth; 0.5 = germination with one or two germ tubes, especially close to the nymph; 1.0 = initial growth of fungus towards the host (Figures 1a–b). FDI value 0.5 represented the beginning of the growth phase and initial viability of either the blastospore or conidia of the fungus. For FDI values 1.5–2.0, once the fungus had contacted or colonized the host this phase was irreversible and the host insect would not recover from the infection (FDI values of 1.5 = first contact between nymph and hyphae is noticed; 2.0 = growth of mycelium on the host: mycelium growth on the surface of nymph and in the area around the nymph; presence of dense mycelium was noted). Conidiogenesis was represented by FDI values 2.5–3.0 (FDI values of 2.5 = initial sporulation, first conidiospore is present on the surface of the nymph; 3.0 = sporulation completed, nymph is covered with mycelium and conidia [Figures 1c–f]).

Each nymph was rated individually as a separate replicate according to the FDI and results were expressed as a mean value for nymphs in each treatment for the duration of the bioassay. Control samples were assessed at the same time. Eclosed adults were recorded for both fungal treatments and control under both photoperiod regimes. All blocks of a given treatment were counted together to determine percent of eclosed whitefly adults. Percent of eclosed adults was determined by dividing the total number eclosed by the total number of nymphs (eclosed and colonized) on all glass slides per treatment times 100.

Determining percent fungal germination

Percent germination was determined by viewing 100 blastospores under the compound microscope (400×) after they had been incubated for 6–8 hours on PDA plates at 25 ± 1 °C. Conidia were viewed 24 hours after incubation. Either blastospores or conidia were considered to have germinated if a germ tube had formed. This procedure was repeated at least twice and a mean was calculated for each fungal strain.

Statistical analysis

In determining which fungal strain had a faster development rate using the FDI, each fourth-instar whitefly nymph was considered as an individual unit for all experiments at both a 24:0 and 16:8 hour LD photoperiod. These FDI values were then used to calculate the mean rating for the individual fungal treatments. Mean FDI values among each fungal treatment were analysed using an ANOVA ($\alpha = 0.05$) to determine any differences among the treatments per day and a two-way repeated measures (RM) ANOVA ($\alpha = 0.05$) was employed to determine any differences among the treatments per observation period. If the ANOVA was significant, then a Sheffé F-test ($\alpha = 0.05$) was employed to identify significant differences among treatment means on a daily basis. If growth trends for strains were not found significant using the RMANOVA ($\alpha = 0.05$) between experiments, means were calculated and statistically analysed as described above. The effect various strains had on the eclosure of the whitefly adults was assessed using a an ANOVA and then a two-way RMANOVA ($\alpha = 0.05$) as described above. Treatments were then separated using a Fisher's Protected Least Significant Difference (PLSD; $\alpha = 0.10$) for both photoperiods. The RMANOVA ($\alpha = 0.05$) was also used to determine if photoperiod length had any effect on the rate of infection and colonization of the whitefly nymphs. All statistical tests were done using the Statview® II: Statview® SE + Graphics from Abacus Concepts software 1991.

Results

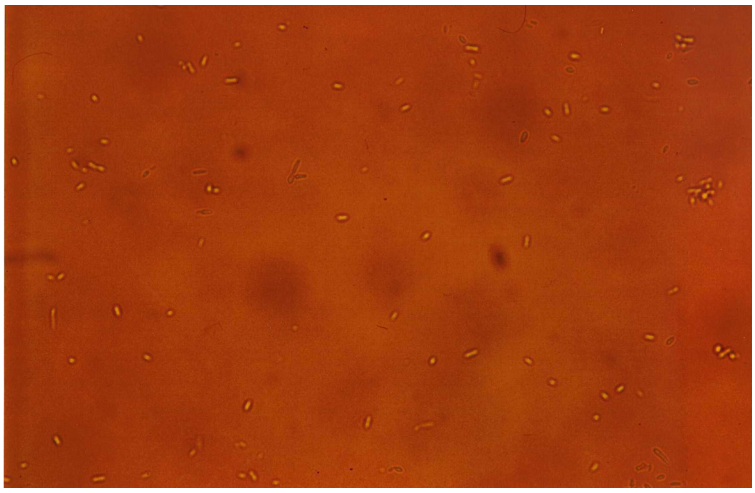
FDI for blastospore treatments on nymphs under both photoperiod regimes

The FDI values did not differ significantly when blastospores of strain T ($F = 2.6$; df = 1, 60; $P = 0.11$), T10 ($F = 2.1$; df = 1, 62; $P = 0.15$) and T11 ($F = 0.4$; df = 1, 61; $P = 0.51$) were suspended in water versus 0.01 % Triton X-100 (v/v). Therefore, the surfactant did not influence the growth of the blastospores.

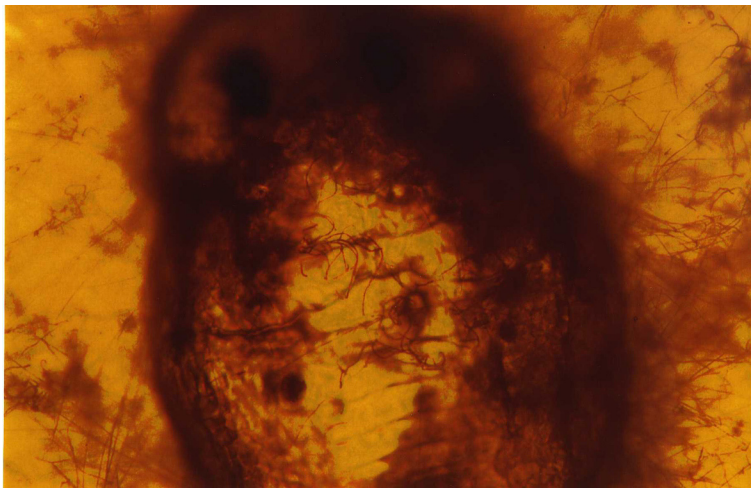
FDI values for the replicate experiments per treatment showed the same trends over the 8 day observation period at 25 ± 0.5 °C under a 24:0 hour LD photoperiod (RMANOVA: $F = 1.3$; df = 2, 6; $P = 0.27$) and were therefore pooled (Table 1). FDI values were significantly higher for blastospores of strains T ($F = 13.15$; df = 1, 45; $P < 0.001$), T10 ($F = 11.37$; df = 1, 45; $P = 0.0013$) and T11 ($F = 14.32$; df = 1, 44; $P < 0.001$) 3 days post-treatment under a 24:0 hour LD photoperiod, compared to a 16-hour photophase. The FDI values for strains T and T11 were significantly higher under a longer photoperiod until day 8, whereas with strain T11 the difference in FDI values was still observed on day 8 ($F = 5.16$; df = 1, 44; $P = 0.023$). The longer photophase had a significant positive effect on the colonization of the nymphs by strain T (RMANOVA: $F = 18.0$; df = 1, 45; $P < 0.001$), T10 (RMANOVA: $F = 9.94$; df = 1, 45; $P = 0.0025$) and T11 (RMANOVA: $F = 19.1$; df = 1, 44; $P < 0.001$) over the observation period. The interactive effect between photoperiod and colonization of the nymphs over time was also significant for strain T (RMANOVA: $F = 10.1$; df = 5, 225; $P < 0.001$), T10 (RMANOVA: $F = 16.7$; df = 5, 300; $P < 0.001$) and T11 (RMANOVA: $F = 15.5$; df = 5, 220; $P < 0.001$).

Over the 24 hour photophase, the mean percentage nymphs colonized was 100%, 84%, and 84% for blastospore treatments T11, T10, and T, respectively. Percent mortality was 17% and 50% for the controls in experiments 1 and 2, respectively, however, 100%

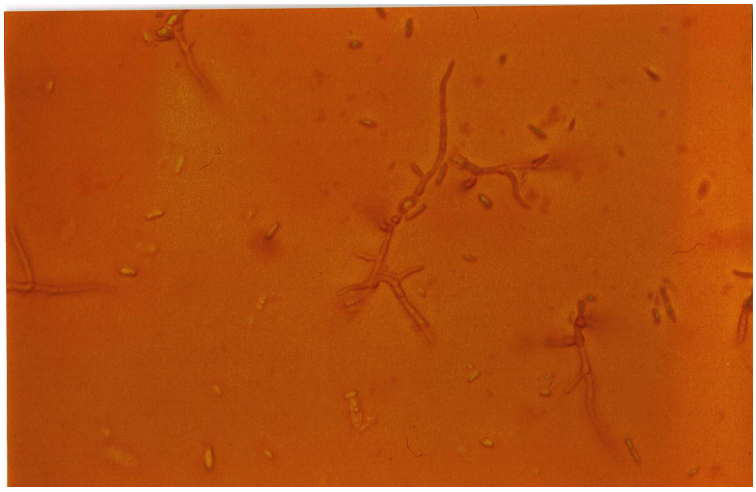
Figures 1a–f. Fungus Development Index (FDI) for Trinidadian strains of *Paecilomyces fumosoroseus* (Pf) on fourth-instar nymphs of *Trialeurodes vaporariorum*.



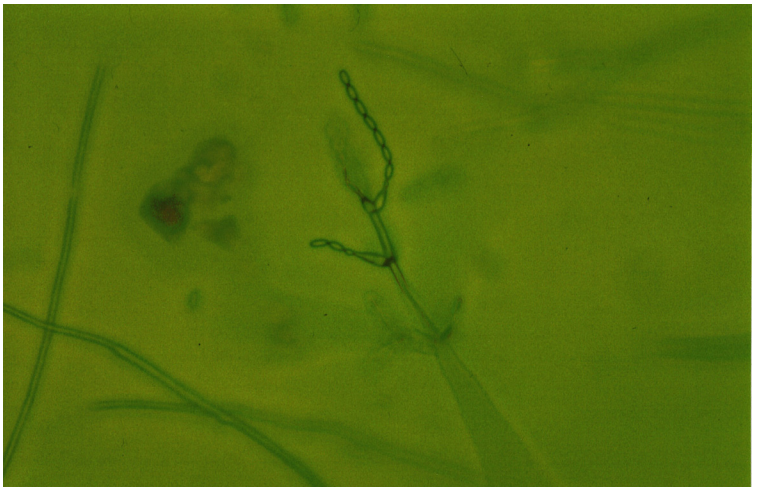
(a) Nongerminated blastospores of Pf in suspension drop which surrounds nymph. FDI = 0.0 (magnification, 200×)



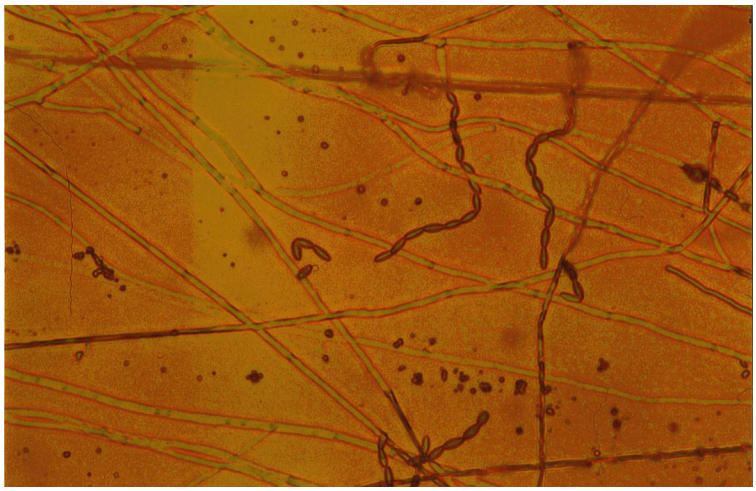
(d) Initial fungal outgrowth from killed host. Nymph infected with Pf fungal hyphae with conidia. Red eyes are still visible. FDI = 2.5 (magnification, 100×)



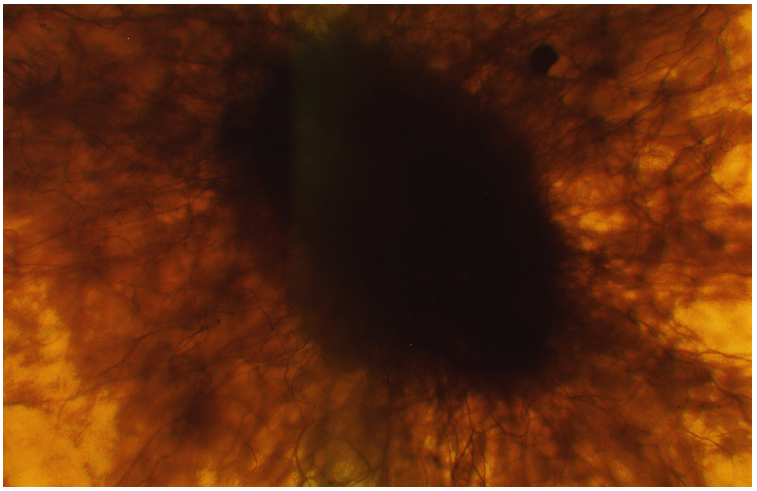
(b) Germination of Pf blastospores with one or more germ tubes. FDI = 0.5 (magnification, 400×)



(e) Pf conidia developed on phailides. Note whorled branching of hyphae with conidia. Sporulation completed on at least 4 areas on nymph where conidia have developed on phailides. FDI = 3.0 (magnification, 400×)



(c) Initial sporulation, first conidiospore is present on the surface of the nymph and in the in area surrounding the nymph. Note the hyphal branching with conidia. FDI = 2.5 (magnification, 400×)



(f) Fungal outgrowth from killed host. Sporulation completed. Nymph is covered with mycelium and conidia. Note eyes and nymph are not visible. FDI = 3.0 (magnification, 50×)

same FDI values from day 2 to day 8, whereas the FDI values of the same strain exposed to the 16:8 photoperiod increased during the experimental period, so that by day 8 the FDI value was significantly greater than that for the 24:0 photoperiod ($F = 9.44$; df = 1, 46; $P = 0.004$). Over the observation period, significant interactive positive effect of photoperiod on the colonization of the whitefly nymphs was found for strains T (RMANOVA: $F = 2.91$; df = 4, 184; $P = 0.023$) and T11 (RMANOVA: $F = 9.74$; df = 4, 184; $P < 0.001$), but not for strain T10 (RMANOVA: $F = 0.39$; df = 4, 184; $P = 0.813$).

The natural mortality for those reared under constant light was 30% (range 25–38%) and the corrected mean mortality for treatments T, T10, and T11 was 59%, 34%, and 29%, respectively. After 8 days the FDI assessment was discontinued because there were no observable changes apparent after days 7 and 8, and the maximum eclosion of adults (70%) occurred in the control treatments.

Under a 16 hour photophase, the natural mortality was 7% and the corrected mortality for treatments T, T10 and T11 was 12%, 5%, and 3%, respectively. The percentage of nymphs covered with both mycelium and conidia (FDI value of 3.0) after an 8 day period of observation was 13%, 8%, and 4% for treatments T,

Table 3. Effect of *Paecilomyces fumosoroseus* blastospore fungal treatment strains T, T10 and T11 for percent eclosion of whitefly adults from fourth-instar nymphs on a glass slide placed inside a PDA Petri dish plate at 25 ± 0.5 °C for 8 days with a 24:0 and 16:8 hour L:D photoperiod at ~100% RH.

	Mean % eclosion ± SEM values observed for blastospore treatments per day[1]						
Days	1	2	3	4	5	6	8
Treatment (L:D)							
strain T (24:0)	9.4 ± 5.98a	15.6 ± 9.38b	25.0 ± 8.84b	25.0 ± 8.84b	25.0 ± 8.84b	31.3 ± 13.01b	31.3 ± 13.01b
strain T (16:8)	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a
strain T10 (24:0)	6.3 ± 6.25a	15.6 ± 5.98b	25.0 ± 10.21b	28.1 ± 12.89b	28.1 ± 12.89b	28.1 ± 12.89b	28.1 ± 12.89b
strain T10 (16:8)	0.0 ± 00.0a	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a
strain T11(24:0)	3.2 ± 3.13a	12.5 ± 5.10a	15.6 ± 5.98a	18.8 ± 3.61a	18.8 ± 3.61a	25.0 ± 5.10a	25.0 ± 5.10a
strain T11(16:8)	6.7 ± 6.67a	6.7 ± 6.67a	6.7 ± 6.67a	6.7 ± 6.67a	6.7 ± 6.67a	13.3 ± 13.30a	13.3 ± 13.30a
Control (24:0)	25.0 ± 14.44a	37.5 ± 14.24c	58.4 ± 8.35c	66.7 ± 13.62c	66.7 ± 13.62c	66.7 ± 13.62c	66.7 ± 13.62c
Control (16:8)	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a	58.3 ± 4.17c	58.3 ± 4.17c	91.7 ± 4.17c	91.7 ± 4.17c

[1]Means followed by different letters in a column are significantly different (Fisher's PLSD test, $p < 0.10$). n = 32 nymphs for T , T10; n = 31 nymphs for T11 for each fungal strain and n = 24 nymphs for control at each daily period of observation under a 24:0 hour L:D photoperiod. n = 24 nymphs for each fungal strain and n = 24 nymphs for control at each daily period of observation under a 16:8 hour L:D photoperiod.

Table 4. Effect of *Paecilomyces fumosoroseus* blastospore fungal treatment strains T, T10 and T11 for percent eclosion of whitefly adults from fourth-instar nymphs on a glass slide placed inside a PDA Petri dish plate at 25 ± 0.5 °C for 8 days with a 24:0 and 16:8 hour L:D photoperiod at ~100% RH.

	Mean % eclosion ± SEM values observed for blastospore treatments per day[1]						
Days	1	2	3	4	5		8
Treatment (L:D)							
strain T (24:0)	0.0 ± 0.00a	0.0 ± 0.00a	20.8 ± 15.02a	29.2 ± 16.70a	29.2 ± 16.70a	29.2 ± 16.70a	29.2 ± 16.70a
strain T (16:8)	8.3 ± 4.17a	8.3 ± 4.17a	8.3 ± 4.17a	50.0 ± 14.43ab	50.0 ± 12.50ab	50.0 ± 11.02a	54.2 ± 11.02a
strain T10 (24:0)	0.0 ± 0.00a	0.0 ± 0.00a	29.2 ± 8.33ab	37.5 ± 14.43a	37.5 ± 14.43a	37.5 ± 14.43a	41.7 ± 15.02ab
strain T10 (16:8)	4.2 ± 4.17a	4.2 ± 4.17a	8.3 ± 4.17a	20.8 ± 11.02a	20.8 ± 11.02a	20.8 ± 18.16a	37.5 ± 19.09a
strain T11(24:0)	0.0 ± 0.00a	0.0 ± 0.00a	16.7 ± 4.17b	45.8 ± 16.70a	45.8 ± 16.70a	45.8 ± 16.70a	45.8 ± 16.70b
strain T11(16:8)	0.0 ± 0.00a	0.0 ± 0.00a	20.8 ± 11.0ab	45.8 ± 22.05ab	45.8 ± 22.05ab	45.8 ± 22.05ab	54.2 ± 22.05b
Control (24:0)	0.0 ± 0.00a	0.0 ± 0.00a	43.5 ± 3.62ac	65.5 ± 7.80ab	65.5 ± 7.80ab	65.5 ± 7.80ab	69.6 ± 3.72b
Control (16:8)	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a	58.3 ± 4.17b	58.3 ± 4.17b	58.3 ± 4.17b	91.7 ± 4.17b

[1]Means followed by different letters in a column are significantly different (Fisher's PLSD test, $p < 0.10$). n = 32 nymphs for T , T10; n = 31 nymphs for T11 for each fungal strain and n = 24 nymphs for control at each daily period of observation under a 24:0 hour L:D photoperiod. n = 15 nymphs for each fungal strain and n = 16 nymphs for control at each daily period of observation under a 16:8 hour L:D photoperiod.

Table 1. Comparison of Fungal Development Index (FDI) for blastospore treatments of *Paecilomyces fumosoroseus* Trinidadian strains T, T10 and T11 infecting fourth-instar nymphs of *Trialeurodes vaporariorum* on a glass slide placed inside a PDA Petri dish plate at 25 ± 0.5 °C for 8 days with a 24:0 and 16:8 hour L:D photoperiod at ~100% RH.

	Mean FDI ± SEM values observed for blastospore treatments per day[1]					
Days	1	2	3	4	6	8
Treatment (L:D)						
strain T (24:0)[a]	1.4 ± 0.04b	1.6 ± 0.05b	1.8 ± 0.04b	2.3 ± 0.08b	2.8 ± 0.09b	2.8 ± 0.09a
strain T (16:8)	1.2 ± 0.07a	1.5 ± 0.07a	1.5 ± 0.08a	1.6 ± 0.07a	2.0 ± 0.14a	2.6 ± 0.13a
strain T10 (24:0)[a]	1.4 ± 0.04a	1.7 ± 0.04a	2.1 ± 0.06b	2.5 ± 0.08b	2.8 ± 0.07b	2.9 ± 0.07a
strain T10 (16:8)	1.5 ± 0.00a	1.8 ± 0.05a	1.8 ± 0.05a	1.9 ± 0.04a	2.4 ± 0.10a	2.7 ± 0.10a
strain T11 (24:0)[a]	1.6 ± 0.04a	1.9 ± 0.04a	2.3 ± 0.06b	2.8 ± 0.05b	2.9 ± 0.04b	3.0 ± 0.00b
strain T11 (16:8)	1.5 ± 0.00a	1.9 ± 0.06a	1.9 ± 0.07a	2.1 ± 0.11a	2.5 ± 0.15a	2.7 ± 0.13a

[1]Means followed by different letters in a column are significantly different (Sheffé F-test, $p < 0.05$). n = 32 nymphs for strains T and T10; n = 31 nymphs for strain T11 assessed at each daily period of observation under a 24:0 hour L:D photoperiod. n = 24 nymphs assessed for each fungal strain at each daily period of observation under a 16:8 hour L:D photoperiod.
[a]Data from replicate experiments were pooled

Table 2. Comparison of Fungal Development Index (FDI) for conidial treatments of *Paecilomyces fumosoroseus* Trinidadian strains T, T10 and T11 infecting fourth-instar nymphs of *Trialeurodes vaporariorum* on a glass slide placed inside a PDA Petri dish plate at 25 ± 0.5 °C for 8 days with a 24:0 and 16:8 hour L:D photoperiod at ~100% RH.

	Mean FDI ± SEM values observed for conidial treatments per day[1]				
Days	2	3	4	6	8
Treatment (L:D)					
strain T (24:0)	1.5 ± 0.03b	1.5 ± 0.12a	1.6 ± 0.06a	1.9 ± 0.13a	1.9 ± 0.14a
strain T (16:8)	1.3 ± 0.07a	1.5 ± 0.09a	1.7 ± 0.16a	2.0 ± 0.18a	2.1 ± 0.19a
strain T10 (24:0)	1.5 ± 0.04a	1.6 ± 0.05a	1.7 ± 0.06a	1.8 ± 0.12a	1.8 ± 0.12a
strain T10 (16:8)	1.4 ± 0.07a	1.5 ± 0.09a	1.6 ± 0.14a	1.8 ± 0.15a	1.8 ± 0.15a
strain T11 (24:0)	1.5 ± 0.07a	1.5 ± 0.07a	1.5 ± 0.08a	1.5 ± 0.09a	1.5 ± 0.09a
strain T11(16:8)	1.5 ± 0.06a	1.6 ± 0.08a	1.6 ± 0.09a	1.7 ± 0.09a	2.0 ± 0.11b

[1]Means followed by the same letter in a column are not significantly different (Sheffé F-test, $p > 0.05$). n = 24 nymphs assessed for each fungal strain at each daily period of observation under a 24:0 and 16:8 L:D photoperiod.

of all nymphs in all blastospore treatments were colonized by day 7 in experiment 2. The combined corrected mean mortality for blastospore treatments T11, T10 and T, was 100%, 81%, and 81%, respectively.

In the 16 hour photophase experiment, the total percentage of colonized nymphs with a FDI value of 3.0 (covered with mycelium and conidia) after 8 days post-treatment was 47%, 73%, and 79% for blastospore strains T, T10, and T11, respectively. Natural mean mortality was 7% (range 0–12%), and corrected mean mortality was 34%, 71%, and 77% for strains T, T10, and T11, respectively.

FDI for conidial treatments on nymphs under both photoperiod regimes

The FDI values for the conidial treatments were similar for both photoperiods over the observation period for strain T (RMANOVA: $F = 0.163$; df = 1, 46; $P = 0.688$), T10 (RMANOVA: $F = 0.166$; df = 1, 46; $P = 0.686$) and T11 (RMANOVA: $F = 2.49$; df = 1, 46; $P = 0.122$) (Table 2). However, after 2 days post-treatment, with strain T the FDI values were higher in the 24:0 photoperiod than in the 16:8 photoperiod ($F = 6.68$; df = 1, 46; $P = 0.013$), but by day 3 the FDI values were similar for both photoperiods. Strain T11 exposed to the 24:0 photoperiod had the

T10, and T11, respectively.

Eclosion of adults in blastospore treatments under both photoperiod regimes

Under constant light, whitefly adults eclosed 24 hours after being placed in a drop of water on the glass slide in the control, and eclosion continued for three more days (Table 3). After 4 days post-treatment, no more eclosion of the whitefly adults occurred in the control. Blastospores of all three strains under constant light had a lower (one fifth to one third) number of whitefly adults eclosing compared to the control throughout the 8 day observation period (T = RMANOVA: $F = 7.42$; df = 1, 6; $P = 0.034$; T10 = RMANOVA: $F = 7.29$; df = 1, 6; $P = 0.036$; T11 = RMANOVA: $F = 17.67$; df = 1, 4; $P = 0.006$). Fungal strain T11 had the lowest percent (25%) of whitefly eclosed at the end of the observation period, compared to strain T (31%) or T10 (28%) (Table 3).

The eclosion of whitefly adults under 16 hour photophase began four days after being placed on glass slides in drops of water incubated at 25 ± 0.5 °C at ~100% RH and continued for a total of four more days in the control treatments (Table 3). Although the observation period was over 8 days, no further eclosion occurred after day 6.

Over the observation period, the eclosion of whitefly adults from nymphs treated with blastospores was similar among strains under a 16 hour photophase (Table 3). When compared to the control, the eclosion of adults was found to be lower (50%) within four days and above 75% after eight days for strains T (RMANOVA: $F = 480.57$; df = 1, 4; $P < 0.001$), T10 (RMANOVA: $F = 480.57$; df = 1, 4; $P < 0.001$) and T11 (RMANOVA: $F = 11.96$; df = 1, 4; $P < 0.001$).

Eclosion of adults in conidial treatments under both photoperiod regimes

Under constant light, eclosion of the whitefly adults did not begin until 3 days post-treatment and continued until day 8 in the control (Table 4). Eclosion of adults did not begin until after day 2 and continued only two more days for strains T and T11; adults continued to eclose until day 8 for strain T10. On day 3, only strain T11 had a significantly lower (26%) percent of whitefly adults eclosing compared to the control ($F = 23.6$; df = 1, 4; $P = 0.0083$). At the end of the observation period under a 24:0 hour photoperiod, all conidial strains had a lower (24 to 40%) percent of whitefly eclosing compared to the control, but only strain T was significant ($F = 5.62$; df = 1, 4; $P = 0.077$).

Under a 16 hour photophase, eclosion of the whitefly did not start until day 4 in the control. In the conidial treatments eclosion began 1 day post-treatment for strains T and T10 and on day 4 for strain T11. Four days post-treatment, the percent eclosion was significantly lower for strain T10 ($F = 10.1$; df = 1, 4; $P = 0.034$); whereas it was lower for strain T ($F = 10.1$; df = 1, 4; $P = 0.034$) on day 6 compared to the control. This trend continued throughout the observation period for strain T and T10; whereas the eclosion rate for strain T11 was similar to the control. Eclosion of whitefly adults in conidial treatments T and T11 were similar on day 8, both being 37% lower than the control under a 16 hour photophase. Only strain T10 after 3 days post-treatment had a significantly (RMANOVA: $F = 10.07$; df = 1, 4; $P = 0.034$) lower percentage of whitefly eclosed compared to the control under a 16 hour photophase. Over the 8 days, a significant interactive positive effect of photoperiod on the colonization of whitefly nymphs was found for strain T10 (RMANOVA: $F = 2.79$; df = 5, 20; $P = 0.045$).

Overall, in comparing the conidial treatments at either photoperiod regime, the percent eclosion of the whitefly nymphs for strains T, T10, and T11 were similar, but were also lower than the controls.

Discussion

The glass slide bioassay

Natural mortality (0–30%), caused by wounding the insects with the probe or other stress-related conditions, was accounted for by using Abbott's formula (Abbott 1925) and corrected mortality values were compared at different photoperiods. No fungal growth was observed in or on any of the nymphs placed in the control drops of 0.01 % Triton X-100 or distilled water in studies conducted under either photoperiod regime. In studies where the nymphs were incubated under a 16:8 hour LD photoperiod regime, 50% of the nymphs were found contaminated in all treatments, including the control by the saprophytic fungi, *Cladosporium* sp., because infested leaves were not washed prior to having nymphs removed from the leaf. However, even though the nymphs were contaminated by *Cladosporium* sp., the *P. fumosoroseus* fungal treatments progressed naturally to sporulation. Some nymphs contaminated with *Cladosporium* sp., either in the control or fungal treatments, progressed naturally to adult eclosion. In the control treatments, more than 90% of the adults eclosed indicating that the contamination had a minimal effect on the survival of the nymphs under these conditions. Thus, leaving some leaves unwashed allowed observation of different *P. fumosoroseus* strains in competition with a saprophytic fungus present on the leaf surface and insect cuticle.

FDI of greenhouse whitefly nymphs for both photoperiod regimes

Drummond *et al*. (1987) found after assessing the efficacy of *V. lecanii* (isolate A) against the greenhouse whitefly fourth-instar nymphs, that high pathogenicity was associated with its more rapid development on the host cuticle during the first 16 hours in high humidity. In glass-slide bioassay studies conducted by Landa *et al*. (1994), the limiting factor for the application of fungal isolate PFR 97 was the relative humdity. In addition, Landa *et al*. (1994) discovered that the fastest development of isolate PFR 97 occurred at 100% RH which must be maintained for at least 12 hours post-inoculation. In the present study, because the relative humidity was maintained at ~100% for at least 12 hours, the efficacy for all Trinidadian strains of *P. fumosoroseus* could be compared equally using a FDI index.

When incubated under constant light and temperature with ~100% RH, blastospores of all fungal strains had higher FDI values compared with a shorter 16:8 hour LD photoperiod. After producing Mexican isolates of *P. fumosoroseus* conidia in submerged culture, de la Torre and Cárdenas-Cota (1996) determined that light is required for, and promotes sporulation. Sakamoto *et al*. (1985) indicated that the conidiation of *P. fumosoroseus* growing on a culture medium at 25 °C was induced by light. Gillespie (1984) indicated that light affects the last phase of the conidiogenesis and noted a

significant increase in the production of conidia when cultures of *P. fumosoroseus* and *V. lecanii* were exposed to fluorescent light. Based on the results of our glass-slide bioassays increasing the photophase resulted in an increase in colonization of the nymphs by the blastospores of the Trinidadian strains of *P. fumosoroseus*.

In contrast, however, the mean FDI values for the conidial treatments would indicate that the infection and colonization of the whitefly hosts appeared not to be affected by the length of the photophase. In both photoperiod regimes, the mean FDI values for all conidial treatments never reached much above 2.0, which indicated that mycelia were only beginning to grow on some nymphs after 8 days post-inoculation. However, the conidiogenesis stage, represented by FDI values of 2.5 (2.5 = initial sporulation, first conidiospore is present near the surface of the nymph), was first recorded 3 and 4 days post-treatment under a 24 and 16 hour photophase, respectively. Under these optimum conditions (e.g., 25 °C and 100% RH), during the 8 day observation period, the maximum percent conidial development (FDI value of 3.0) with strain T at a 24 hour photophase (65%) was faster than at the 16 hour photophase (22%). However, none of the Trinidadian strains of *P. fumosoroseus* were as efficient in colonizing the greenhouse whitefly nymphal hosts on glass-slides as the PFR 97 isolate of *P. fumosoroseus* (Landa *et al*. 1994). Under optimum conditions (e.g., 25 °C and ~100% RH), with a 16:8 hour LD photoperiod regime, isolate PFR 97 was able to colonize the whitefly nymphs in less than 5 days post-inoculation. In our study, none of the *P. fumosoroseus* Trinidadian conidial treatments reached the mean FDI value of 2.5 after 8 days.

These differences in pathogenicity could be because the concentrations used in our study (2.5×10^5 conidia ml^{-1}) were lower than that used by Landa *et al*. (1994; 1.0×10^7 conidia ml^{-1}). By increasing the dose from 1.0×10^5 to 10^7 conidia ml^{-1}, the potential contact with the host would be increased. Landa *et al*. (1994) also used early fourth-instars (sub-stage 1), whereas in this study late fourth-instars (sub-stages 2 and 3) were used. Younger greenhouse whitefly larval instars are more susceptible to infection by the entomopathogen *A. aleyrodis*, than older instars or pupae (Fransen *et al*. 1987). Therefore, by increasing the potential for faster colonization of the nymphs, the FDI values will be higher. Lastly, Trinidadian conidia were placed in water with no surfactant added to the droplet suspension, whereas Landa *et al*. (1994) added Tween 80 to the suspension which could have increased the contact droplets would have had with the insect host cuticle due to increased spreading. Surfactants are used to help spread spores in suspension and increase the surface area covered, thereby increasing the potential for host contact and subsequent infection on the insect cuticle. In a preliminary experiment, aggregation of conidia were observed by the author (unpublished data) in fungal suspensions without the addition of the surfactant Triton X-100. Therefore, suspending the conidia in just distilled water may have increased the probability of aggregates and thus decreased both the contact the fungal strains would have had with the host and the pathogenicity of the strains.

Eclosion of greenhouse whitefly adults for both photoperiod regimes

Landa *et al*. (1994) found that greenhouse whitefly adults began eclosing 48 hours post-treatment when early fourth-instar nymphs were placed in control drops of 0.05 % Tween 80 on glass slides incubated under constant light at 25 °C with 100% RH. In our study some whitefly adults began eclosing 24 hours after being incubated under constant light in either a droplet of Triton X-100 or in distilled water. This could be because some nymphs were late fourth-instars (2nd and 3rd substages), requiring less than 48 hours for adults to eclose. Earlier eclosion of the whitefly adults could also be due to the fact that development occurred in constant light.

Treatments with blastospores of *P. fumosoroseus* effectively lowered the percent eclosion of the whitefly adults at both photoperiod regimes and the percent eclosion of adults for blastospore treatments was much lower than the conidial treatments. Conidial treatments were observed to be less virulent than the blastospore treatments at both light regimes, which could also account for higher percent adult eclosion.

According to our FDI results, fungal penetration of the insect cuticle (FDI value 2.0) requires about 24–36 hours at a 24 hour photophase. If the whitefly adult emerged earlier than 24 hours post-treatment, the inoculum would not have been able to penetrate the insect cuticle and therefore would result in a higher percentage of adults eclosing. The percent eclosion seems to be directly associated with the speed of infection of the *P. fumosoroseus* blastospore or conidial treatment and the photoperiod regime selected.

P. fumosoroseus conidia and blastospore treatments against greenhouse whitefly nymphs

Both germination and infection of *P. fumosoroseus* influence greenhouse whitefly nymphal colonization and adult eclosion. In these studies, blastospore treatments of all strains infected and colonized the whitefly nymphs faster under constant light, but FDI values at the end of 8 days was comparable at the shorter 16 hour photophase. Also, under a shorter photophase an extremely low number (0–13%) of whitefly adults eclosed which indicated that the fungal hyphae was able to germinate quickly, penetrate, infect and ultimately colonize the host due to a prolonged nymphal development and eclosion time. Vega et al. (1999), after assessing the germination rates of conidia and blastospores of *P. fumosoroseus* on the cuticle of the silverleaf whitefly cultivated on PDA and Noble agar plates, found that 4 hours after spraying more than 37% of blastospores had germinated compared to 0% for the conidia on the whitefly cuticle. In general, infection and colonization of the nymphal hosts by conidial treatments did not seem to be affected by the length of photoperiod as compared with the blastospore treatments; however higher FDI values were recorded under a 16 hour photophase.

In comparison with other hyphomycetes, except isolate PFR 97, the FDI values of the Trinidadian conidial strains were comparable to isolates of *V. lecanii* and *B. bassiana* tested against the silverleaf whitefly after 5 days on glass slides (Landa *et al*. 1994). Overall, the colonization and sporulation processes for the blastospore treatments on the greenhouse whitefly nymphal host were observed approximately 3 and 6 days post-inoculation, respectively, which is comparable to other *P. fumosoroseus* isolates and hyphomycetes.

In summary, *P. fumosoroseus* blastospore strain T11 was shown to offer the most potential of the three Trinidadian strains against the greenhouse whitefly fourth-instar nymphs under

laboratory conditions, especially under constant light. Blastospore treatments (all strains) under optimum conditions (25 °C and ~100% RH) and a 16:8 hour LD photoperiod light regime allowed for the least eclosion and caused the most mortality (86–100%). The longer photophase had a significant positive effect on FDI for the blastospores; however, a lesser effect was observed for the conidia at either light regime.

Successful development of entomopathogenic fungi as potential microbial insecticide agents requires careful assessment and eventual appropriate selection of the most efficacious species and isolates. The data from our study indicate that this laboratory bioassay technique can play a crucial role in screening different isolates for determining the pathogenicity and efficacy of various fungal preparations to be selected against aleyrodid pests prior to being tested in field trials. This technique will allow for a sensitive and relatively rapid assay of fungal preparations for both viability and pathogenicity and can be used as a simple assessment for the commercial development of a fungal biopesticide designed for controlling *T. vaporariorum* or other aleyrodid pests.

Acknowledgements

We thank the technical assistance of Jenny Smale. The Trinidadian fungal strains of *P. fumosoroseus* were made available by Drs. Penelope Smith and Paul Bridge CABI BioScience, Egham, UK. The greenhouse whitefly *T. vaporariorum* populations were obtained from Biological Crop Protection Ltd, Acorn Nurseries, Chapel Lane, West Wittering, Chichester, PO20 8QG, UK. Statistical analysis assistance and suggestions by Stephen P. Wraight at the USDA-ARS-Plant Protection Research Unit in Ithaca, New York was greatly appreciated. Reviews by Drs. H. D. Burges at Goring-by-the-Sea, UK and S. P. Wraight provided constructive criticism for improving the manuscript.

References

Abbot WS. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18: 265–267.

Antonious GF, Snyder JC. 1995. Pirimiphos-methyl residues and control of greenhouse whitefly (Homoptera: Aleyrodidae) on seven vegetables. *Journal of Entomological Science* 30: 191–201.

Avery PB. 2002. Tritrophic interactions among *Paecilomyces fumosoroseus*, *Encarsia formosa* and *Trialeurodes vaporariorum* on *Phaseolus vulgaris* and *Pelargonium* spp. PhD thesis, University of London, UK.

Bell JV. 1975. Production and pathogenicity of the fungus *Spicaria rileyi* from solid and liquid media. *Journal of Invertebrate Pathology* 26: 129–130.

Bidochka MJ, Pfeifer TA, Khachatourians GG. 1987. Development of the entomopathogenic fungus *Beauveria bassiana* in liquid cultures. *Mycopathologia* 99: 77–83.

Byrne DN, Bellows Jr TS, Parrella MR. 1990. Whiteflies in agricultural systems. In: Gerling D., editor. *Whiteflies: Their bionomics, pest status and management*, 10: 227–261. Hants, UK Intercept Ltd.

Castañé C, Albajes R. 1992. *Pelargonium* cultivar selection by adults of greenhouse whitefly (Homoptera: Aleyrodidae). *Environmental Entomology* 21: 269–275.

Castañé C, Albajes R. 1994. Mortality of immature stages of *Trialeurodes vaporariorum* (Homoptera:Aleyrodidae) on regal geranium, *Pelargonium x domesticum*. *Environmental Entomology* 23: 1443–1448.

Drummond J., Heale JB., Gillespie AT. 1987. Germination and effect of reduced humidity on expression of pathogenicity in *Verticillium lecanii* against the glasshouse whitefly *Trialeurodes vaporariorum*. *Annals of Applied Biology* 111, 193–201.

de la Torre M, Cárdenas-Cota HM. 1996. Production of *Paecilomyces fumosoroseus* conidia in submerged culture. *Entomophaga* 41: 443–453.

EWSN (European Whitefly Studies Network) 2003. Whitefly review. *European Whitefly Studies Network Newsletter*, No. 15. http://www.whitefly.org

Fang QX, Yang SF, Hu YM, Zhou YY, Gong YX, Kang ZJ. 1986. Control of whitefly, *Trialeurodes vaporariorum*, by *Paecilomyces fumosoroseus* var. *beijingensis* Fange et Q.T. Chen in greenhouses. *Chinese Journal of Biological Control* 3: 129–131.

Feng MG. 1998. Interaction between the aphid-specific fungus *Pandora neoaphidis* and the pea aphid *Acyrthosiphon pisum* at regimes of varying temperature and photoperiod simulating variable seasonal patterns. *Acta Entomologica Sinica* 41: 145–152.

Feng MG, Poprawski TJ, Nowierski RM, Zeng Z. 1999. Infectivity of *Pandora neoaphidis* (Zygomycetes: Entomophthorales) to *Acyrthosiphon pisum* (Hom., Aphididae) in response to varying temperature and photoperiod regimes. *Journal of Applied Entomology* 123: 29–35.

Fransen JJ, Winkelman K, van Lenteren JC. 1987. The differential mortality at various life stages of the greenhouse whitefly, *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae), by infection with the fungus *Aschersonia aleyrodis* (Deuteromycotina: Coelomycetes). *Journal of Invertebrate Pathology* 50: 158–165.

Fransen JJ. 1990. Natural enemies of whiteflies: Fungi. In: Gerling D., editor. *Whiteflies: Their bionomics, pest status and management*, 8: 187–210. Hants, UK: Intercept Ltd.

Fransen JJ. 1993. Integrated pest management in glasshouse ornamentals in the Netherlands: a step by step policy. *IOBC/WPRS Bulletin* 16: 35–38.

Gillespie AT. 1984. The potential of entomogenous fungi to control glasshouse pests and brown planthoppers of rice. PhD thesis, University of Southampton.

Hajek AE, Wraight SP, Vandenburg JD. 2001. Control of arthropods using pathogenic fungi. In: Pointing, SB, Hyde, KD, editors. *Bio-exploitation of filamentous fungi*, 13: 309–347. Fungal Diversity Research Series 6.

Hall RA. 1982. Control of whitefly, *Trialeurodes* and cotton aphid, *Aphis gossypii* in glasshouses by two isolates of the fungus, *Verticillium lecanii*. *Annals of Applied Biology* 10: 1–11.

Hall RA. 1985. Whitefly control by fungi. In: Hussey NW, Scopes NEA, editors. *Biological pest control–The glasshouse experience*, pp.116–118. Ithaca: Cornell University Press.

Hoffman CJ, Byrne DN. 1986. Effects of temperature and photoperiod upon adult eclosion of the sweet potato whitefly *Bemisia tabaci*. *Entomologia Experimentalis et Applicata* 42: 139–144.

Kanagaratnam P, Hall RA, Burges HD. 1982. Control of glasshouse whitefly, *Trialeurodes vaporariorum*, by an 'aphid' strain of the fungus *Verticillium lecanii*. *Annals of Applied Biology* 100: 213–219.

Lacey LA, Fransen JJ, Carruthers R. 1995. Global distribution of naturally occurring fungi of *Bemisia*, their biologies and use as biological control agents. In: Gerling D, Mayer R, editors. *Bemisia 1995: Taxomomy, biology, damage control and management*, 35: 401–433. Hants, UK: Intercept Ltd.

Landa Z. 1984. Ochrana proti molici sklenikove (*Trialeurodes vaporariorum* Westw.) V Programech integrovane ochrany sklenikovych okurek. *Sbornik UVTIZ*, Zahradnictvi 11: 215–228.

Landa Z, Osborne L, Lopez F, Eyal J. 1994. A bioassay for determining pathogenicity of entomogenous fungi on whiteflies. *Biological Control* 4: 341–350.

Lane BS, Trinci APJ, Gillespie AT. 1991. Influence of cultural conditions on the virulence of conidia and blastospores of *Beauveria bassiana* to the green leafhopper, *Nephotettix virescens*. *Mycological Research* 95: 829–833.

Manzano MR, van Lenteren JC, Cardona C, Drost YC. 2000. Developmental time, sex ratio, and longevity of *Amitus fuscipennis* MacGown & Nebeker (Hymenoptera: Platygasteridae) on the greenhouse whitefly. *Biological Control* 18: 94–100.

Mier T, Rivera F, Bermudez JC, Dominquez Y, Benavides C, Ulloa M. 1991. First report in Mexico on the isolation of *Verticillium lecanii* from whitefly and *in vitro* pathogeniity tests on this insect. *Revista Mexicana de Micologia* 7: 149–156.

Omer AD, Leigh TF, Granett J. 1992. Insecticide resistance in field populations of greenhouse whitefly (Homoptera: Aleyrodidae) in the San Joaquin Valley, California cotton cropping system. *Journal of Economic Entomology* 85: 21–27.

Orozco OL, Abella F, Pinzon C. 1995. Imidacloprid, a future chemical tool in integrated management of greenhouse whitefly. *Revista Colombiana de Entomologia* 21: 87–90.

Osborne LS, Landa Z. 1992. Biological control of whiteflies with entomopathogenic fungi. *Florida Entomologist* 75: 456–471.

Ramakers PMJ, Samson RA. 1984. *Aschersonia aleyrodis*, a fungal pathogen of whitefly. II. Application as a biological insecticide in glasshouses. *Journal of Applied Entomology* 97: 1–8.

Ravensburg WJ, Malais M, van der Schaaf DA. 1990. *Verticillium lecanii* as a microbial insecticide against glasshouse whitefly. In: *Proceedings of the Brighton Crop Protection Conference : BCPC Pests and Diseases–1990*. Vol 1: 265–268.

Sakamoto M, Inoue Y, Aoki J. 1985. Effect of light on the conidiation of *Paecilomyces fumosoroseus*. *Transactions of the Mycological Society of Japan* 26: 499–509.

Samson RA, Rombach MC. 1985. Biology of the fungi *Verticillium* and *Aschersonia*. In: Hussey NW, Scopes NEA, editors. *Biological pest control–The greenhouse experience*, 34–42. Ithaca: Cornell University Press.

Smith PA. 1996. Development of *Paecilomyces fumosoroseus* (Wise) Brown and Smith (Hyphomycete:Deuteromycotina) for control of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). PhD thesis, Imperial College, London, England, UK.

van der Schaaf DA, Ravensburg WJ, Malais M. 1991. *Verticillium lecanii* as a microbial insecticide against whitefly. *IOBC/WPRS Bulletin* 14: 120–123.

Vandenburg JD, Jackson MA, Lacey LA. 1998. Relative efficacy of blastospores and aerial conidia of *Paecilomyces fumosoroseus* against the Russian wheat aphid. *Journal of Invertebrate Pathology* 72: 181–183.

Vega FE, Jackson MA, McGuire MR. 1999. Germination of conidia and blastospores of *Paecilomyces fumosoroseus* on the cuticle of the silverleaf whitefly, *Bemisia argentifolii*. *Mycopathologia* 147: 33–35.

Webb RE, Smith FF, Boswell AL, Fields ES, Waters RM. 1974. Insecticidal control of the greenhouse whitefly on greenhouse ornamental and vegetable plants. *Journal of Economic Entomology* 67: 114–118.

Wraight SP, Carruthers RI 1999. Production, delivery, and uses of mycoinsecticides for control of insect pests on field crops. In: Hall FR, Menn JJ, editors. *Methods in biotechnology, Vol. 5: Biopesticides: Use and delivery*, 14: 233–269. Totowa, NJ: Humana Press Inc.