Bioassay of Potential Entomopathogenic Fungi for the Control of Silverleaf Whitefly

Adhanom Negasi
Awassa Research Centre
PO Box 6, Awassa, Ethiopia

Bruce L. Parker, Michael Brownbridge
Entomology Research Laboratory
University of Vermont
PO Box 53400, Burlington, VT 05405-3400 USA

Abstract

Strains of *Beauveria bassiana* (Balsamo) Vuillemin, *Paecilomyces farinosus* (Holm ex Ef Gray) Brown and Smith and *Verticillum lecanii* (Zimmermann) Viegas were evaluated for their pathogenicity to adults, eggs, first and third instars of silverleaf whitefly (SLWF), *Bemisia argentifolli* (Bellows & Perring). Conidia dilutions were prepared in 0.05% Tween^{R} and leaves treated by immersion in these suspensions. Assessment of nymphal infections were made 2, 3, 5, 7, and 10 days post treatment. Infection rates assessment in adults and eggs were made 14 d post-treatment.

All strains were pathogenic to nymphs and adults. None of the strains was pathogenic to eggs. Strains L3444 *(P. farinosus)* and L3009 *(B. bassiana)* were the most pathogenic to first instars and adults with LC_{50} and LC_{90} values of $7.3X10^4$ and $3.1X10^6$ spores/ml, and $1.3X10^6$ and $1.0X10^7$ spores/ml, respectively. Strain FR20 *(V. lecanii)* was the most pathogenic strain for third instars with LC_{50} and LC_{90} values of $4.8X10^3$ and $7.4X10^5$ spores/ml, respectively. Results imply that L3444 and L3009 are the most efficient strains based on their pathogenicity to first instars and adults. Results under laboratory conditions are discussed in relation to their potential for management of silverleaf whitefly in greenhouses.

Introduction

In many parts of the world economic and domestic crops such as cotton, tomato, tobacco, soybean, cassava, cucurbits, and lettuce incur dramatic losses from sweet potato whitefly (SPWF), *Bemisia tabaci* (Gennadius), and silverleaf whitefly (SLWF), *Bemisia argentifolli* (Bellows & Perring) (Homoptera: Aleyrodidae), [formerly known as SPWF, strain B] (McHugh 1991, Sanderson & Ferrentino 1991). Crop losses result from physical tissue damage, viral transmission, and pathogenic contamination associated with honeydew excretion (Lopez-Avila & Cock 1986, Lenteren & Noldus 1990). The SLWF has a long history as a major field crop pest, but it is also becoming an increasingly serious pest of greenhouse crops (McHugh 1991, Osborne & Landa 1992, Sanderson & Ferrentino 1991).

The greenhouse environment is ideal for the survival and reproduction of SLWF (Brownbridge et. al. 1992). Adults and nymphs feed on the underside of leaves where they are protected from conventional contact insecticides (Matthews 1986). Repeated use of sprays has resulted in SLWF insecticide resistance (Osborne & Landa 1992). In general, entomopathogenic fungi have a strong potential as viable control agents for many insect pests (Reineke 1990). For example, recently Naturalis-O, *B. bassiana* JW1-based product reached market for the control of SLWF immatures (Troy Biosciences Incorporated) and a biological insecticide of *Paecilomyces fumosoroseus*, PFR- 97 ™ 20% WDG, is also registered as a potential product for the control of all life stages of SLWF (Grace & Co. Conn).

The increasing problem of resistance to conventional pesticides emphasised the imperative for isolating and testing microbial organisms to develop a long-term management strategy for SLWF.

This study investigates the pathogenicity of three species (four strains) of entomopathogenic fungi under laboratory conditions against adults, eggs, and first and third instars of SLWF.

Materials and Methods

Preparation of Inoculum

Fungal isolates for all assays were cultured on quarter strength sabouraud dextrose agar medium containing 0.25% w/v yeast extract and incubated at 20°C for 12-14 d before harvesting. Spores were harvested by flooding plates with 2.5% Tween. The resulting suspension was filtered through coarse-meshed cheese cloth to remove hyphal debris. Suspensions were centrifuged and the supernatant removed by pipette. Pellets were resuspended in 2-3 ml sterile distilled water. Conidial concentration in stock suspension was determined using a hemocytometer. Viability tests were performed 24 hr before each assay (Hall 1976) and spore batches with greater than 95% viability were used.

Bioassay

Four fungal isolates obtained from an initial screening of 21 strains of *V. lecanii, M. anisopliae, B. bassiana,* and *P. farinosus* selected from the entomopathogenic fungal collections at the University of Vermont, Entomology Research Laboratory were bioassayed for activity against adults, eggs, first and third instars of SLWF. Leaf dip bioassay method using the box type technique was used. Bean leaves (*cv* 'Royal Burgundy') were inoculated with 10 pairs adult SLWF. Adults were allowed to oviposit for 24 hr and then removed to provide age homogeneity in the developing life stages for the bioassay study. Each leaf was maintained in a growing cube (Magi-cube^R , Smithers-Oasis Co, Kent, Ohio, USA) placed in a petri dish in 2 mm depth of tap water to maintain the leaves and conditions of high humidity (>95%) during the assay. The leaves were held in vented plastic boxes measuring 35 mm X 90 mm X 150 mm.

Eggs: Implanted leaves with SLWF sessile eggs of the same age were dipped in 40 ml spore suspension of each isolate at 0 (control, 0.05% Tween), $1.0X10^5$, $1.0X10^6$, $1.0X10^7$, and $1.0X10^8$ spores/ml for 20 sec. After treatment, leaves were removed and allowed to air dry before transfer to the plastic boxes. Four replicates/strain at each dosage level were set-up on each of four consecutive days in a balanced incomplete block design. Treated leaves were held in plastic boxes at 23 ± 1^0C for 14 d. Efficacy on eggs was measured by egg hatch at 14 d after treatment.

First and third instar larvae: Implanted leaves with SLWF first and third instar larvae of the same age were dipped in 40 ml spore suspension of each isolate at 0 (control, 0.05% Tween^R), $1.0X10^4$, $1.0X10^5$, $1.0X10^6$ and $1.0X10^7$ spores/ml for 20 sec. After treatment, leaves were removed and allowed to air dry before transfer to the plastic boxes. Leaf dips were replicated four times for each strain at each dosage level on each of four consecutive days in a balanced incomplete block design. Treated leaves were held in plastic boxes at 23 ± 1^0C. Efficacy measured by observing the mycelial growth over the bodies of the first and third instars at 2, 3, 5, 7, and 10 d after treatment. Data obtained from d 7 and 10 were subjected to probit analyses using Statistical Analyses System (SAS 1987) to determine Lethal Concentration (LC), LC_{50} and LC_{90} values for each strain.

Adults: Implanted bean leaves in a Magi-cube were dipped in 40 ml spore suspension of each isolate at 0 (control, 0.05% Tween^R), $1.0X10^4$, $1.0X10^5$, $1.0X10^6$ and $1.0X10^7$ spores/ml for 20 sec. Bean leaves were allowed to air drying before eclosed adults were introduced onto bean leaves in plastic boxes. Leaf dips were replicated four times for each strain at each dosage level on each of four consecutive days in a balanced incomplete block design. Adults were anesthetised with CO_2 for approximately 2 sec

before being placed onto treated leaves. A parafilm cover was placed over the petri dish to prevent adults loss by drowning in the water reservoir in the plastic box. Efficacy (please decide where to refer to) was measured by observing the mycelial growth over the bodies of the adults 14 d after treatment. Data were subjected to probit analyses using SAS to determine LC_{50} and LC_{90} values for each strain.

Results

Eggs

Egg eclosion ranged from 82.3 to 98.4% after 14 d (Table 1). No fungal hyphae were observed on the egg cuticle for any spore concentration. Percent hatch of eggs exposed to L3444 and FR20 were significantly lower than any of the other strains tested.

First Instar Larvae

Bioassay data on first instar larvae were subjected to probit analyses (SAS 1987) to obtain LC_{50} and LC_{90} values for each strain. The LC_{50} and LC_{90} values were significantly different from each other for all isolates at d 7 and 10 (Tables 2, 3). L3444 had the lowest LC_{50} value at both time periods of all the strains. Strain L3009, however, had the lowest LC_{90} value.

Table 1. Percent hatch of *Bemisia argentifolli* eggs after treatment with four fungal strains

Fungal species	Strain	Mean percent hatch*
Paecilomyces farinosus	L3444	82.3b
Beauveria bassiana	L3009	96.0a
Beauveria bassiana	MIC3	98.4a
Verticillium lecanii	FR20	85.6b
Control (0.05% Tween^R	Check	93.0a

Means followed by the same letter are not statistically different from each other at the 5% probability level (Duncan's multiple range test).

Table 2. LC_{50} and LC_{90} values of four fungal strains tested against first instar *Bemisia argentifolli*, 7 d after treatment

Fungal species	Stain	LC_{50} Conidia/ml*	LC_{90} Conidia/ml*	Chi-square X^2
Beauveria bassiana	L3009	$6.8X10^5$b	$8.0X10^5$d	0.015
Beauveria bassiana	MLC3	$1.7X10^6$a	$5.7X10^7$a	0.193
Paecilomyces farinosus	L3444	$8.0X10^4$d	$2.0X10^7$b	0.033
Verticillium lecanii	FR20	$6.0X10^5$c	$1.1X10^7$c	0.260

LC values within a column followed by the same letters are not significantly different at Z=2.64.

Table 3. LC_{50} and LC_{90} values of four fungal strains against first instar *Bemisia argentifolli*, 10 d after treatment

Fungal species	Strain	LC_{50} Conidia/ml*	LC_{90} Conidia/ml*	Chi-square X^2
Beauveria bassiana	L3009	$1.4X10^5$c	$3.1X10^6$d	0.260
Beauveria bassiana	MLC3	$9.4X10^5$a	$2.3X10^7$a	0.125
Paecilomyces farinosus	L3444	$7.3X10^4$d	$7.6X10^6$b	0.254
Verticillium lecanii	FR20	$4.5X10^5$b	$6.2X10^6$c	0.027

LC values within a column followed by the same letters are not significantly different at Z=2.64.

Third Instar Larvae

Bioassay data on third instar larvae were subjected to probit analyses to determine LC_{50} and LC_{90} values for each fungal strain. The LC_{50} calculated for L3009 and FR20 were significantly lower than other isolates tested. FR20 had the lowest LC_{90} value (Table 4). By d 10 the lowest concentration at both LC_{50} and LC_{90} was provided by FR20 (Table 5).

Adults

Bioassay data collected on adults were subjected to probit analyses (SAS 1987) to determine LC_{50} and LC_{90} values for each strain. According to the analyses made, the lowest LC_{50} and LC_{90} values were obtained for L3009 and L3444 (Table 6).

Table 4. LC_{50} and LC_{90} values of four fungal strains against third instar *Bemisia argentifolli*, 7 d after treatment

Fungi species	Strain	LC_{50} Conidia/ml*	LC_{90} Conidia/ml*	Chi-square X^2
Beauveria bassiana	L3009	$7.5X10^{4}c$	$1.4X10^{7}b$	0.853
Beauveria bassiana	MLC3	$2.0X10^{5}b$	$9.4X10^{6}c$	0.889
Paecilomyces farinosus	L3444	$2.6X10^{5}a$	$2.4X10^{7}a$	0.500
Verticillium lecanii	FR20	$8.3X10^{4}c$	$6.7X10^{6}d$	0.000

**LC values within a column followed by the same letters are not significantly different at Z=2.64.*

Table 5. LC_{50} and LC_{90} values of four fungal strains against third instar *Bemisia argentifolli*, 10 d after treatment

Fungal species	Strain	LC_{50} Conidia/ml*	LC_{90} Conidia/ml*	Chi-square X^2
Beauveria bassiana	L3009	$7.8X10^{3}b$	$2.0X10^{6}b$	0.020
Beauveria bassiana	MLC3	$4.0X10^{4}a$	$2.0X10^{6}b$	0.080
Paecilomyces farinosus	L3444	$1.1X10^{4}b$	$3.4X10^{6}a$	0.080
Verticillium lecanii	FR20	$4.8X10^{3}c$	$7.4X10^{5}c$	0.110

*LC values within a column followed by the same letters are not significantly different at Z=2.64.

Table 6. LC_{50} and LC_{90} values of four fungal strains tested on newly eclosed adult *Bemisia argentifolli*, 14 d after treatment

Fungal species	Strain	LC_{50} Conidia/ml*	LC_{90} Conidia/ml*	X^2
Beauveria bassiana	L3009	$1.3X10^{5}c$	$1.3X10^{7}c$	0.427
Beauveria bassiana	MLC3	$1.4X10^{7}b$	$5.8X10^{8}a$	0.000
Paecilomyces farinosus	L3444	$1.6X10^{6}c$	$1.0X10^{7}c$	0.795
Verticillium lecanii	FR20	$3.2X10^{7}a$	$4.3X10^{8}b$	0.323

**LC values within a column followed by the same letters are not significantly different at Z=*

Discussion

The efficacy of a range of entomopathogens against SLWF has not been extensively studied. Though several fungal pathogens have been tested against the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), *V. lecanii* and *Aschersonia aleyrodis* in particular, have been evaluated as potential biocontrol agents (Drummond & Heale 1988, Fransen et al. 1987). These studies have shown that fungal pathogens can effectively control greenhouse whitefly, and

commercially available formulations have been developed for *V. lecanii* (Hussey 1985). The data reported herein suggest a similar control approach with fungal pathogens could be effectively pursued for SLWF.

The most pathogenic strains in this study were at least as effective against first and third instar SLWF as those reported in other studies. For example, LC_{50} values of $8.0X10^4$ spores/ml for *P. farinosus* against first instar SLWF and $8.3X10^4$ spores/ml for *V. lecanii* against the third instar SLWF 7 d after treatment are considerably lower than the respective values of $1.3X10^5$ and $4.2X10^7$ spores/ml reported for the most virulent *V. lecanii* against greenhouse whitefly (Drummond et al. 1987). An LC_{50} value of $0.57X10^5$ spores/ml on d 8 was calculated for the most virulent *B. bassiana* isolate active against the aphid, *Diuraphis noxia* (Mordvilko) (Feng & Johnson 1990). The current study demonstrates the potential of fungal pathogens for biocontrol of SLWF under laboratory conditions. A critical phase in the evaluation process involved access to a sufficiently broad range of fungal isolates for initial screening. In this study, only three strains were found to be suitable for bioassay out of 21 initial candidates, representing a 14% success rate. The effectiveness of a fungal strain is measured in terms of pathogenicity and the spread with which it kills the target host. Taking these variables into account, only two strains, L3444 and L3009 were effective against all developmental stages of the whitefly although eggs were not significantly affected by any of the strains tested. Though infection of adult greenhouse whitefly by *A. aleyrodis* (Fransen et al. 1987) is quite rare, *V. lecanii* (Ekbom 1979), *V. fusisporum* (Ekbom & Ahman 1982), and *P. fumosoroseus var beiiingensis* (Osborne & Landa 1992) were quite pathogenic. The time taken for the host to die following exposure is a critical factor because the insect may remain active during this period. For example, adults remaining active for the time being could lay eggs which could be a potential source of infestation to the host plant. In this study, mortality was observed in the whitefly immatures 3 to 5 d after exposure to some strains. This pattern falls well within the range of 3 to 7 d of inoculation in other insects (Aguda & Rombach 1986).

Immatures are in general the most susceptible developmental stage to infection by hyphomycetous fungi (Osborne & Landa 1992), and this is confirmed here for SLWF. Perhaps the most significant result of this study is that the first instar can readily be infected and killed, being the most susceptible stage to target in a control strategy. While eggs were not affected by the fungi used in this study, we observed that emerged first instars were rapidly infected with *B. bassiana* (L3009), and *P. farinosus* (L3444). Fransen et al. (1987) made similar observation when greenhouse whitefly eggs were sprayed with *A. aleyrodis* newly hatched nymphs rapidly developed symptoms of infection. The two most effective pathogens studied (*B. bassiana* and *P. farinosus*) in this experiment were being tested for their persistence under different humidity and temperature regimes that are applicable to greenhouse and field growing conditions.

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