Screening of Fungal Pathogens Against 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

Twenty-one strains of the entomopathogenic fungi *Beauveria bassisna* (Bolsamo) Vuillemin, *Paecilomyces farinosus* (Holm ex Ef Gray) Brown and Smith, *Metarhizium anisopliae* (Metschnikoff) Sorokin, and *Verticillium lecanii* (Zimmermann) Viegas were evaluated for pathogenicity and virulence to silverleaf whitefly (SLWF), *Bemisia argentifolli* (Bellows & Perring) first instar larvae using a leaf-dip bioassay method. Conidia dilutions from each strain at 0 (control, 0.05% Tween[R]), $1.0X10^4$, $1.0X10^5$, $1.0X10^6$, and $1.0X10^7$ spores/ml were prepared in 0.05% Tween and leaves were treated by immersion in these suspensions. Assessment of first instar larvae infections were made 7 and 14 d post treatment. All results indicated that all strains infected first instar larvae. Mortality for all strains was $\subseteq$ 76% at d 7, but after 14 d three strains exceeded 90% mortality. The mean percent mortality induced by strains MLC3 and L3009 of *B. bassiana* and strain L3444 of *P. farinosus* was 92.6, 92.3, and 90.5, respectively. These were selected for bioassay of eggs, adults, first and third instars SLWF together with *V. lecanii* (FR20), a whitefly-active strain identified in a prior screening programme.

Introduction

Silverleaf whitefly (SLWF), *Bemisia argentifolli* (Bellows & Perring) (Homoptera: Aleyrodidae), formerly known as *Bemisia tabaci* (Gennadius) Strain B, is a serious insect pest of several economically important crops around the world. It has a wide host-plant range. Record shows up to 506 plant species attacked by SLWF(McHugh 1991). In the protected greenhouse environment, large populations of SLWF can build-up quickly (Brownbridge et al. 1992). Intensive insecticide spraying contributes to the SLWF population build-up. SLWF feeds on the underside of leaves. Its feeding habit renders difficulty to target with conventionally applied contact insecticides (Matthews 1980). Thus, intensive spraying is usually done. However, resistance develops quickly by this method (Osborne & Landa 1992). Moreover production and distribution of plants by large scale propagators using intensive spraying regime significantly contributes to the development and spread of resistant strains of SLWF. The heavy reliance on pesticides illustrates the urgency for development of effective biological management strategies. Entomopathogenic fungi offer a viable biocontol option as they are particularly well suited to humid greenhouse conditions and some species are easy to mass produce (Reinecke 1990).

Several species of entomopathogenic fungi have been found to show pathogenicity in varying levels on different pests under greenhouse environment. *Verticillium lecanii*, has been shown effective against the greenhouse whitefly, *Trialeurodes vaporariorum* (Ekbom 1979, Hall 1982, Kanagaratnam et al. 1982). Variation in pathogenicity and epizootic potential of different V. *lecani*i isolates have been documented (Drummond et al. 1987, Drummond & Heale 1988). Other fungal strains such as *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces farinosus* are considered of great potential. Variance in the pathogenicity of these fungal strains for particular target pests has been demonstrated (Feng et al. 1990). Undoubtedly, there is also variation between isolates of the same fungal species. Entomopathogenic fungi offer great potential for long term management, but their research and development lags far behind than chemical control. In this study effective and suitable entomopathogenic fungi from a range of strains initially collected from Vermont forest soils, insects, and infected greenhouse pests against SLWF first nymphal stage were identified.

Table 1. Origin of fungal isolates used in the screening assay against the first instar whitefly, *Bemisia argentifolli*

Fungal species	Source host			
	Strain*	Order	Family	Species
Paecilomyces farinosus	L3006	Thysanoptera	Thripidae	*Taeniothrips inconsequens*
Metarhizium anisopliae	L3012	Thysanoptera	Thripidae	*T.inconsequens*
Verticillium lecanii	L3114	Thysanoptera	Thripidae	*T.inconsequens*
V.lecanii	L3122	Thysanoptera	Thripidae	*T.inconsequens*
V.lecanii	L3119	Thysanoptera	Thripidae	*T.inconsequens*
M.anisopliae	L3377	Thysanoptera	Thripidae	*T.inconsequens*
V.lecanii	L3371	Thysanoptera	Thripidae	*T.inconsequens*
V.lecanii	L3430	Thysanoptera	Thripidae	*T.inconsequens*
M.anisopliae	L3387	Thysanoptera	Thripidae	*T.inconsequens*
V.lecanii	L3431	Thysanoptera	Thripidae	*T.inconsequens*
V.lecanii	L3440	Thysanoptera	Thripidae	*T.inconsequens*
P.farinosus	L3444	Thysanoptera	Thripidae	*T.inconsequens*
Beauveria bassiana	L3009	Thysanoptera	Thripidae	*T.inconsequens*
V.lecanii	L3441	Thysanoptera	Thripidae	*T.inconsequens*
B.bassiana	MLC2	Lepidoptera	Incurvariidae	*Paraclemensia acerifoliella*
V.lecanii	L3578	Thysanoptera	Thripidae	*T.inconsequens*
V.lecanii	L3680	Thysanoptera	Thripidae	*T.inconsequens*
B.bassiana	MLC3	Lepidoptera	Incurvariidae	*P.acerifoliella*
V.lecanii	L3682	Thysanoptera	Thripidae	*T.inconsequens*
V.lecanii	L3708	Thysanoptera	Thripidae	*T.inconsequens*
P.farinosus	L3730	Thysanoptera	Thripidae	*T.inconsequens*

**Numbers assigned to each strain by the Entomology Research Laboratory at the University of Vermont.*

Materials and Methods

An initial screening was carried out for 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 (Table 1). These strains were selected at random and tested against first instar SLWF at controlled temperatures and humidity. Those strains producing 90% or greater mortality were selected for dose mortality assays against eggs, adults, first and third instar SLWF.

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 then resuspended in 2-3 ml sterile distilled water. Conidial concentration in the stock suspension was then determined using a hemocytometer. Viability tests were performed 24 h before each assay (Hall 1976) and spore batches with > 95% viability were used.

Screening Assay

Bean leaves, (*cv* 'Royal Burgundy') were inoculated with 10 pairs adult SLWF. Adults were allowed to oviposit for 24 h and then removed to provide age homogeneity of first instar SLWF. 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 a vented plastic boxes measuring 35 mm X 90 mm X 150 mm (Fig. 1).

A standard dose of $1.0X10^7$ spores in 0.05% Tween was prepared for each strain tested. Implanted leaves with first instar of the same age were dipped in 40 ml spore suspension for 20 sec, then removed and allowed to air dry before transfer to plastic boxes (Fig. 1). Control leaves were dipped in 0.05% Tween only. Leaf dips were replicated four times for each strain at one dose level ($1.0X10^7$ spores/ml) with 0.05% Tween as a control check 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. Data on infection rates were taken by observing mycelial growth on the body of the instars 7 and 14 d after treatment. Based on the infection rate, mean percent mortality was calculated. Strains causing $\supseteq$90% mortality after 14 d were selected for bioassay study.

Results

All strains infected SLWF first instar larvae. At d 7, mortality for all strains was $\subseteq$ 76% (Fig. 2), but after 14 d three isolates exceeded 90% mortality (Fig. 3). The mean percent mortality induced by stains MLC3 and L3009 of *B. bassiana* and strain L3444 of *P. farinosus* was 92.6, 92.3, and 90.5, respectively. These were selected for bioassay of eggs, adults, first and third instar SLWF together with *V. lecanii* (FR20), a whitefly-active strain identified in a prior screening programme.

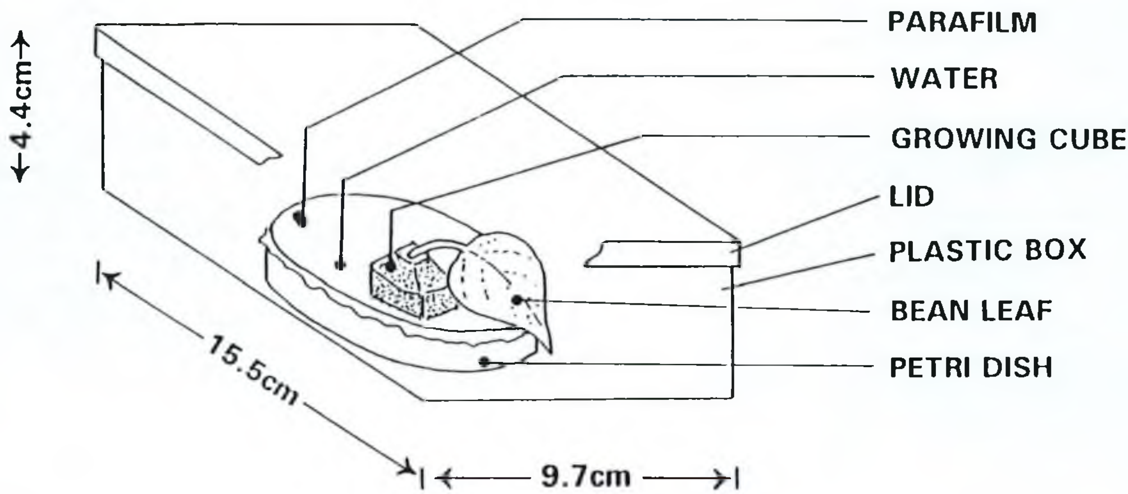

Fig. 1. Petri dish covered with parafilm to prevent mobile adults from being immersed in water.

Discussion

The current study demonstrates the potential of fungal pathogens for biocontrol of SLWF under laboratory conditions. A critical phase in the evaluation process involves access to a sufficiently broad range of 21 strains from four fungal species for initial screening. Accordingly, 12 strains of *V. lecanii*, 3 strains of *P. farinosus*, 3 strains of *M. anisopliae*, and 3 strains of *B. bassiana* were evaluated.

The percent mortality of first instar SLWF obtained from the different fungal strains ranged from about 2% to 76% 7 d after treatment, the highest mortality being obtained from strain L3009, followed by MLC3 and MLC2, all *B. bassiana*. However, most of the strains achieved mortality between 40 to 50 percent over 7 d period (Fig. 2). In general, percent mortality progressed with all the strains 14 d after treatment. In fact, strains L3009 and MLC3 of *B. bassiana* and strain L3444 of *P. farinosus* achieved mortality of over 90% (Fig. 3) within 14 d after treatment. Increase of percent mortality after 14 d suggests that the incubation period for all the strains was longer than 7 d. This result is in agreement with the findings of other workers. According to Aguda and Rombach (1986), fungal infection can best be evaluated after 3 to 7 d and up to 2 months or longer after application.

Variation in the levels of virulence among fungal species was observed as measured by percent mortality of the first instar SLWF in 7 and 14 d after treatment in this study. Similarly, variability in pathogenicity was also observed between different strains or isolates of a single species (Figs. 2, 3). Variation in pathogenicity and epizootic potential of different fungal species have also been documented (Drummond & Heale 1988, Drummond et al. 1987). According to Drummond and Heale (1988), seven strains or isolates of *V. lecanii* showed intermediate to low levels of pathogenicity against the greenhouse whitefly, *Trialeurodes vaporariorum* (Wood) as compared to the wild potential isolates. In this study, only three strains, *viz*. MLC3 and L3009 of *B. bassiana* and L3444 of *P. farinosus* with 92.6, 92.3, and 90.5 percent mortality, respectively, were found to be suitable for bioassay out of 21 initial candidates, representing a 14% success rate 14 d after treatment. The effectiveness of a fungal strain is measured in terms of pathogenicity and the spread with which it kills the target host.

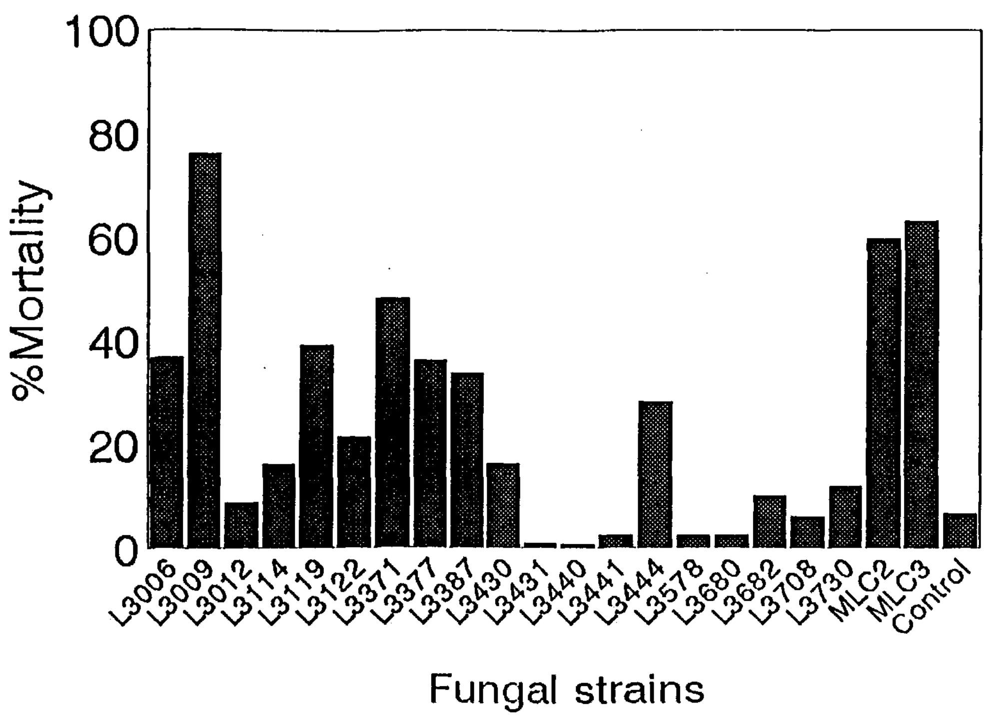

Fig. 2. Efficacy of 21 fungal strains against first instar siiverleaf whitefly 7 d post treatment. The concentration of each strain was 1.0X 10^7 spores/ml.

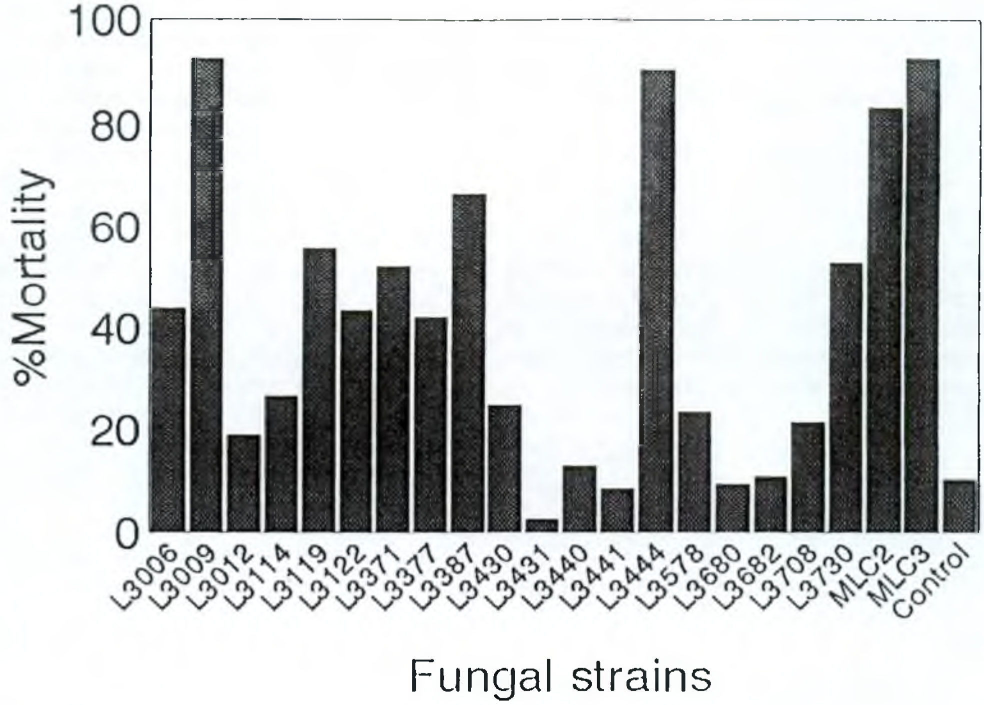

Fig. 3. Efficacy of 21 fungal strains against first instar silverleaf whitefly 14 d post treatment. The concentration of each strain was 1.0X10^7 spores/ml

Acknowledgments

We gratefully acknowledge Dr. John R. Grehan for his comments on an earlier draft of this paper. A Summer Research Scholarship from the Graduate College, University of Vermont is also appreciated and allowed us to complete this study.

References

Aguda RM, Rombach MC. 1986. Handling of insect fungi, pp. 117-123. In *Proceedings of the Southeast Asia regional training course on microbial control of insect pest and plant diseases in the tropics.* National Institute of Biotechnology and Applied Microbiology (BIOTECH), UP, Los Baños, Laguna, Philippines. October 19-26, 1986.

Brownbridge M, Parker BL, Skinner M. 1992. Development of insect-killing fungi for greenhouse use: A more sustainable pest management approach, pp. 39-40. In Sustaining a Vermont Way of Life: Research and Education in Sustainable Agriculture at the University of Vermont, Plant and Soil Science Department and Extension System. E. Seyler (ed.) with the assistance from F. Magdoff, K. Dueterburg, D. Helaba, B. Holtzman, B. Fardleman.

Drummond J, Heale JB. 1988. Genetic studies on the inheritance of pathogenicity in *Verticillium lecanii* against *Trialeurodes vaporariorum*. Journal of Invertebrate Pathology **52**:57-65.

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

Ekbom BS. 1979. Investigation on the potential of parasitic fungus (*Verticillium* *lecanii*) for biological control of greenhouse whitefly (*Trialeurodes vaporariorum*). Swedish Journal of Agricultural Research **9**:129-138.

Feng MG, Johnson J, Kish LP. 1980. *Verticillium lecanii* and an aphid derived isolates of *Beauveria bassiana* (Fungi: Hyphomycetes) for six species of cereal infesting aphids (Homoptera: Aphididae) Environmental Entomology **19**:815-820.

Hall RA. 1982. Control of whitefly, *Trialeurodes vaporariorum* and cotton aphid, *Aphis gossypii*, in glasshouses by two isolates of the fungus *Verticillium lecanii*. Annals of Applied Biology **101**:1-11.

Hall RA. 1976. A bioassay of the pathogenicity of *Verticillium lecanii* conidiospores on the aphid, *Macrosiphonielea sanborni*. Journal of Invertebrate Pathology **27**:41-48.

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.

Matthews GA. 1986. Overview of chemical control with special reference to cotton crops. In *Bemisia tabaci - a literature survey on the cotton whitefly with annotated bibliography*, ed. MC Cock, pp. 51-54. Silwood Park, UK: CAB International.

McHugh JB. 1991. Yes, you can win the war on whiteflies. Greenhouse Grower. Spring **1**:2.

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

Reinecke P. 1990. Biological control products: demands of industry for successful development. In *pesticides and alternatives: innovative chemical and biological approaches to pest control,* ed. JE Casida, pp. 99-108. Amsterdam: Elsevier