The Potential of Entomopathogenic Fungi to Control the Lettuce Root Aphid, *Pemphigus bursarius*

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The lettuce root aphid, *Pemphigus bursarius*, is a commercially important pest of lettuce and occupies a niche amenable to the action of entomopathogenic fungi. As the first stage of a biological control programme, a bioassay was developed against adult apterous *P. bursarius*. An isolate of *Metarhizium flavoviride* was pathogenic to *P. bursarius* and sporulated abundantly upon aphid cadavers. However, two isolates of *Verticillium lecanii*, pathogenic against glasshouse whitefly and aphids, were only weakly pathogenic to *P. bursarius*.

INTRODUCTION

Entomopathogenic fungi offer exciting possibilities as biological control agents because of their damaging effects on the target insect host and their ability to cause epizootics (Heale *et al.*, 1989). However, abiotic variables, especially temperature and humidity, have a profound effect on fungal efficacy and until improvements are made in formulation development, entomopathogenic fungi should be used only in conditions of high humidity and moderate temperatures. Thus suitable target pests in tropical regions include spittle bugs, stem borers and pests of the wet rice paddy (Gillespie, 1988), and in temperate regions include root-feeding pests and pests of protected crops. Within the UK, successful control has been demonstrated of the glasshouse whitefly, *Trialeurodes vaporariorum*, and glasshouse aphids using *Verticillium lecanii* (isolates 19.79 and 1.72, respectively) (Hall, 1981), and of the black vine weevil, *Otiorhynchus sulcatus*, on protected ornamentals using *Metarhizium anisopliae* (Moorhouse *et al.*, 1990). However, the potential of entomopathogenic fungi to control pests of UK field crops remains largely unrealised.

The lettuce root aphid, *Pemphigus bursarius*, is a commercially important pest of field-grown lettuce and provides a suitable model for the development of entomopathogenic fungi as biological control agents. This paper describes a bioassay of the pathogenicity of conidiospores of entomopathogenic fungi against *P. bursarius*.

MATERIALS AND METHODS

Production of fungal conidia

Metarhizium flavoviride isolate 99.82 and *V. lecanii* isolates 1.72 and 19.79 were obtained from the culture collection of HRI (Table 1). Conidia were harvested from 10-day spread-plate cultures grown at 22±1°C on Sabouraud's Dextrose Agar (SDA) in 0.01% (v/v) Triton X-100 and filtered through sintered glass thimble filters (BDH size 2, pore size 40-100 μm). Spore suspensions were counted using a haemocytometer and the concentration was adjusted to $10^7 ml^{-1}$.

TABLE 1. Fungal isolates used in this study

Isolate no.	Species	Origin	Host
1.72	*Verticillium lecanii*	UK	*Macrosiphoniella sanborni*
19.79	*Verticillium lecanii*	UK	*Trialeurodes vaporariorum*
99.82	*Metarhizium flavoviride*	UK	*Pemphigus trehernei*

Insect rearing

Pemphigus bursarius was obtained from P.R. Ellis, HRI Wellesbourne. Lettuce plants, variety 'Webbs Wonderful', were raised for 21 days in vermiculite in the glasshouse at 20±5°C and a photoperiod of 16 h. Plants were washed in distilled water, then the roots and stem of one plant were placed on a filter paper disc (Whatman No. 1, 9 cm diameter) and 'sandwiched' between two glass plates (10 cm x 9 cm) separated by cocktail sticks (2 mm thickness) at the sides of the plates, and by nonabsorbent cotton wool at the top and bottom. Lettuce leaves thus protruded from the top of the rearing chamber. Adult apterous *P. bursarius* were placed in the rearing chamber, five per plant, and chambers were closed with rubber bands and the glass plates covered by aluminium foil to exclude light from the roots and stem. Individual chambers were held in Hewitt's nutrient solution (1 cm depth) at 22±1°C and a photoperiod of 16 h. Parent aphids were removed after 5 days, and apterous adults for bioassays were recovered after 14 days.

Bioassay procedure

Apterous adult *P. bursarius* (30 per assay) were collected from rearing chambers and placed on a filter paper disc (Whatman No.1, 9 cm diameter) within the lid of a 9 cm petri dish. Aphids were then sprayed with 10 ml of conidial suspension in a Potter Tower (Potter, 1952), left to air-dry for 1 h, and then the 15 most active individuals were transferred to a newly-prepared rearing chamber. Chambers were incubated at 22±1°C as above, and the numbers of live and dead aphids were counted daily for 10 days. Progeny were counted and

removed from chambers at 3, 7 and 10 days after treatment. Dead aphids were removed from chambers 10 days after treatment and placed on damp filter paper within petri dishes (9 cm diameter) and incubated at 22±1°C for 4 days. Individual cadavers supporting sporulating mycelia were transferred to 1 ml 0.05% Triton X-100 in Eppendorf tubes, vortex-mixed for 30 s, and the concentration of spores in each tube was counted using a haemocytometer.

The insects not selected for inclusion in the bioassay chamber after treatment with conidial suspension were placed together in 1 ml 0.05% Triton X-100 within an Eppendorf tube, macerated with a sterilised dissecting needle, and then vortex-mixed for 30 s. Suitable serial dilutions were prepared and aliquots (0.1 ml) were plated onto a selective medium (2.5%, w/v, Malt Extract Agar plus dodine (12.5 $\mu g.ml^{-1}$, w/v), chloramphenicol (50 $\mu g.ml^{-1}$, w/v) and copper sulphate (200 $\mu g.ml^{-1}$, w/v). Colony counts were recorded after 7 days' incubation at 22±1°C.

Data analysis

Estimates of the time to kill 50% of the sample population (eLT_{50}) were made by probit analysis using the Maximum Likelihood Program (MLP) (Ross, 1980; Moorhouse, 1990). In addition, the number of spores adhering to a single insect 1 h after treatment, and the number of spores produced per insect 14 days after treatment, were calculated.

RESULTS

The bioassay developed here was simple and convenient. Control mortality, assessed as the number of dead aphids recorded 10 days after treatment with 0.01% Triton X-100, showed a mean mortality of 11% (range 8-15%). Of the three fungal isolates studied, 1.72 and 19.79 were of low pathogenicity and did not reduce the number of aphid offspring, whereas isolate 99.82 was pathogenic (eLT_{50}=158 h) and reduced the number of progeny by 70% (Table 2 and Fig. 1).

TABLE 2. Pathogenicity of *Metarhizium flavoviride* and *Verticillium lecanii* to adult apterous *Pemphigus bursarius*

Isolate	eLT_{50} (h)	Acquired Dose[z]	No. of Progeny[y]
Control	—	—	202 (±26)
V. lecanii	1.72	>400	410 (±23)
V. lecanii	19.79	305 (±8)[x]	360 (±55)
M. flavoviride	99.82	158 (±3)	113 (±39)

[z] Refers to the number of spores adhering to a single insect 1 hour after treatment.
[y] Refers to the number of progeny produced per assay chamber 10 days after treatment.
[x] Figures in parentheses refer to the S.E.M. of three replicates.

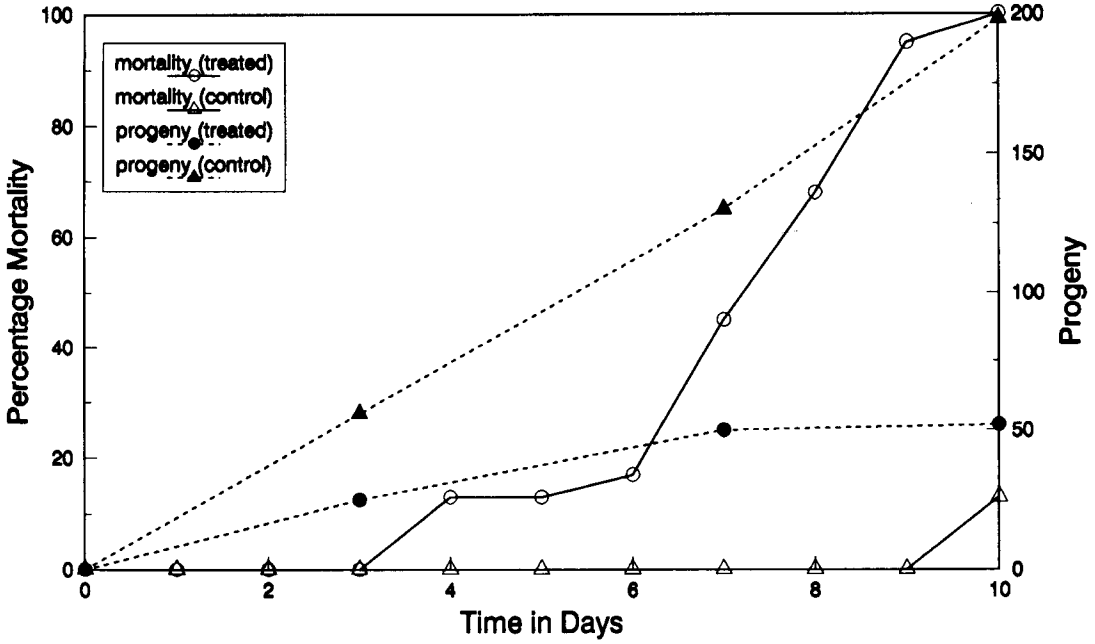

FIG. 1. Pathogenicity of isolate 99.82 to *Pemphigus bursarius*.

The number of spores produced on cadavers was assessed 14 days after treatment. Isolate 99.82 produced 3.14 x 10^6 (±3.72 x 10^5) spores per insect, whereas isolates 1.72 and 19.79 produced <10^5 spores per insect.

DISCUSSION

The selection of the most suitable fungal isolate is vital for the control of a given pest (Gillespie, 1988) and an effective bioassay is essential for isolate screening prior to glasshouse and field trials. Consistency and accuracy must be balanced with simplicity and convenience. Hall and Papierok (1982) considered entomopathogenic fungi, of all microbiological control agents, to provide the greatest difficulties for bioassay design because infectious conidia must be delivered to the cuticle of the host insect in a standardised manner. In this study, the Potter Tower (Potter, 1952) provided the most suitable method of spore application; other methods, such as total immersion (Hall, 1976) or the use of aerosol spray-propellents (Jackson *et al.*, 1985), gave an unacceptably high control mortality (data not shown). Hall (1976) and Yokomi and Gottwald (1988) used detached leaf systems to assay the pathogenicity of *V. lecanii* to the aphids *Macrosiphoniella sanborni* and *Myzus persicae*, respectively. However, Fransen (1987) rejected such assays for not reflecting field or glasshouse conditions. The bioassay developed in this study reflects the field situation; indeed, the use of whole plants was essential for insect survival (data not shown).

V. lecanii isolate 1.72 is an effective pathogen of glasshouse aphids such as *M. sanborni* and *M. persicae* (Hall, 1981) and its weak pathogenicity to *P. bursarius* was initially unexpected. However, soil-inhabiting insects encounter a wider range of entomopathogens than foliar species and *P. bursarius* may, therefore, have evolved more effective defence mechanisms than other aphid species. The pathogenic fungal isolate, *M. flavoviride* 99.82, originated from *Pemphigus trehernei,* resident on the salt-marsh halophyte *Aster tripolium* (Foster, 1975), although it has been suggested (Roberts and Humber, 1981) that the selection of isolates from non-related hosts may be a more effective strategy than choosing isolates from, and which may be in equilibrium with, near-target hosts.

REFERENCES

Foster, W.A. (1975) The life history and population of an intertidal aphid, *Pemphigus trehernei* Foster. *Transactions of the Royal Entomological Society London* 127(3), 193-207.

Fransen, J.J. (1987) *Aschersonia aleyrodes* as a microbial control agent of greenhouse whitefly. Ph.D. Thesis, Landbouwuniversiteit te Wageningen, the Netherlands.

Gillespie, A.T. (1988) Use of fungi to control pests of agricultural importance. In: Burge, M.N. [Ed.] *Fungi in Biological Control Systems.* pp. 37-60. Manchester Univ. Press, Manchester, UK.

Hall, R.A. (1976) A bioassay of the pathogenicity of *Verticillium lecanii* conidiospores on the aphid *Macrosiphoniella sanborni. Journal of Invertebrate Pathology* 27, 41-48.

Hall, R.A. (1981) The fungus *Verticillium lecanii* as a microbial insecticide against aphids and scales. In: Burges, H.D. [Ed.] *Microbial Control of Pests and Plant Diseases 1970-1980.* Academic Press, New York, NY.

Hall, R.A.; Papierok, B. (1982) Fungi as biological control agents of arthropods of agricultural and medical importance. *Parasitology* 84, 205-240.

Heale, J.B.; Isaac, J.E.; Chandler, D. (1989) Prospects for strain improvement in entomopathogenic fungi. *Pesticide Science* 26, 79-92.

Jackson, C.W.; Heale, J.B.; Hall, R.A. (1985) Traits associated with virulence to the aphid *Macrosiphoniella sanborni* in eighteen isolates of *Verticillium lecanii. Annals of Applied Biology* 105, 39-48.

Moorhouse, E.R. (1990) The potential of the entomogenous fungus *Metarhizium anisopliae* as a microbial control agent of the black vine weevil, *Otiorhynchus sulcatus.* Ph.D. Thesis, University of Bath, UK.

Moorhouse, E.R.; Gillespie, A.T.; Charnley, A.T. (1990) The progress and prospects for the control of the black vine weevil *Otiorhynchus sulcatus* by entomogenous fungi. In: *Proceedings of the 5th International Colloquium on Invertebrate Pathology and Microbial Control* (Adelaide, Australia), pp. 381-385.

Potter, C. (1952) An improved laboratory apparatus for applying direct sprays and surface films, with data on the electrostatic charge on atomized spray fluids. *Annals of Applied Biology* 39,1-29.

Roberts, D.W.; Humber, R.A. (1981) Entomogenous Fungi. In: Cole, G.T. and Kendrick, B. [Eds.] *Biology of Conidial Fungi.* pp. 201-236. Academic Press, New York, NY.

Ross, G.J.S. (1980) Maximum Likelihood Program Manual. Rothamsted Experimental Station, Harpendon, Herts, UK.

Yokomi, T.K.; Gottwald, T.R. (1988) Virulence of *Verticillium lecanii* isolates in aphids determined by detached-leaf bioassay. *Journal of Invertebrate Pathology* 51, 250-258.