COMPARATIVE PATHOGENICITY OF *BEAUVERIA BASSIANA* ISOLATES TO ADULT ARGENTINE STEM WEEVIL IN THE LABORATORY

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SUMMARY

Isolates of the entomopathogenic fungus *Beauveria bassiana* from Argentine stem weevil (*Listronotus bonariensis*) hosts were evaluated for their pathogenicity to adult Argentine stem weevil. Weevil mortality was assessed following topical application in aqueous suspensions containing 10^2 to 10^8 conidia per ml and incubation at 11-25°C for 17 to 29 days. Inoculum source, concentration, and temperature significantly influenced weevil mortality.

Keywords: biological control, entomopathogen, *Beauveria bassiana*, *Listronotus bonariensis*

INTRODUCTION

The Argentine stem weevil (ASW) (*Listronotus bonariensis* (Kuschel)) is an important pest in pastures throughout New Zealand. The stem boring larve may cause a depression in grass growth and death of infested grass during summer when moisture-stressed plants are unable to compensate for tiller damage. Larval damage to grass results in open, weedy or clover-dominant pasture.

The entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin is ubiquitous, occurring on a number of insect hosts (Samson *et al* 1988). *B. bassiana* can cause epizootic density-dependent mortality in overwintering Waikato populations of adult ASW but these occur too late in the season to prevent pasture damage by larval stages of the weevil during summer (Barker *et al* 1989).

B. bassiana is readily cultured on artificial media, and hence amenable to commercial production. Current research is aimed at utilising *B. bassiana* as a biological control agent for ASW. The approach is reliant on applying sufficient *B. bassiana* spores of high viability and pathogenicity to pasture in late spring to induce disease in the adult weevil population prior to significant pasture damage by the larvae. This paper describes initial screening of *B. bassiana* isolates from different ASW populations for pathogenicity to this host and experiments to determine factors which influence the pathogenicity.

MATERIALS AND METHODS

Experiment 1 and 2

B. bassiana was isolated from ASW and maintained in pure culture on potato dextrose agar (PDA) under 12 h light/12 h dark cycle for 2 to 4 weeks at $20 \pm 2^\circ\text{C}$. A conidial suspension was obtained from each isolate by pipetting 12-15 ml of sterilised 0.5% Tween 80 onto the culture plate and dislodging the spores with a flamed hockey stick. Ten millilitres of the suspension were pipetted, vortexed and the spores counted with a haemocytometer. Serial dilutions were made in sterilised 0.5% Tween 80 to give 10^8 , 10^6 , 10^4 and 10^2 conidia/ml. Thirty weevils, collected from Waikato pastures during January 1991, were placed in 10 ml of each dilution and agitated for 30 seconds. Ten inoculated weevils were then placed in each of three replicate petri dishes lined with moistened filter paper. Similarly, 30 control weevils were agitated for 30 seconds in 0.5% Tween 80 solution. Each Petri dish was supplied with fresh leaf segments of ryegrass (*Lolium multiflorum* L.) cv. Concord at 2 to 4 day intervals as food for the weevils.

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Ten isolates of *B. bassiana* (ASW 1 to ASW 10) from different Waikato ASW populations were utilised for Experiment 1. Following inoculation, the weevils were maintained in an incubator at a mean temperature of 15 °C for 27 days. In Experiment 2, *B. bassiana* from Waikato (seven isolates, ASW 5, ASW 11-16) and Canterbury (three isolates, ASW 17-19) ASW populations were used and the mean post-inoculation temperature over the 29 day experimental period was 11 °C. ASW 5 and ASW 17 were chosen as standard isolates for comparison with other isolates.

The numbers of dead weevils and cadavers bearing *B. bassiana* spore structures were recorded at 2 to 4 day intervals. At each assessment, dead weevils were removed from the petri dishes.

Experiment 3

Five *B. bassiana* isolates, one from Canterbury (ASW 17) and four from Waikato (ASW 4, ASW 5, ASW 7, ASW 12) were evaluated at four temperatures against ASW adults for 17 days. Spore suspensions of 10^8 conidia/ml were prepared and 160 weevils inoculated as described above. Inoculated weevils were placed in Petri dishes lined with moistened filter paper and supplied with fresh ryegrass leaf segments at 2 to 4 day intervals as described above. Four replicate dishes containing 10 weevils were placed in each of three incubators maintained at 15, 20 and 25 °C respectively. Four additional replicates of 10 weevils were held at room conditions of mean ambient temperature 22 °C (range 19 ° to 28 °C). To maintain 100% relative humidity, all petri dishes were held in closed vessels containing open water. Numbers of dead weevils were recorded at 5 to 7 day intervals. At each assessment, dead weevils were removed from the dishes.

Statistical analyses

The proportions of weevils killed by *B. bassiana* inoculation were corrected for mortality in untreated controls (which never exceeded 0.20) using Abbott's formula and then analysed by generalised linear regression using a binomial distribution with logit link.

RESULTS

Significant differences ($P < 0.001$) in pathogenicity between Waikato *B. bassiana* isolates were observed in Experiments 1 and 2. These isolate differences were apparent at day 14 and were maintained until the end of the experiment. In Experiment 2, the Canterbury isolates did not differ significantly ($P > 0.05$) from Waikato isolates in their pathogenicity to ASW (Table 1).

TABLE 1: Proportions of adult Argentine stem weevil dead 20 days after various isolates of *Beauveria bassiana* were topically applied at 1×10^8 conidia/ml.

Experiment 1 (15 °C)			Experiment 2 (11 °C)		
Isolate	Source	Proportional mortality ¹ (s.e.)	Isolate	Source	Proportional mortality (s.e.)
ASW 1	Waikato	0.75 (0.08)	ASW 5	Waikato	0.48 (0.11)
2	Waikato	0.72 (0.08)	11	Waikato	0
3	Waikato	0.73 (0.08)	12	Waikato	0.46 (0.11)
4	Waikato	0.90 (0.05)	13	Waikato	0.30 (0.11)
5	Waikato	0.67 (0.09)	14	Waikato	0.27 (0.11)
6	Waikato	0.73 (0.08)	15	Waikato	0.61 (0.12)
7	Waikato	0.69 (0.08)	16	Waikato	0.27 (0.11)
8	Waikato	0.53 (0.09)	17	Canterbury	0.73 (0.09)
9	Waikato	0.66 (0.09)	18	Canterbury	0.48 (0.11)
10	Waikato	0.76 (0.08)	19	Canterbury	0.54 (0.10)
untreated		0	untreated		0.10 (0.05)

¹Proportion dead, corrected for mortality in untreated

No significant interaction between *B. bassiana* isolates and inoculum concentration on the levels of weevil mortality was observed in Experiments 1 and 2. At both incubation temperatures, inoculum containing 10^8 conidia/ml effected significantly higher mortality than inoculum concentrations of 10^2 , 10^4 and 10^6 conidia/ml (Table 2).

TABLE 2: Proportions of adult Argentine stem weevil dead 20 days after topical inoculation with *Beauveria bassiana* isolates at various inoculum strengths.

Inoculum concentration conidia/ml	Proportional mortality ¹			
	Experiment 1 (15 °C)		Experiment 2 (11 °C)	
	All isolates†	ASW 5	All isolates†	ASW 5
10^2	0.20 (0.02)	0.21 (0.08)	0	0
10^4	0.17 (0.02)	0.10 (0.05)	0	0
10^6	0.25 (0.02)	0.24 (0.08)	0	0
10^8	0.71 (0.02)	0.67 (0.09)	0.41 (0.05)	0.48 (0.09)
untreated	0		0.10 (0.05)	

¹Proportion dead (standard error), corrected for mortality in untreated

† including ASW 5

In Experiment 3, temperature had a marked effect on the rate of mortality in weevils following topical inoculation with *B. bassiana* (Table 3). While all inoculated weevils were dead at day 17, earlier assessments showed higher temperatures increased the mortality rate (Table 3). A significant ($P < 0.001$) temperature effect on weevil mortality was observed at day 5. At day 10, there were significant temperature x isolate interactions ($P < 0.01$), reflecting the lower pathogenicity of some isolates at 15 °C.

TABLE 3: Proportion of adult Argentine stem weevil dead at 5 and 10 days after topical inoculation with *Beauveria bassiana* isolates and incubated at various temperatures.

Temperature °C	Proportional mortality ¹					
	All isolates†		ASW 5 (Waikato)		ASW 17 (Canterbury)	
	Day 5	Day 10	Day 5	Day 10	Day 5	Day 10
15	0	0.60 (0.02)	0	0.80 (0.04)	0.02 (0.02)	0.93 (0.03)
20	0.10 (0.01)	0.79 (0.01)	0.19 (0.04)	0.97 (0.02)	0.05 (0.03)	0.93 (0.03)
25	0.72 (0.01)	0.85 (0.01)	0.90 (0.03)	1.00 (0)	0.78 (0.04)	1.00 (0)
22 (ambient)	0.40 (0.02)	0.82 (0.01)	0.68 (0.05)	1.00 (0)	0.42 (0.05)	0.99 (0.01)

¹Proportion dead (standard error), corrected for mortality in untreated

† including ASW 5 and ASW 17

DISCUSSION

The results of the present study indicate that *B. bassiana* naturally infecting ASW is composed of several strains heterogenous in their pathogenicity even within a geographical region of New Zealand. The basis of these isolate differences is not presently known but isolate specificity can occur at several stages of disease development (Payne 1988). The differences in pathogenicity between isolates particularly at lower temperatures, are likely to be important in the exploitation of *B. bassiana* as a mycoinsecticide applied late in the Waikato spring, when temperatures are in the order of 15 °C.

The dose-response data presented indicates that weevils must contact high numbers of fungal conidia to effect mycosis. Several authors (Ferron 1981; Zacharuk and Tinline 1968) have reported direct relationships between dosage of *Beauveria* conidia and pathogenicity in the host. This may limit the potential of *B. bassiana* as a mycoinsecticide. The absence of interactions between dosage and isolate source

indicates that isolate selection is unlikely to improve the requirement for high spore numbers. The requirement for high inoculum levels may be overcome if the factors influencing virulence can be understood and manipulated.

The dependence of the mycosis in ASW on high inoculum levels and its sensitivity to temperature, provides an insight to the autumn, density-dependent epizootic processes in Waikato weevil populations described by Barker *et al* (1989). Such information, when coupled with isolate screening for high virulence and pathogenicity, provides a sound basis for the field evaluation of late spring applications of *B. bassiana* conidia as a mycoinsecticidal inoculum. Factors affecting persistence of applied fungal conidia, and formulations to improve this, are subjects of current research.

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