SUSCEPTIBILITY OF LIFE STAGES OF *PAROPSIS CHARYBDIS* TO *BEAUVERIA BASSIANA*

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ABSTRACT

Paropsis charybdis (Coleoptera: Chrysomelidae) is a pest of eucalypts and is responsible for increasing problems in some New Zealand plantations. In bioassays at 25°C, isolates of the entomopathogenic fungus, *Beauveria bassiana*, were tested against larvae, pupae and adults of *P. charybdis*. Using a topical spray application of 10⁵ and 10⁷ conidia/ml, larvae and pupae were highly susceptible to two isolates of the fungus and a commercially available *B. bassiana* product. In a second experiment, an isolate from the commercial product and a larval-active isolate were less pathogenic to adults than larvae or pupae. First to second instar larvae died more rapidly than older instars and pupae after infection.

Keywords: *Paropsis charybdis*, Coleoptera, Chrysomelidae, eucalyptus tortoise beetle, *Beauveria bassiana*, microbial control.

INTRODUCTION

Eucalyptus tortoise beetle, *Paropsis charybdis* (Coleoptera: Chrysomelidae), was first discovered in New Zealand in 1916 after accidental introduction from Australia (Thompson 1922). Both adults and larvae feed on leaves, causing frequent and severe defoliation of some eucalypt species, sometimes leading to tree death. Insecticides are normally not suitable for controlling *P. charybdis* populations because they are regarded as harmful to the environment and require frequent applications. Biological control by parasites has been attempted with the introduction of one species and examination of several others. However, past releases of parasites have been unsuccessful in reducing populations of *P. charybdis* (Kay 1990).

Biological control using microbial pathogens has been used against beetle pests in some countries. In New Zealand, the entomopathogenic fungus *Beauveria bassiana* and the bacterium *Serratia marcescens* have been recorded as naturally occurring pathogens of *P. charybdis* (Glare *et al.* 1993). *B. bassiana* is of particular interest as a single isolate has recently been developed into several successful commercial products in the USA. Several of these products produced by Mycotech (Butte, Montana) have been imported to New Zealand by Elliot Chemicals (Willoughby *et al.* 1998). Although *B. bassiana* has a wide recorded host range, individual isolates are usually restricted in host preference. In this study, we investigated the pathogenicity of three isolates of *B. bassiana* against developmental stages of *P. charybdis*.

MATERIALS AND METHODS

Paropsis charybdis rearing

Adults were obtained from a laboratory culture at Forest Research, Rotorua, or field-collected from around Lincoln University, Canterbury. Adults were held at 22.5°C (20°–25°) under 16 hour photoperiod in nylon cages (550 x 550 x 550 mm) and fed with fresh young eucalyptus (e.g., *Eucalyptus nitens*, *E. viminalis*) leaves, changed every two days. Larvae were reared in ventilated plastic containers (300 x 210 x 140 mm) and also fed eucalyptus foliage, renewed daily. Prepupae were removed from the

rearing containers and placed on plastic trays (270 x 175 x 20 mm). Some pupae were reared through to adults.

Fungal isolates and culturing

Three isolates were used against *P. charybdis*. F235 was originally isolated from *Leptinotarsa decemlineata* (Col., Chrysomelidae) (ex Dr E. Groden, University of Maine, isolate 726); F238 was originally isolated from *L. decemlineata* from Maine, USA and accessioned into the USDA pathogens culture collection as ARSEF252 (Humber 1992); F305 was isolated from the commercially available product BotaniGard ES, obtained from Mycotech Corporation (supplied by Elliot Chemicals, Pukekohe). BotaniGard is based on only one isolate of *B. bassiana*. All isolates are held in the AgResearch Insect Pathogen Culture Collection (Canterbury Agriculture and Science Centre, Lincoln) at -80°C.

Isolates were maintained on Potato Dextrose Agar (PDA, Gibco, Paisley, Scotland) at 25°C in constant light and subcultured approximately every two weeks. Conidia were harvested from the surface of PDA plates and suspended in 5 ml of 0.05% Triton X-100. Conidial concentration was estimated using a haemocytometer and adjusted to conidial concentrations of 10^5 and 10^7 conidia/ml.

Bioassay design

P. charybdis larvae, pupae and adults were inoculated with conidia using a cylindrical spray tower measuring 11 (dia.) x 29 (height) cm. For each treatment, ten *P. charybdis* were placed into each of three Petri dishes lined with moistened filter paper (30 insects/treatment). Each Petri dish was exposed to 100 µl of conidial suspension (10^5 or 10^7 conidia/ml) applied through a Paasche H single action airbrush (Paasche Airbrush Co., Harwood Heights, Illinois), using approximately 69 kPa pressure. Even distribution of the conidial suspension was checked by spraying with distilled water on filter paper prior to treatments. Controls received 100 µl of 0.05% Triton X-100. PDA plates were also sprayed with 100 µl of each conidial suspension to check conidial viability.

In experiment 1, the commercial preparation of *B. bassiana*, BotaniGard ES (Emulsifiable Suspension containing 2×10^{13} viable *B. bassiana* conidia/quart [0.95 litre) and F235 and F238 were used to treat small (1st-2nd instar) larvae, large (3rd-4th instar) larvae and pupae of *P. charybdis*. Two concentrations of conidia for all treatments, including BotaniGard, (10^5 and 10^7 conidia/ml) were used at 100 µl/dish.

In experiment 2, F235 was compared with the isolate from BotaniGard (F305) grown on PDA under the same conditions as for F235. The insect developmental stages used in this experiment were first instar larvae, pupae and adults, treated with two concentrations as in experiment 1.

Following inoculation, eucalypt leaves were added before the dishes were enclosed in a plastic bag with wet tissue paper within the bag to maintain a saturated environment for the first 24 h at 25°C. Subsequently dishes were incubated without added humidity at 25°C. *P. charybdis* were monitored and fed with fresh foliage daily for 21 days. Dead individuals were transferred to a Petri dish lined with moistened filter paper and kept at room temperature to determine the proportion of cadavers which supported *B. bassiana* sporulation.

Statistical analysis

Mortality results were analysed by one-way ANOVA using the program, Statistix (Anon 1985). Probit analysis and a log-time transformation were used to analyze the time-mortality data using the program POLO (Finney 1971; Robertson *et al.* 1980). Adjustments to account for control mortality were made using Abbott's formula (Abbott 1925). The lethal time (LT₅₀) and 95% fiducial limits were calculated using the maximum likelihood procedure after probit transformation. The probability integral of the χ^2 distribution was used to test the hypotheses of equality and parallelism among test groups.

RESULTS

In experiment 1, all three isolates/products of *B. bassiana* caused high mortality of larvae and pupae of *P. charybdis* (Table 1). Very few dead larvae developed external

signs of sporulation and it appeared that most died soon after the fungus penetrated through the cuticle (Table 1). Mortality at the end of the experiment (21 days) was between 60-100% and concentration had a significant ($t = 2.45$; 16 df; $P = 0.026$) effect on eventual mortality. After 7 days, small larvae showed significantly higher mortality ($F = 14.5$; df = 2, 17; $P = 0.0003$) than large larvae and pupae in both concentrations, whereas after 14 days no significant difference was observed between developmental stages.

TABLE 1: Percentage mortality and sporulation in brackets at 21 days and LT₅₀s for *Paropsis charybdis* larvae and pupae treated with two isolates of *Beauveria bassiana* (F235 and F238) and the *B. bassiana*-based product, BotaniGard (25°C, 30 individuals/treatment).

Insect stage/ Inoculum (conidia/ml)	F235 % Mortality (% sporulation ¹)	F238 % Mortality (% sporulation)	BotaniGard % Mortality (% sporulation)	Control % Mortality (% sporulation)
Small larvae				
10 ⁵	90 (25.9)	80 (8.3)	93.3 (10.7)	33.3 (-)
10 ⁷	93.3 (28.6)	86.7 (15.4)	100 (40)	
Large larvae				
10 ⁵	60 (-)	73.3 (-)	76.7 (-)	13.3 (-)
10 ⁷	83.3 (-)	80 (-)	90 (37.04)	
Pupae				
10 ⁵	83.3 (-)	73.3 (-)	90 (-)	16.7 (-)
10 ⁷	90 (-)	86.7 (-)	96.7 (13.8)	

¹% sporulation was based on the number of cadavers supporting conidial production.

By comparing the 95% fiducial limits (Table 2), a highly significant difference ($F = 17.98$; df = 2, 35; $P < 0.001$) was observed in LT₅₀ for the developmental stages. Mortality was most rapid for small larvae, although differences were not significant between the LT₅₀ values for small and large larvae with different isolates. Conidial concentration did significantly affect time to death. BotaniGard at the higher concentration (10⁷ conidia/ml) resulted in a higher daily rate of mortality in large larvae of *P. charybdis* than F235 and F238, and in all cases 21 day mortality was higher using BotaniGard than F235 or F238.

TABLE 2: LT₅₀s for *Paropsis charybdis* larvae and pupae treated in Table 1 with two isolates of *Beauveria bassiana* (F235 and F238) and the *B. bassiana*-based product, BotaniGard (25°C, 30 individuals/treatment).

Insect stage/ Inoculum (conidia/ml)	F235	LT ₅₀ (FL 95%) (days) F238	BotaniGard
Small larvae			
10 ⁵	4.8 (3.5 - 6.1)	10.3 (8.5 - 12.1)	7.5 (5.8 - 9.1)
10 ⁷	3.8 (2.8 - 4.8)	7.3 (5.7 - 8.7)	3.2 (2.7 - 3.6)
Large larvae			
10 ⁵	15.5 (14.3 - 17.0)	13.0 (11.9 - 14.1)	12.3 (11.3 - 13.4)
10 ⁷	12.4 (11.4 - 13.2)	10.6 (9.5 - 11.6)	9.3 (8.3 - 10.1)
Pupae			
10 ⁵	9.5 (8.1 - 10.7)	12.6 (11.2 - 14.1)	9.2 (8.1 - 10.3)
10 ⁷	8.1 (7.0 - 9.1)	10.6 (9.5 - 11.6)	8.1 (7.2 - 8.9)

In experiment 2, F235 and F305 were highly pathogenic to larvae and pupae at 10^5 and 10^7 conidia/ml concentrations, with both concentrations resulting in >90% mortality. However, adults were significantly less susceptible ($F = 155.09$; $df = 2, 11$; $P < 0.001$) (Table 3). Adult mortality was not significantly different between isolates, but there was significant effect of conidial concentrations ($t = 8.66$; 82 df ; $P < 0.001$). The LT_{50} values of first instars and pupae in experiment 2 were significantly different ($t = 5.34$; 14 df ; $P = 0.0001$) (Table 3). Adults did not reach 50% mortality at either concentration, so LT_{50} estimates are very high (Table 3).

TABLE 3: Percentage mortality at 21 days and LT_{50} s of *Paropsis charybdis* larvae, pupae and adults treated with *Beauveria bassiana* isolates F235 and F305 (BotaniGard isolate) (25°C, 30 individuals/treatment).

Insect stage/ Inoculum (conidia/ml)	F235		F 305		Control
	% Mortality (% sporulation ¹)	LT_{50} (FL 95%) (days)	% Mortality (% sporulation ¹)	LT_{50} (FL 95%) (days)	% Mortality (% sporulation ¹)
1 st instar larvae					
10^5	90 (33.3)	3.6 (2.5-4.6)	93.3 (46.4)	3.3 (2.4-4.0)	16.7 (-)
10^7	100 (26.7)	2.9 (2.2-3.5)	100 (46.7)	2.7 (2.3-3.1)	
Pupae					
10^5	93.3 (17.9)	6.9 (5.8-7.8)	100 (23.3)	5.4 (4.9-5.8)	13.3 (-)
10^7	100 (20)	6.0 (5.6-6.4)	100 (16.7)	4.7 (4.3-5.0)	
Adults					
10^5	6.7 (50)	548.7 (-) ²	6.7 (100)	1233.9 (-) ²	0 (-)
10^7	23.3 (42.9)	78.3 (43.6-297.1)	26.7 (62.5)	85.3 (44.3-400.7)	

¹% sporulation was based on the number of cadavers supporting sporulation.

²Fiducial limits not calculated.

More cadavers supported sporulation in experiment 2 than experiment 1. Of first instars that died within three days of inoculation, 10-31% of cadavers supported sporulation. Among first instars which died later than three days after inoculation, 45-89% of cadavers supported sporulation. Few (17-23%) pupae supported sporulation after death and of adults which died 43-100% of cadavers supported conidial production.

DISCUSSION

P. charybdis was susceptible to all *B. bassiana* isolates used under laboratory conditions. First and second instar larvae appear to be the most susceptible stage for all isolates tested and adults were relatively tolerant. Mortality was rapid, with about 60-70% of 1st-2nd instar larvae dying within 3 days of inoculation using high concentrations of *B. bassiana*. Older (3rd and 4th instar) larvae took longer to die than either younger larvae or pupae, although final mortalities were similar. This study indicates that *B. bassiana* and in particular the product BotaniGard (F305) could be a useful control for *P. charybdis*.

The results of these studies confirm those obtained with chrysomelids in other studies. For example, Colorado potato beetles (Hajek *et al.* 1987) were highly susceptible to *B. bassiana*. In comparison with other coleopterans, similar LT_{50} s as those for *P. charybdis* have been found. For example, Soper and Ward (1981) tested 50 isolates of *B. bassiana* on the Colorado potato beetle and obtained LT_{50} values of between 2 and 10 days. Delarosa *et al.* (1997) and Barson (1977) obtained with LT_{50} values between 4.3 and 7.5 d and an LT_{50} value of 6 d at 25°C with the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) and larvae of elm bark beetle *S. scolytus* (Coleoptera: Scarabaeidae), respectively.

Further research is now required to determine if infection can result from leaf contamination, as well as surface application and on the environmental variables which might restrict field performance.

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REFERENCES

- Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265-266.
- Anonymous, 1985. Statistix: An interactive statistical analysis program for microcomputers. NH Analytical Software, New York. 324 pp.
- Barson, G., 1977. Laboratory evaluation of *Beauveria bassiana* as a pathogen of the larval stage of the large elm bark beetle, *Scolytus scolytus*. *J. Invertebr. Path.* 29: 361-366.
- Delarosa, W., Alatorre, R., Trujillo, J. and Barrera, J.F., 1977. Virulence of *Beauveria bassiana* (Deuteromycetes) strains against the coffee berry borer (Coleoptera: Scolytidae). *J. Econ. Ent.* 90: 1534-1538.
- Finney, D.J., 1971. Probit Analysis. 3rd edition. Cambridge University Press. Cambridge. 333 pp.
- Glare, T.R., O'Callaghan, M. and Wigley, P.J., 1993. Checklist of naturally occurring entomopathogenic microbes and nematodes in New Zealand. *N.Z. J. Zool.* 20: 95-120.
- Hajek, A.E., Soper, R.S., Roberts, D.W., Anderson, T.E., Biever, K.D., Ferro, D.N., Lebrun, R.A. and Storch, R.H., 1987. Foliar applications of *Beauveria bassiana* (Balsamo) Vuillemin for control of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae): An overview of pilot tests results from the Northern United States. *Can. Ent.* 119: 959-974.
- Humber, R.A., 1992. Collection of entomopathogenic fungal cultures: Catalog of strains. U.S. Department of Agriculture, Agricultural Research Service, ARS-110, 177 pp.
- Kay, M., 1990. Success with biological control of eucalyptus tortoise beetle, *Paropsis charybdis*. *What's New in Forest Research* 184: 4 pp.
- Robertson, J.L., Russell, R.M and Savin, N.E., 1980. POLO: a Users Guide to Probit or Logit Analysis. Berkley, Pacific Southwest Forest and Range Experimental Station. 14 pp.
- Soper, R.S. and Ward, M.G., 1981. Production, formulation and application of fungi for insect control. Pp 161-180. *In: Biological Control of Crop Production*. G.C. Papavizas (ed.), BARC Symposium No. 5. Allaheld, Osmum, Totowa.
- Thompson, H.G.M., 1922. The Naturalisation of Animals and Plants in New Zealand. The University Press, Cambridge. 607 pp.
- Willoughby, B.E., Glare, T.R., Kettlewell, F. and Nelson, T.L., 1998. *Beauveria bassiana* as a potential biocontrol agent against the clover root weevil, *Sitona lepidus*. *Proc. 51st N.Z. Plant Prot. Conf.*: 9-15.