

EFFECTS OF TEMPERATURE AND HUMIDITY ON THE SUSCEPTIBILITY OF *PAROPSIS CHARYBDIS* TO *BEAUVERIA BASSIANA*

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ABSTRACT

The effect of relative humidity on pathogenicity of the fungus *Beauveria bassiana* to the Eucalyptus tortoise beetle, *Paropsis charybdis* (Coleoptera: Chrysomelidae) was examined using saturated salt solutions to maintain constant relative humidity (RH) at 25°C. An RH of 92.5% had no effect on mortality compared to 100% RH. However RH of 53, 75.5 and 85% increased the time taken to kill and reduced the overall mortality. Lower humidities encouraged more fungal sporulation on cadavers compared to those killed at a constant 100% RH. The fungus was pathogenic at all temperatures tested (15–30°C). Temperature altered the LT₅₀ significantly, with the lowest LT₅₀ of 2.5 days at 25°C.

Keywords: *Paropsis charybdis*, eucalyptus tortoise beetle, *Beauveria bassiana*, humidity, temperature.

INTRODUCTION

Eucalyptus tortoise beetle, *Paropsis charybdis*, was first recorded in New Zealand from the Port Hills, Christchurch, in November 1916 (Thompson 1922). With few natural control agents, it has become an abundant pest on a wide range of eucalypt species. White (1973) reported that this beetle fed on 59 species of *Eucalyptus* in the field in New Zealand. Both larvae and adults feed on young foliage and shoots, and the adults also feed on the more mature foliage, giving the leaves a scalloped appearance.

Introduction of a parasitoid from Australia has had little impact and few natural enemies have been recorded attacking this species in New Zealand. Microbial pathogens may have some potential, especially for use as inundative agents to replace chemical controls presently used. Two pathogens, the fungus *Beauveria bassiana* and the bacterium *Serratia marcescens*, have been recorded naturally killing *P. charybdis* in New Zealand (Glare *et al.* 1993). In a previous paper (Hastuti *et al.* 1999), we showed that isolates of *B. bassiana* are pathogenic to all stages of *P. charybdis* although adults were relatively tolerant compared to other stages. In addition, a commercially produced *B. bassiana*-based product, BotaniGard (Mycotech, Butte, Montana, USA), was as pathogenic as two isolates from Coleoptera.

Herein, we report on laboratory investigations into the influence of temperature and humidity on the mortality of *P. charybdis* caused by the BotaniGard isolate of *B. bassiana*.

MATERIALS AND METHODS

Insects and fungal culturing

P. charybdis were obtained from Forest Research, Rotorua, or field-collected around Lincoln University, Lincoln and maintained as described in Hastuti *et al.* (1999). *B. bassiana* isolate F305 (isolated from the BotaniGard product) was used in all experiments. All culturing and conidial collection was performed as described in Hastuti *et al.* (1999).

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Inoculation of *P. charybdis*

First instar larvae, shown by Hastuti *et al.* (1999) to be very susceptible to *B. bassiana*, were used in all experiments. Larvae were sprayed with 100 μ l of F305 at a concentration of 10^7 conidia/ml and then maintained at different relative humidities and temperatures. Inoculation was performed in a spray tower, as described previously (Hastuti *et al.* 1999). For each treatment, ten first instars in each of three Petri dishes (30 larvae/treatment) were treated and maintained as previously described. Larvae were kept in Petri dishes and fed eucalypt leaves for the duration of the experiment. A control treatment of 30 insects received 100 μ l of 100mM phosphate buffer (pH 7.2). Statistical analyses of results were performed as described in Hastuti *et al.* (1999).

Maintenance of humidity and temperature

A saturated atmosphere (100% RH) was maintained using distilled water. Relative humidities lower than 100% were maintained in airtight containers (220 x 135 x 80 mm) using saturated solutions of: potassium nitrate (KNO₃-92.5%), potassium chloride (KCl-85%), sodium chloride (NaCl-75.5%), and magnesium nitrate (Mg(NO₃)₂.6H₂O-53%) (Solomon 1951; Winston and Bates 1960). After inoculation, Petri dishes containing larvae were put in the containers suspended above the appropriate salt solutions and maintained in a constant temperature cabinet at 25°C for 21 days.

To examine the effect of temperature, Petri dishes containing inoculated larvae were transferred to constant temperature cabinets set at 15°, 20°, 25° and 30°C (\pm 1°C) and 16:8 L:D photoperiod with ambient RH. All larvae were checked daily and dead removed to assess sporulation on cadavers.

RESULTS

Effect of relative humidity

B. bassiana killed first instar *P. charybdis* at all humidities tested, but mortality levels reduced and larvae took longer to die at lower RH (Fig. 1; Table 1). There was a highly significant difference in mortality of larvae maintained under different RH. At 92.5% RH and 100% RH, the daily mortality was similar but significantly higher than that recorded at 85, 75.5 and 53% RH. LT₅₀s reduced with increasing RH, ranging from 13.1 days at 53% RH to 2.3 days at 100%RH.

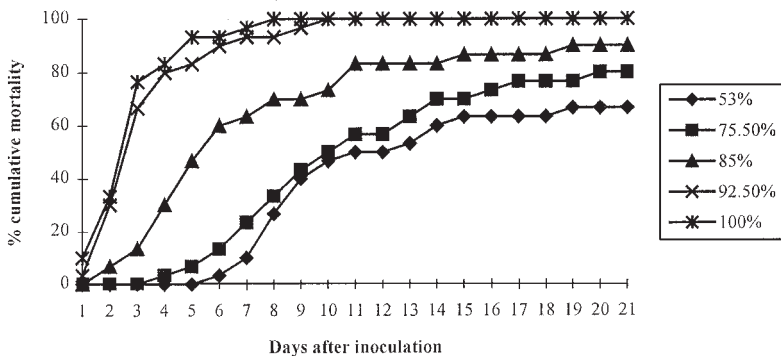


FIGURE 1: Cumulative mortality of *Paropsis charybdis* first instar larvae inoculated with *Beauveria bassiana* (F305) incubated at various RH at 25°C (30 individuals/treatment, corrected for control mortality).

The percentage of cadavers that supported fungal sporulation ranged from 26.7% - 60%, increasing with decreasing humidity (Table 1). Among larvae that died within three days of inoculation, only a small proportion (8.7-25%) developed external

sporulation, whereas the fungus sporulated on the majority of first instars (45.8-85.7%) that died four days after inoculation until the end of observation (21 days).

TABLE 1: Probit analysis of mortality data¹ for *Paropsis charybdis* first instar larvae inoculated with *Beauveria bassiana* (F305, 10⁷ conidia/ml) incubated at different relative humidities at 25°C (30 larvae/treatment).

Treatment (% RH)	% mortality (% sporulation ²)	Slope ± SE	LT ₅₀ (FL 95%) (days)
53	67 (60)	3.7 ± 0.3	13.1 (12.2 - 14.1)
75.5	80 (45.8)	3.8 ± 0.3	10.9 (10.2 - 11.8)
85	90 (51.8)	2.7 ± 0.2	5.9 (5.2 - 6.6)
92.5	100 (40)	3.9 ± 0.3	2.7 (2.3 - 3.0)
100	100 (26.7)	4.0 ± 0.4	2.3 (1.9 - 2.6)

¹Analysis was processed after probit transformation.
²Sporulation represents the % cadavers supporting sporulation.

Effect of temperature

B. bassiana infected larvae of *P. charybdis* at all temperatures tested (Fig. 2; Table 2). By 21 days after inoculation 100% of larvae incubated at 20°, 25° and 30°C had died, while approximately 93% of larvae were killed at 15°C. No significant difference was detected between 20°, 25° and 30°C, and only at 15°C was the comparative daily percentage mortality significantly different from those recorded at the other temperatures.

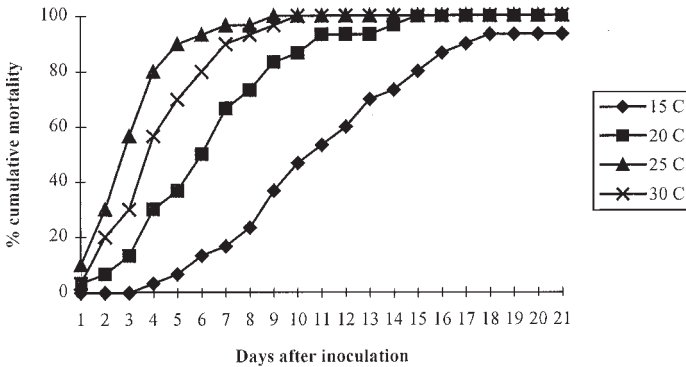


FIGURE 2: Cumulative mortality of *Paropsis charybdis* first instar larvae inoculated with *Beauveria bassiana* (F305) at different temperatures (30 larvae/treatment; corrected for control mortality).

By comparing the 95% fiducial limits, a highly significant difference ($F = 43.68$; $df = 3,7$; $P = 0.00016$) was observed between the LT₅₀ values at various temperatures (Table 2), although not at 25° and 30°C.

Sporulation from cadavers was fairly constant between treatments, with between 33-46.4% of cadavers supporting sporulation (Table 2). The fungus sporulated more often (34.6-76.9%) on larvae which had died on day 4 until the end of the observation period (21 days after inoculation), while 11.1-25% cadavers formed new conidia on those that died quickly (within 3 days after inoculation).

TABLE 2: Probit analysis of mortality data¹ for *Paropsis charybdis* first instar larvae inoculated with *Beauveria bassiana* (F305) and incubated at various temperatures.

Treatment (°C)	% mortality (% sporulation ²)	Slope ± SE	LT ₅₀ (FL 95%) (days)
15	93 (46.4)	5.2 ± 0.4	10.3 (9.7 - 10.9)
20	100 (33.3)	4.2 ± 0.3	5.3 (4.6 - 6.0)
25	100 (43.3)	4.0 ± 0.4	2.5 (2.2 - 2.8)
30	100 (40.0)	4.4 ± 0.4	3.5 (3.1 - 3.9)

¹Analysis was processed after probit transformation.

²Sporulation represents the % cadavers supporting sporulation.

DISCUSSION

Previously (Hastuti *et al.* 1999), we demonstrated that the BotaniGard product and the isolate on which it is based, F305, kills *P. charybdis* larvae. In this study, two environmental parameters, RH and temperature, were evaluated in the laboratory. Using a high inoculum level (10⁷ conidia/ml), F305 killed *P. charybdis* larvae at all humidities tested (53-100%), however, total mortality declined with reducing humidities. The most favourable temperatures for F305 to infect *P. charybdis* larvae were 25°-30°C, although close to 100% mortality was recorded at all temperatures from 15°-30°C, with only the speed of kill reducing significantly at 15° and 20°C.

High humidity (>90%) is required by many fungi for conidial germination and also for sporulation (Roberts and Humber 1981). Infection at RH as low as 53% would appear to contradict this requirement, although other studies have shown laboratory infections by *B. bassiana* at low humidity (e.g. Dunn and Mechalas 1963; Ramoska 1984; Marcandier and Khachatourians 1987). Conidia of *B. bassiana* in isolation do not germinate below ~90% RH (e.g. Walstad *et al.* 1970). It is unclear whether infection at lower humidities was due to a microclimate effect on the cuticle or due to residual moisture from a high inoculum dose formulated in Triton X-100. Previously, Dunn and Mechalas (1963) found that when massive inoculum concentrations of *B. bassiana* were applied, infection at lower humidity occurred more readily than when low inoculum densities were used.

B. bassiana is known to have a wide thermal tolerance, which may explain the broad climatic adaptation of this species. In general, growth of *B. bassiana* can occur between 5° and 35°C, with the optimum between 20° and 30°C (Roberts and Yendol 1981). Virulence of F305 at 15-30°C and 53-100% RH indicates climatic variables in New Zealand may not be limiting to the use of this fungus as a biocontrol agent against *P. charybdis*. The next stage in assessing *Beauveria*-based biocontrol of *P. charybdis* is to conduct field trials.

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