**PATHOGENICITY OF *BEAUVERIA*, *METARHIZIUM*,
PAECILOMYCES AND *FUSARIUM* ISOLATES AGAINST
EARLY INSTAR GRASS GRUB (*COSTELYTRA*
ZEALANDICA) LARVAE**

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Grass grub larvae (*Costelytra zealandica* White) continues to be viewed by farmers as New Zealand's most serious pasture pest (Dymock and Ford 1989). Environmental and human health hazards associated with the use of insecticides has encouraged the development of naturally occurring entomopathogens as a more economically and environmentally sustainable control technology.

This study reports on an initial laboratory screening of a range of naturally occurring fungal entomopathogens against early instar grass grub larvae.

Isolates were cultured from species of the entomopathogenic fungi *Beauveria* Vuillemin, *Metarhizium* Sorokin, *Paecilomyces* Bainier and *Fusarium* Link collected from a variety of pasture pests (Table 1). These pure cultures were maintained on PDA agar at 20 °C under 12 h light/12 h dark cycle.

Isolates were cultured for 2-4 weeks, the spores were aseptically removed and placed in a water suspension. Spore concentration was estimated from haemocytometer counts. Subsequent dilutions (in sterile 0.5% Tween 80) were made to produce a spore concentration for all screened isolates of 10^8 conidia/ml.

Field collected grass grub larvae were placed in McCartney bottles, three quarters filled with unsterilised soil (Taupo sandy loam). Soil moisture was determined gravimetrically. A 15 μ l drop of conidial suspension (i.e. 15×10^8 spores) was placed on each larva. Untreated larvae received a 15 μ l drop of 0.5% Tween 80. When the larvae had burrowed into the soil a moist cotton wool plug was placed in the neck of the bottle. This was re-moistened periodically, to limit soil moisture loss. Each treatment was replicated ten times.

In Experiment 1, isolates were tested on first instar larvae at 20 ± 2 °C and a soil moisture content of 60% Mw. In Experiment 2, isolates that caused greater than 50% larval mortality in Experiment 1 were further evaluated for their pathogenicity against second instar larvae at three temperatures (10, 15 and 20 °C) and a soil moisture of 21% Mw.

Assessments of mortality and microscopic examinations of the cadavers were made at 21 (Experiment 1 only) and 42 days post treatment. Larval mortality was not adjusted for control mortality in Experiment 1 and 2.

The experimental parameters in Experiment 1 of relatively high temperature (20 °C), high soil moisture content (60% Mw) and the use of first instar grass grub larvae were chosen to give optimum conditions for pathogenicity in the initial screening experiment. From the original 19 isolates, 13 achieved at least 50% larval mortality (Table 1).

Soil moisture content in Experiment 2 (21%) approximated field conditions in the central North Island during February/March on free draining pumice soils. Only three of the 15 isolates had the same degree of pathogenicity (20 °C at 21 days) against second instar as against first instar larvae. This had increased to nine by the second assessment (42 days). As the conidia must germinate on the host before penetrating the cuticle, environmental conditions are of great importance.

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TABLE 1: Host or source of entomopathogenic fungi and percent grass grub (first instar) larval mortality at 20°C and 21 days incubation.

Fungal genera	Isolate	Host/source	% mortality (se)
<i>Metarhizium</i>	M1	Porina (<i>Wiseana cervinata</i> (Walker))	50 (16)
	M2	Porina (<i>W. cervinata</i>)	80 (13)
	M3	Porina (<i>W. cervinata</i>)	40 (15)
	M4	Porina (<i>W. cervinata</i>)	80 (13)
	M5	Porina (<i>W. cervinata</i>)	50 (16)
	M6	CBS 289.67	60 (15)
	M7	Black beetle (<i>Heteronychus arator</i> F.)	50 (16)
	M8	Black beetle (<i>H. arator</i>)	10 (9)
<i>Beauveria</i>	B1	Black beetle (<i>H. arator</i>)	50 (16)
	B2	Black beetle (<i>H. arator</i>)	40 (15)
	B3	Black vine weevil (<i>Otiorhynchus sulcatus</i> F.)	20 (13)
	B4	Wasp (<i>Vespula germanica</i> F.)	50 (16)
	B5	Wasp (<i>V. germanica</i>)	60 (15)
	B6	Wasp (<i>V. germanica</i>)	60 (15)
	B7	Wasp (<i>V. germanica</i>)	70 (14)
	B8	Hoverfly (<i>Syrphus novaezealandiae</i> Macquart)	30 (14)
	B9	Grass grub (<i>Costelytra zealandica</i>)	—
<i>Paecilomyces</i>	P1	Grass grub (<i>C. zealandica</i>)	60 (15)
	P2	Grass grub (<i>C. zealandica</i>)	40 (15)
	P3	Grass grub (<i>C. zealandica</i>)	70 (14)
<i>Fusarium</i>	F1	Grass grub (<i>C. zealandica</i>)	—
Untreated			20 (13)

TABLE 2: Percentage mortality of second instar grass grub larvae at 10, 15 and 20°C and 21% soil moisture content.

Isolate	% larval mortality (21 days)			% larval mortality (42 days)		
	10°C (se)	15°C (se)	20°C (se)	10°C (se)	15°C (se)	20°C (se)
M1	70 (14)	90 (9)	50 (16)	80 (13)	90 (9)	90 (9)
M2	50 (14)	70 (14)	30 (14)	60 (15)	100 (0)	90 (9)
M4	30 (14)	30 (14)	30 (14)	40 (15)	40 (15)	80 (13)
M5	40 (15)	50 (16)	40 (15)	30 (14)	70 (14)	90 (9)
M6	30 (14)	0 (0)	30 (14)	30 (14)	30 (14)	40 (15)
M7	40 (15)	30 (14)	50 (16)	40 (15)	80 (13)	100 (0)
B1	10 (9)	10 (9)	50 (16)	50 (16)	20 (13)	90 (9)
B4	10 (9)	0 (0)	10 (9)	10 (9)	30 (14)	10 (9)
B5	20 (13)	40 (15)	30 (14)	50 (16)	60 (15)	70 (14)
B6	0 (0)	10 (9)	30 (14)	10 (9)	50 (16)	80 (13)
B7	0 (0)	20 (13)	40 (15)	20 (13)	50 (16)	50 (16)
B9	30 (14)	30 (14)	50 (16)	40 (15)	40 (15)	90 (9)
P1	30 (14)	10 (9)	10 (9)	40 (15)	10 (9)	20 (13)
P3	60 (15)	10 (9)	30 (14)	70 (14)	20 (13)	90 (9)
F1	40 (15)	60 (15)	100 (0)	70 (14)	80 (13)	90 (9)
Untreated	20 (9)	35 (11)	10 (7)	45 (11)	55 (11)	35 (11)

At 10 °C there was no significant increase in pathogenicity between the 21 day and 42 day assessments. Only three isolates, two *Metarhizium* species (M1, M2) and one *Paecilomyces* (P3) were significantly different ($P < 0.01$) from the untreated. The high larval mortalities in the untreated control (42 day assessment) were probably due to starvation. First instar grass grub larvae survive and develop by ingesting organic matter in the soil. By the second instar, the larvae require living organic material for survival and development. This was not provided.

The current study confirmed that in general the optimum temperatures for the deuteromycete fungi tend to be high at between 20-30 °C (Ferron 1981; Payne 1988). Low temperature isolates of *Metarhizium anisopliae* have been reported (Rath 1989). Host ranges and pathogenicities do vary considerably within fungal species (Payne 1988), thus the selection and isolation of cold tolerant strains of fungi pathogenic to grass grub larvae could form a valuable part of an integrated biocontrol programme.

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