**SUSCEPTIBILITY OF DIAMONDBACK MOTH (*PLUTELLA*
XYLOSTELLA (L.)) LARVAE TO THE
ENTOMOPATHOGENIC FUNGUS, *ZOOPHTHORA*
RADICANS (BREFELD) BATKO**

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ABSTRACT

The fungus *Zoophthora radicans* is being developed as a biopesticide for control of diamondback moth (*Plutella xylostella*). A series of laboratory, dose-response bioassays was used to determine the virulence of five isolates of *Z. radicans* to diamondback moths indigenous to New Zealand. A reference isolate from Malaysia was compared to four isolates from New Zealand. Late second and early third instar diamondback moth larvae were exposed to discs of broccoli leaves that had previously been inoculated with increasing densities of fungal conidia. Isolates from New Zealand were consistently more infective than the Malaysian isolate. LC₅₀ values ranged from 0.92 conidia/mm² for isolate NW337 (New Zealand) to 6.97 conidia/mm² for NW250 (Malaysia). Resting spores were found in up to 30% of the total number of larvae that succumbed to infection with the New Zealand isolates but were never found in larvae that succumbed to infection with isolate NW250. A single isolate was identified with potential for future testing under field conditions.

Keywords: *Plutella xylostella*, *Zoophthora radicans*, microbial control, bioassays, resting spores.

INTRODUCTION

The diamondback moth *Plutella xylostella* (L.) is considered the most important lepidopteran pest of brassica crops throughout the world (Talekar & Shelton 1993). Many populations are now resistant not only to insecticides, but to other control agents such as *Bacillus thuringiensis* Berliner (Talekar & Shelton 1993). In New Zealand, recent control failures have been attributed to insecticide resistance (Cameron et al. 1997). For this reason increasing interest in sustainable alternative control strategies, such as the use of entomopathogenic fungi, has developed (Furlong et al. 1995).

The entomophthoralean fungus *Zoophthora radicans* (Brefeld) Batko has shown potential in a lure and infect strategy against diamondback moth in Malaysia (Furlong et al. 1995). Conidia land on an insect and, under the correct environmental conditions, germinate and penetrate the host cuticle. The fungus proliferates in the host, eventually killing it and then produces new conidia on the dead insect which are available to infect new hosts. Some isolates produce resistant resting spores (RS) for long term survival. An essential component in the development of pest control strategies is the initial identification of pathogenic isolates. Pell et al. (1993) described a laboratory bioassay which was used to determine the virulence of several isolates of *Z. radicans*

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to diamondback moth larvae. A single isolate from Malaysia (isolate NW250) was identified as the most virulent isolate to diamondback moth larvae. The objective of the current study was to determine the virulence of four new isolates of *Z. radicans* collected in New Zealand to indigenous diamondback moths in comparison to isolate NW250.

MATERIALS AND METHODS

Diamondback moth

Diamondback moth pupae were collected from brassicas on two organic farms in Christchurch, New Zealand, in February 1999. The adult moths that emerged were used to establish a laboratory colony. Larvae were reared on excised broccoli leaves in ventilated boxes (20°C, 12:12 h light:dark photoperiod) and were either used in experiments when they reached late second/early third instar (12 days post oviposition) or contributed to colony maintenance.

Fungal isolates

Five isolates of *Z. radicans* were used in bioassays against larvae. Isolates NW331, NW334, NW337 and NW328 were from New Zealand populations of diamondback moth and isolate NW250 was from Malaysian populations. After retrieval from long term storage in liquid nitrogen all isolates were maintained at 20°C in darkness on SEMA (Sabouraud Dextrose Agar supplemented with egg and milk (Wilding & Brobyn 1980)) and never subcultured more than three times at monthly intervals before use in bioassays.

Bioassay methods and design

Petri dishes containing sporulating mycelia on water agar were used to shower the upper surface of broccoli leaf discs (50 mm diameter) with primary conidia. The density of conidia on the leaf was estimated by counting the number of conidia in a sample area on a glass coverslip placed on the leaf during inoculation. Individual leaf discs were inoculated for periods between one second and ten min to give a range of estimated densities of conidia, from 0.06 to 21.56 conidia/mm². Ten to sixteen leaf discs were inoculated in this way for each isolate.

Each leaf disc was maintained individually at 100% RH and 20°C overnight during which time the primary conidia produced capilliconidia. Fifteen larvae were introduced into the centre of each leaf disc and left for 30 min to become inoculated with capilliconidia. After inoculation larvae were incubated on fresh broccoli leaf discs at 100% RH and 20°C for a further 24 h to encourage infection. All surviving larvae were then transferred to ventilated plastic boxes with food and mortality assessed daily for five days. All dead larvae were removed and fungal sporulation and the presence or absence of resting spores determined. The bioassay was repeated on three occasions.

Statistical analysis

Mortality data were analysed using general linear regression with Probit analysis (Finney 1971) in the statistical package POLO. The LC₅₀ values (concentration of conidia on the leaf which causes 50% mortality of larvae) and associated standard errors were calculated for each isolate. Data on the sporulation or production of resting spores in larvae which succumbed to infection with the different isolates were analysed using logistic regression in Genstat 5 (v4.1).

RESULTS

There were large differences between isolates in LC₅₀ values (Table 1). The isolates from New Zealand were consistently more virulent than the isolate from Malaysia. It was not possible to calculate an LC₅₀ for isolate NW334 as data were too variable over the range of doses tested. However, this isolate still infected a large proportion (up to 93%) of larvae. Isolate NW337 was the most virulent isolate with an LC₅₀ value of less than one conidium/mm².

TABLE 1: Virulence of five isolates of *Zoophthora radicans* to diamondback moth larvae.

Isolate	Country of origin	LC ₅₀ (No. of conidia/mm ² leaf disc)	Confidence limits of LC ₅₀	Slope ± SE
NW250	Malaysia	6.97	4.20 – 16.70	0.76 ± 0.12
NW328	New Zealand	2.43	1.93 – 3.15	0.66 ± 0.04
NW331	New Zealand	1.74	1.00 – 2.81	0.94 ± 0.11
NW337	New Zealand	0.92	0.27 – 1.90	0.76 ± 0.10

*No LC₅₀ calculated for NW334 as data were too variable.

The proportion of larvae that sporulated having succumbed to infection was not related to the concentration of conidia ($\chi^2_1=0.003$, $P=0.956$), but was significantly different between isolates ($\chi^2_4=43.219$, $P<0.001$). Significantly more larvae infected by isolate NW337 contained resting spores compared to other isolates and no larvae infected with isolate NW250 contained resting spores (Table 2). Fewer larvae infected with isolate NW328 contained resting spores compared with the other isolates from New Zealand.

TABLE 2: Percentage of *Zoophthora radicans*-infected diamondback moth larvae containing resting spores (RS).

Isolate	Country of origin	Percentage with RS (average for 3 bioassays)	SE of larvae with RS	Percentage with RS (for each of 3 bioassays)		
NW250	Malaysia	0	-	0	0	0
NW328	New Zealand	7.90	1.96	0	16	9
NW331	New Zealand	13.76	2.30	7	29	8
NW334	New Zealand	12.13	1.95	8	28	5
NW337	New Zealand	19.07	2.56	15	30	11

DISCUSSION

Isolates from New Zealand were consistently more infective to indigenous diamondback moth larvae than the isolate from Malaysia (NW250). The LC₅₀ value obtained for NW250 (6.97 conidia/mm² leaf disc) in this study was greater than that obtained in a previous study by Pell et al. (1993) with the same isolate (0.02–0.52 conidia/mm²). Diamondback moth larvae used by Pell et al. (1993) were from the Philippines and had been in continuous culture at IACR-Rothamsted for several years whilst those used in the current study were from New Zealand and were only a few generations from field collected individuals. It is possible that larvae in continuous culture were inherently more susceptible than those collected recently from the field. Isolates of *Z. radicans* have been obtained from a wide range of hosts but they are generally most virulent to the hosts from which they were originally isolated. However, Milner (1982) demonstrated that different biotypes of the pea aphid (*Acyrtosiphon pisum* Harris) varied in their susceptibility to infection by the entomophthoralean fungus *Erynia neoaphidis* Remaudière & Hennebert. It is possible that geographically distinct populations of the same host may also exhibit differential susceptibility to the same pathogen.

Not all isolates of *Z. radicans* produced resting spores, which is in agreement with previous studies (Pell et al. 1993). The formation of resting spores can be an undesirable characteristic in fungi intended for use as swift acting mycoinsecticides, since these

spores are not available to infect new hosts immediately. It is unclear why resting spores were formed in larvae infected with New Zealand isolates but not in larvae infected with isolate NW250. More research is needed to determine the factors which affect formation of resting spores in individual hosts.

In conclusion, isolate NW337 was selected for further studies as the most consistently virulent isolate. A screening programme to include more isolates would enable the most promising isolate to be selected for field testing. Future assays should be based not only on the virulence of isolates to the host but also on their ability to kill insects over a range of conditions that may be experienced in the field. This will enable selection of isolates for use in an integrated programme to control diamondback moth in New Zealand.

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