THE FUNGI *BEAUVERIA* SPP. CAUSE EPIZOOTICS IN GRASS GRUB POPULATIONS IN WAIKATO

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ABSTRACT

Grass grub (*Costelytra zealandica*) larvae infected with a white fungus, identified as *Beauveria* sp., were collected from pastures near Karapiro from 1993 to 1995. Up to 30% mortality occurred in the population during spring 1993. Another epizootic observed in 1994 near Matangi resulted in a 99% mortality. At both sites most disease was observed during spring in late third instar larvae. The Karapiro epizootic was caused by a strain of *Beauveria brongniartii* and the Matangi outbreak by a strain of *Beauveria bassiana*. Bioassays have shown an isolate of the Karapiro strain can cause 100% mortality at 15°C only 48 days from inoculation. Both strains requirements and efficacy will be investigated.

Keywords: *Costelytra zealandica*, *Beauveria brongniartii*, *Beauveria bassiana*, biological control, epizootic

INTRODUCTION

The fungal genus *Beauveria* (Deuteromycetes) contains species which are often pathogenic to insects. The two most common species, *B. bassiana* and *B. brongniartii* are both present in New Zealand and have been recorded as pathogens of a number of insects (Glare *et al.* 1993). *B. bassiana* was the first insect pathogenic fungus described and occurs in most parts of the world. The origin of this species in New Zealand is unknown, but it could have entered with several of the exotic insects now found in New Zealand, or it could be endemic. *B. bassiana* has been described from over 20 species in New Zealand, including grass grub (Helson 1965; Glare *et al.* 1993). In contrast, *B. brongniartii* is generally associated with scarab species (Glare 1992), although it has also been described from several other insect groups in New Zealand (Laird 1991). *B. brongniartii* was the first documented example of an introduction of a microbial insect pathogen for the control of an insect pest in New Zealand. In the 1890's, a "*Botrytis*" from white grubs in France, produced by Alfred Giard, was imported by an "old farmer" and applied to insects in New Zealand (Anon. 1893). It is possible that this material, almost certainly a scarab-active strain of *B. brongniartii*, is the origin of this species in New Zealand.

Beauveria infected grass grub larvae are routinely found in pasture although, in our experience, rarely in large numbers (Glare and Townsend, unpubl. data). Grass grub are regarded as one of the major pasture pests throughout New Zealand and the farming community recognises the need for ecologically sound and sustainable biological control options. Therefore, when two separate incidences of *Beauveria* causing high mortalities in late instar grass grub larvae were noted during field sampling in Waikato in 1993 and 1994, a programme was set up to monitor the outbreaks and investigate the classification and efficacy of the two strains.

MATERIALS AND METHODS

Field epizootics

As part of a wider survey of grass grub populations, four paddocks on a property at Karapiro (Waikato) were sampled in April and September of 1993, 1994 and 1995.

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Sampling consisted of twenty random 15 x 15 cm spade squares dug to a depth of 25 cm in each paddock. Samples were sorted and recorded in the field with larvae returned to the laboratory to be assessed for fungal infection. Mummified cadavers were recorded from September 1993 when they were first observed in the field.

Grass grub populations in five adjacent 0.5 ha paddocks at Matangi, near Hamilton, were sampled in the winter and spring of 1994. Sampling consisted of twenty random 19 x 19 cm spade squares dug to a depth of 25 cm in each paddock. The paddocks were similar in topography and aspect and had been subdivided and sown in pasture 3 years previously following 14 years in maize. Soil samples were taken from the paddock in which the epizootic developed at weekly intervals from October 1994 to December 1995 to monitor fungal spore densities.

Fungal classification

Two semi-selective media, modified Veen's medium based on chloramphenicol and cycloheximide (Doberski and Tribe 1980) and JC medium based on streptomycin and tetracycline (Joussier and Catroux 1976), were used to isolate *Beauveria* spp. from several larval and soil samples collected from the Waikato epizootic sites. Once isolated pure strains were maintained on potato dextrose agar (PDA) (GIBCO, Paisley, Scotland), at 15°-25°C, by subculturing every 2 to 4 weeks.

Determination of species affiliation was by microscopic examination of conidiophores and conidia. Two isolates of known species were also examined for comparative purposes. Morphological examinations were conducted on material mounted in water or lactophenol cotton blue. Conidial dimensions were determined using eye-piece micrometers in an Olympus BH5 microscope at 1000x.

Bioassays against grass grub

Field collected second and third instar grass grub larvae were placed in trays with carrot squares overnight at 15°C to select actively feeding larvae for use in bioassays. Bioassays were conducted in 10 g of heat-sterilised soil (80°C for 24 h). Conidia were collected directly either from PDA plate cultures grown at 25°C for 1-2 weeks, or from fungus on rice grown for over 1 month, and suspended in 0.01% triton x-100. Conidial density was determined by plating dilution series on PDA. Soil was mixed with conidial suspensions to give a final soil moisture of 20% (w/v). For each assay 15 larvae were placed individually in containers with soil to which fungal conidia had been added at loadings of 10^8 for isolate F156 and 10^7 for F186. A carrot square was placed in the tube for food and was replaced each time the assay was assessed. Containers were incubated at a constant temperature (15°C or 20-22°C) in environmental cabinets with daylength set at 16 h. Assays were regularly assessed by direct observation of larvae. Dead larvae were incubated at ambient temperature and 100% RH for several days and the cause of death was then confirmed by microscopic examination of the cadaver and outgrowth.

RESULTS

Field epizootics

Karapiro results are an average for the four paddocks monitored. Grass grub populations declined from a peak of 179/m² (117 to 220) in the autumn of 1993 to 30/m² (25 to 36) in autumn 1995 (Table 1). Numbers of mummified grass grub recovered were as high as 32% and 19% of the population in the spring of 1993 and 1994, respectively, declining to 2% and 6% in the autumn of 1994 and 1995. No mummified cadavers were observed in April 1993. Healthy larvae returned to the laboratory did not develop any visual symptoms of fungal infection.

In the Matangi paddock in which an epizootic occurred a population density of 170/m² in June 1994 had fallen to 1/m² healthy grass grub and 34/m² mummified cadavers by September 1994 (Table 1). Over the same period populations in the other four paddocks sampled, starting at the same mean density of 170/m² in June, fell to a mean of 115/m² in September 1994. No mummified cadavers were recovered from these paddocks.

TABLE 1: Density of grass grub and of mummified cadavers and the percentage population reduction associated with two *Beauveria* epizootics monitored in Waikato.

Site	Month	Year	Grass grub /m ²	Mummified cadavers /m ²	% population reduction
Karapiro ¹	April	1993	179	0	0
	Sept	1993	63	30	32
	May	1994	136	3	2
	Sept	1994	33	8	20
	May	1995	30	2	6
Matangi ²	June	1994	170	0	0
	Sept	1994	1	34	99

¹ mean of four paddocks except May 1995 when only three paddocks sampled, one resown

² Data from the epizootic paddock only

Fungal isolation and classification

Veen's medium, a standard medium used for isolation of *Beauveria*, failed to detect any *Beauveria* spp. in soil samples from Matangi and only three colonies were found in soil from Karapiro. However the use of an alternative medium, JC, was more successful. An examination of the effect of these media on *Beauveria* isolates from Matangi and Karapiro showed Veen's to be inhibitory for all isolates tested and especially inhibitory for F156 (Table 2). For *B. bassiana* (F180 and F186) JC medium only inhibited up to 20% of colonies. This medium has since been successfully used for the isolation of *B. brongniartii* from Karapiro.

TABLE 2: Growth of *Beauveria* spp. on selective media at 25°C after 4 days.

Isolate	PDA	No. colonies and (average colony diameter [mm])			
		Veen's	% PDA ²	JC	% PDA ²
F156	220.8 (4) ¹	21.5 (>1)	9.7	NA	NA
F180	185.5 (3-5)	135 (1-2)	72.8	196 (1.5-2)	106
F186	156.5 (2-3)	48.5 (>1)	31.0	125 (2)	79.9

¹ Left for 5 days

² Number of colonies expressed as a % of colonies growing on PDA

Soil samples from Matangi, screened for *Beauveria* spp. using JC medium, ranged between 10³ and 2.8 x 10⁴ CFU (colony forming units) per gram soil identified as *Beauveria* sp. and up to 3 x 10³ CFU/g as *Metarhizium* sp., another common entomopathogenic fungus. No *Metarhizium* infected grass grub were recorded.

Cadavers from both areas infected by *Beauveria* spp. displayed extensive synnematal outgrowths but little sporulation. Cadavers generally had to be maintained in the laboratory for several weeks under moist conditions before sporulation would occur. Spore morphology of isolates from Karapiro and Matangi was variable immediately following isolation. From fresh cadavers conidia of all isolates were elongate to bullet shaped. However, after culturing, the isolates from Matangi reverted to producing mainly spherical conidia as shown by spore dimensions (Table 3) while those from Karapiro remained elongate to ellipsoidal. The two isolates of known species, a *B. bassiana* (F180) from the Chatham Islands and a Chinese isolate of *B. brongniartii* (F190), included for comparison purposes exhibited conidial dimensions similar to the Matangi and Karapiro isolates respectively.

TABLE 3: *Beauveria* spp. isolates used in this study.

Isolate	Species	Host	Site and date isolated	Spore dimensions (μm) ²	
				Length mean $\pm$ SD	Width mean $\pm$ SD
F190	<i>B. brongniartii</i>	ex soil	Cangzhou, China 10.94	4.3 ± 1.1	2.1 ± 0.3
F156	<i>B. brongniartii</i>	<i>Costelytra zealandica</i>	Karapiro, Waikato 13.9.93	3.9 ± 1.0	2.0 ± 0.5
F180	<i>B. bassiana</i>	ex soil	Chatham Islands, 29.5.94.	2.5 ± 0.3	1.8 ± 0.3
F185	<i>B. bassiana</i>	<i>Costelytra zealandica</i>	Matangi, Hamilton 19.10.94.	2.9 ± 0.6	2.4 ± 0.4
F186	<i>B. bassiana</i>	<i>Costelytra zealandica</i>	Matangi, Hamilton 19.10.94.	2.3 ± 0.3	1.9 ± 0.4

¹ All isolates are held in the AgResearch Insect Pathogens Culture Collection, Lincoln.

² Spore dimensions from PDA cultures

Bioassays against grass grub

Mortalities reported in Fig. 1 are corrected for control mortalities in each assay. At 15°C all grass grub larvae exposed to F156 (*B. brongniartii* ex Karapiro) were dead 48 days after inoculation while at 22°C all larvae were dead after 28 days (Fig. 1a). In the second assay grass grub larvae were exposed to F186 (*B. bassiana* ex Matangi) at 15°C and 20°C (Fig. 1b). At 20°C, 100% mortality was not achieved until 70 days after inoculation while at 15°C only 53% of larvae had died after 70 days.

FIGURE 1: a) The pathogenicity of *Beauveria brongniartii* isolate F156 for third instar grass grub larvae at 15° and 22°C. b) The pathogenicity of *Beauveria bassiana* isolate F186 for third instar grass grub larvae at 15° and 20°C.

DISCUSSION

Traditionally, classification of *Beauveria* into species is largely determined by spore morphology and, to a lesser extent, conidiophore morphology (eg. Brady 1979a; b). Variation in *Beauveria* spore morphology after culturing has been noted by Mugnai *et al.* (1989) who found that *B. bassiana* could form both globose and ellipsoidal conidia from the same strain under different conditions, whereas *B. brongniartii* only formed ellipsoidal or cylindrical conidia. As assigning species in *Beauveria* by conidial morphology alone may be influenced by substrate factors, a comparison was made using direct genetic analysis. Preliminary results using RAPD analysis indicated that the Matangi isolates were similar to *B. bassiana* from other hosts and the Karapiro

isolates were similar to *B. brongniartii* (Glare and Inwood, unpubl. data), confirming the morphological classification in this paper.

Since epizootics occurred at both Matangi and Karapiro the virulence of *Beauveria* spp. isolated was expected to be high. However in our bioassays the Matangi material did not appear as effective as the Karapiro isolate. The fact that the Matangi isolate was used at a lower dose meant no definite conclusion on comparative virulence could be reached. It is also possible that the F186 material collected from a rice-grown culture over a month old may have lacked virulence. In the light of these assays, we are investigating the effect on virulence of prolonged culture on rice. The effect of extended *in vitro* culturing, which may reduce virulence of entomopathogenic fungi (Glare 1992), is also being considered.

The ongoing occurrence of grass grub mortalities attributable to *B. brongniartii* in the four monitored paddocks at the Karapiro site has been a significant factor in a population decline from 179/m² in May 1993 to 30/m² in May 1995. Other factors implicated in the population decline at Karapiro were the application of the bacteria *Serratia entomophila* (Invade) to two of the paddocks in autumn 1993 and climatic factors which appeared to have caused a population decline throughout the Waikato over the summer of 1994-5.

The epizootic in a single paddock at Matangi was clearly attributable to *B. bassiana*. We have been unable to identify why an epizootic did not occur in the adjacent paddocks which had similar histories and spore loadings in the soil. Possibly the strain of *B. bassiana* in the epizootic paddock was different to that in the other four paddocks.

B. bassiana isolate F186 is being trialled in the field in the 1995-96 season against grass grub in Canterbury and the Waikato, and the F156 isolate of *B. brongniartii* will be added to the trial in spring 1995.

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