POTENTIAL OF *BEAUVERIA* AND *METARHIZIUM* AS CONTROL AGENTS OF PINHOLE BORERS (*PLATYPUS* SPP.)

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ABSTRACT

Three species of pinhole borer (*Platypus* spp.) are known in New Zealand. They are pests of beech and some other trees, boring deep into living and dead trees which allows the ingress of sapstain and other fungi. Although the species are native to New Zealand, they can still cause localised problems to trees when populations reach epidemic levels. We investigated the virulence of a selection of New Zealand isolates of three entomopathogenic fungi, *Beauveria bassiana*, *B. brongniartii* and *M. anisopliae*, against *Platypus*. The fungi were mainly from soil in beech forests. All isolates tested could kill and sporulate on *Platypus*. The ability of adult *Platypus* to contaminate larvae by transfer of spores was tested and found to occur in the laboratory. The possibilities of using *Beauveria* for localised *Platypus* control are discussed.

INTRODUCTION

Pinhole borers, *Platypus* spp. (Platypodidae: Coleoptera), are major pests of beech (*Nothofagus* spp.) and other forest trees in New Zealand. Three indigenous species make up a pest complex, *P. apicalis* (White), *P. caviceps* (Broun) and *P. gracilis* (Broun) (Milligan 1979). The beetles may attack apparently healthy beech trees, stumps, freshly felled logs and larger branches, and occasionally green sawn timber (McCracken 1994). The larvae and adults tunnel into the wood, allowing ingress of fungi deep into trees. These beetles depend on their symbiotic fungi as food (Kirkendall et al. 1997), and can utilise pathogenic fungi to kill host trees for colonisation and brood establishment (Faulds 1977). Fungi associated with *Platypus* may also cause stain in wood (Faulds 1973). Chemical control is both ineffective and unattractive due to difficulties accessing larvae and adults in tunnels (galleries). More sustainable control systems are required for this pest complex.

The few predators and parasites of *Platypus* recorded do not appear to be effective control agents (Milligan 1979). Microbial pathogens may offer more hope for use as localised biopesticides, but little is currently known about natural microbial enemies. Milligan (1979) reported an undescribed parasitic nematode attacking all three pest species, but the significance to population size is unknown. More recently, the ubiquitous entomopathogenic fungus, *Beauveria* sp., was recorded as 'regularly found infecting dead or unhealthy larvae and adults' (Ytsma, in McCracken 1994). Several *Beauveria*-based products are available around the world for other pests species, such as scarabs (MELOCONT; Strasser & Keller 2000), making it a potentially attractive fungus to develop for *Platypus* control. However, as most of the *Platypus* life cycle occurs within galleries in beech trees, access of any microbial agent to all *Platypus* life stages is important to consider.

In this study we investigated the virulence of several isolates of *Beauveria* spp. and *Metarhizium anisopliae* (another common insect pathogenic fungus) against larvae and adult *Platypus*. In addition, the ability of adults to transfer disease-causing spores to other larvae and adults after brief exposure was tested.

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MATERIAL AND METHODS

Fungal strains and culturing

Isolates from the AgResearch Insect Pathogen Culture Collection (Lincoln) were used in bioassays and transfer experiments with *Platypus* spp. (Table 1). All isolates were cultured on potato dextrose agar (PDA) (Merck) and incubated at 25°C for 2 – 3 weeks until conidia were produced. For bioassays, conidia were harvested into sterile 0.01% Tween 20 (BDH) to a final concentration of 10^8 conidia/ml. Conidial viability was determined by serial dilution plating onto PDA and the colony forming units were counted 4 – 7 days after incubation at 25°C.

TABLE 1: Fungal isolates used in these experiments. These are held in the AgResearch Insect Pathogen Culture Collection, Lincoln, New Zealand.

Fungus	Host	Date isolated
Beauveria bassiana		
F90	*Listronotus bonariensis* (Col: Curculionidae)	27.7.93
F305	from the product BotaniGard (Mycotech, USA)	20.11.97
F359	Soil isolation from logged beech forest, West Coast, NZ	11.3.99
F361	Soil isolation from un-logged beech forest, West Coast, NZ	11.3.99
F363	Soil isolation from un-logged beech forest, West Coast, NZ	11.3.99
Beauveria brongniartii		
F265	Soil isolation using *Galleria mellonella* (Lep: Galleriidae)[1]	4.4.97
Metarhizium anisopliae		
F142	Soil using *Galleria mellonella* North Island, NZ[1]	2.8.93
F358	Soil isolation from logged beech forest, West Coast, NZ	11.3.99
F360	Soil isolation from logged beech forest, West Coast, NZ	11.3.99
F362	Soil isolation from un-logged beech forest, West Coast, NZ	11.3.99

[1]Using the *Galleria* bait trap method of Zimmermann (1986).

Platypus spp.

For bioassay experiments, larvae and adult *Platypus* were extracted directly from beech logs collected from near Reefton, New Zealand. In most cases, species was not determined. Larvae and adults were stored overnight in sawdust before use in bioassays.

Direct inoculation experiments

For inoculation with fungi directly, larvae or adult beetles were placed into 90 mm plastic Petri dishes. Two methods were used. In method 1 (Fig. 1), 100 µl of the 10^8 conidia/ml suspensions was applied to the Petri dish using a Paasche H single action airbrush (Paasche Airbrush Co, Hartwood Heights, IL, USA) with approximately 48 kg/m^2 pressure. Each dish received 10^7 conidia, while control larvae or adults received 0.01% Triton X-100. In method 2 (Fig. 2) beetles were left to walk over sporulating cultures of *B. bassiana* strains F90 and F305 for 1 min before removal to clean Petri dishes. Control beetles were left walking for the same time on uninoculated PDA. Each Petri dish was then filled with oven-dried beech sawdust dampened with sterile distilled water, along with a 3 x 25 x 40 mm sliver of beech wood, and incubated at 20°C in 16: 8 h light:dark.

Transmission experiments

To study the ability of adult *Platypus* to vector pathogenic fungi to the larvae, adults were allowed to walk (or were rolled in experiment 2) over PDA plates of sporulating *B. bassiana* or *M. anisopliae,* and were then introduced to Petri dishes containing 5-7 larvae or adults and larvae. Control beetles were walked or rolled over clean PDA plates. Each Petri dish had a moist filter paper in the bottom to maintain high humidity,

and was sealed by parafilm. The dishes were incubated at 20°C. Dead beetles and larvae were removed daily and incubated at 100% humidity to check for *B. bassiana* infection.

Three transmission experiments were conducted. In experiment 1, beetles were walked for 1 min across sporulating cultures of F90, F305 and F142, then single adults transferred to dishes containing five larvae (four replicate plates). In experiment 2, adults were rolled over sporulating plates of F90 and F305, and then one adult introduced to a plate with five larvae and five adults (five replicate plates). In experiment 3, beetles were walked over sporulating cultures of seven fungal strains for 15 s, and then single adults placed in Petri dishes with 7-8 larvae (four replicate plates/treatment). In the last experiment five control plates of seven larvae were used.

To examine the potential conidial loading and viability of conidia on beetles, three adults left for 1 min on fungal plates were macerated in 1 ml of 0.05% Triton X-100 (BDH). Conidia were enumerated by haemocytometer counts (for total number of conidia) and tested for viability by dilution plating on PDA.

Statistics

χ^2 analyses were performed on mortality results from the adult transfer to larvae experiments using 1 degree of freedom.

RESULTS

Direct inoculation

Beauveria spp. were pathogenic to *Platypus* after exposure to spray showers of conidial suspensions. It was difficult to keep the larvae alive during assays and mortality from direct exposure of larvae was not significant, due to high and rapid control mortality deaths (Fig. 1a).

For adult beetles, exposure to a spray shower of 10^7 conidia (method 1) resulted in rapid death (Fig. 1b). The *B. brongniartii* isolate F265 and *B. bassiana* isolates F359 and F361 were similar in virulence, causing 100% mortality in 6-8 days. Only F363 failed to kill all exposed beetles at this spore concentration. No statistical analyses were performed as the data was not recorded as separate replicates.

In method 2 where the beetles were left to walk over sporulating fungal cultures, two more generalist *B. bassiana* isolates, F305 and F90, killed 100% of beetles after 8 and 16 days respectively (Fig. 2).

Transmission experiments

In experiment 1, walking beetles for 1 min across sporulating cultures of F90, F305 and F142, then introducing the adults to dishes containing larvae, resulted in 92-100% mortality of larvae after 4 days (data not shown). Both treatments were significantly different to the control ($P<0.01$), but not from each other. In experiment 2, rolling adults over sporulating plates of F90 and F305, then introducing one adult to plates containing adults and larvae resulted in 100% larval mortality after 4 days compared to 28% in controls. There were significant differences between larval mortality in treatments and control ($P<0.01$). For adult beetles there was 70 and 100% mortality for F305 and F90 respectively, after 13 days, which was significantly different ($P<0.05$ for F305 and $P<0.01$ for F90) than the control adult morality of only 32% after 13 days.

In experiment 3, introduction of adults exposed for 15 s to six isolates of *Beauveria* spp. and *Metarhizium anisopliae* resulted in significant mortality of larvae. Although control mortality was high (26% after 3 days), significantly ($P<0.001$-0.05) more larvae died in fungal treatments than untreated controls (Table 2). All adults exposed to the fungi and used as vectors died, usually within 4 days, and most cadavers supported fungal sporulation, indicating infection. For larvae, not all cadavers supported sporulation (Table 2).

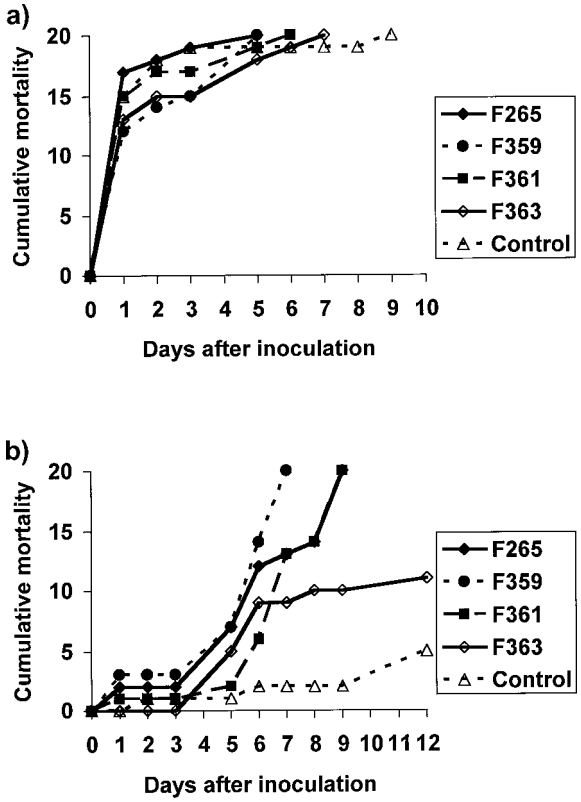

FIGURE 1: Cumulative mortality of *Platypus gracilis* (a) larvae and (b) adults after shower inoculation with 10^7 conidia/ml of *Beauveria* spp. (n=20/ treatment, 20°C).

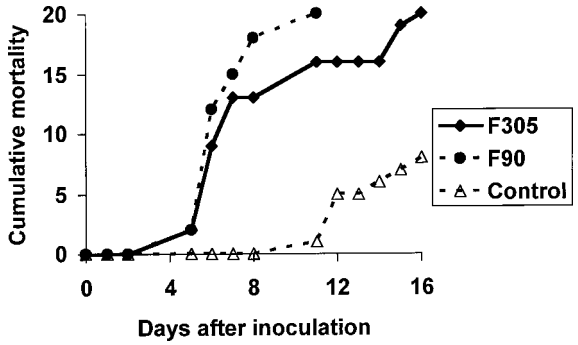

FIGURE 2: Cumulative mortality of *Platypus* sp. when beetles were walked over fungal plates of *B. bassiana* isolates for 1 min (n=20/treatment, 20°C).

TABLE 2: Mortality of larvae after exposure to adult *Platypus* left walking for 15 s on fungal cultures (20°C).

Fungal isolate	Number treated[1]	% larval mortality after 3 days	% cadavers supporting fungal sporulation after 14 days
Beauveria brongniartii			
F265	29	93 (***)[2]	69.0
Beauveria bassiana			
F359	28	89 (***)	100
F361	28	100 (***)	92.9
F363	28	57 (*)	89.9
Metarhizium anisopliae			
F358	29	75 (***)	75.8
F360	29	82 (***)	86.2
F362	29	50 (NS)	68.9
Control	35	26	0

[1]Four replicate plates containing seven-eight larvae/treatment and five plates of seven larvae in the control.
[2]Level of significance for comparison with control: *** = P<0.001; * = P<0.05; NS = not significant.

Viability and spore loadings on beetles

One large *Platypus* beetle carried between 2.5 x 10^4 - 4.3 x 10^5 conidia after 1 min exposure to sporulating cultures, depending on the isolate of fungus (Table 3). The amount of conidia on individual beetles was variable, even when exposed to the same fungus under the same conditions. This was probably due to the level of movement during the minute of exposure. Viability of conidia also varied greatly and was less than 20% for all three insects exposed to *M. anisopliae* F142.

TABLE 3: Conidial viability and quantity of spores carried by an adult *Platypus* beetle after walking for 1 min on sporulating fungal plates.

Strain	Replicate	Quantity carried (10^4 conidia)	Quantity of viable spores (10^4 conidia)	Viability (%)
Beauveria bassiana				
F305	1	3	2	66.7
	2	2.5	0.5	20
	3	3.5	1	28.6
	Average	3	1.2	38.4
F90	1	43	35	81.4
	2	24	5	20.8
	3	16	10	62.5
	Average	27.7	16.7	54.9
Metarhizium anisopliae				
F142	1	26	5	19.2
	2	9	1	11.1
	3	6	0	0
	Average	13.7	2.0	10.1

DISCUSSION

The entomopathogenic fungi *B. bassiana, B. brongniartii* and *M. anisopliae* were pathogenic to adult *Platypus* spp. in bioassays and transfer experiments. Larval assays were inconclusive due to high control mortality. However, *B. bassiana* did infect larvae during experiments and the cadavers supported sporulation. The isolates used included strains from soil around beech trees as well as isolates from other insects, but all showed some virulence. Unfortunately, no strain naturally infecting *Platypus* was available for bioassay. Recently we have isolated *B. bassiana* from an adult *P. gracilis*, but have not compared activity to those isolates used in this study. This study is the first report of *M. anisopliae* and *B. brongniartii* virulent to *Platypus* in New Zealand.

The experiments described also demonstrate that it is feasible to contaminate adults by walking through conidia and transfer infection to larvae and possibly other adults. We found that the larger *Platypus* beetles, *P. apicalis* and *P. caviceps*, could carry approximately 10^4 to 10^5 conidia/beetle, with up to 80% of the recovered conidia viable. This may be important for any control strategy aimed at attracting beetles to fungus-containing traps, and subsequent transfer to larvae and adults in pinhole tunnels. No suitable attractant is yet available, although aggregation pheromones are thought to exist (Milligan et al. 1988). Alternatively, logs or other brood material may be treated using *Beauveria* spray to prevent colonisation by adult *Platypus*.

The two genera of fungi, *Metarhizium* and *Beauveria*, are generally easily cultured and formulated. The infective conidia survive out of direct sunlight for several weeks and in a protected environment such as soil for years. This makes them good targets for developing potential biopesticides. Combined with an attractant, it may be possible to develop a control system where adults contaminated with fungi in traps then carry the infective conidia to the larvae in tunnels. This "lure and infect" strategy has been developed for other insects (Furlong et al. 1995).

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