***Beauveria brongniartii* – a potential biocontrol agent for use against manuka beetle larvae damaging dairy pastures on Cape Foulwind**

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Abstract Manuka beetles (*Pyronota* sp.; Scarabaeidae) are serious and persistent pests of dairy pastures on Cape Foulwind, Westport. When a selection of scarab-active fungal isolates were tested against 3rd instar larvae of two *Pyronota* species, a locally sourced *Beauveria brongniartii* (F636) isolate consistently achieved the fastest and highest levels of larval mortality. Topical application of F636 caused an average of 80% larval mortality 6 weeks post-treatment. Mortality was shown to be dose rate dependent for both *Pyronota* species. When treatments were applied by incorporating rice grains colonised by the fungus into soil, simulating field application, F636 again produced the fastest and highest levels of larval mortalities, averaging 70% 6 weeks post-application. Mortalities of both *Pyronota* species reached 100% after 8 weeks when the assay medium was a grey sand based soil (ex Cape Foulwind). Isolate F636 shows promise as a biological control agent for this pest and field trials have been carried out in the autumn of 2010.

Keywords *Beauveria brongniartii*, *Pyronota festiva*, *Pyronota setosa*, manuka beetle, biocontrol agent.

INTRODUCTION

Pyronota spp. (Coleoptera: Scarabaeidae), commonly called manuka beetle, are related to the New Zealand grass grub, *Costelytra zealandica* (White), which is a major pasture pest on lighter soils throughout New Zealand. Manuka beetle larvae have been reported to cause pasture damage in several regions of New Zealand (Thomson et al. 1978; Miln et al. 1983; Chapman 1984), often associated with grass grub damage in areas close to bush margins (Thomson et al. 1979). Following large scale pasture developments on the West Coast (“hump and hollow”, “flipping”), insect pests have caused significant decreases in pasture

yield and losses of sown pasture species. In recent years high populations of manuka beetles have become the dominant pests causing significant pasture production losses during autumn and winter on dairy farms on Cape Foulwind.

Two *Pyronota* species have been identified from the damaged pastures – *P. festiva* and *P. setosa* (det. Shaun Forgie, Manaaki Whenua Landcare Research). Pathogen surveys of these manuka beetle populations by AgResearch have shown low infection rates by bacteria and protozoans but have recorded moderate levels of infection in 3rd instar larvae by the Deuteromycete fungi,

Metarhizium and *Beauveria*. Isolates of both of these fungi have been used as biocontrol agents against scarab pests (Glare et al. 1994; Kessler et al. 2003). One isolate of *B. brongniartii* (Saccardo) Petch has been highlighted as a potential biocontrol agent for use against manuka beetle larvae (Townsend & Jackson 2008).

This paper reports on a series of bioassays carried out to examine the efficacy of *B. brongniartii* F636 as an entomopathogen of manuka beetle larvae compared to several other known scarab-active fungal isolates.

METHODS

Insects and fungal strains

Third instar larva of both *Pyronota festiva* (F.) and *Pyronota setosa* (Given) were collected from damaged pastures on Landcorp Farming Ltd properties on Cape Foulwind during autumn and winter of 2009 and held at 4°C until required. The average weight of *P. festiva* larvae was 0.077 g, while *P. setosa* was 0.051 g. Two fungal isolates from the AgResearch reference collection, *Metarhizium anisopliae* (F605) and *Beauveria bassiana* (F227), plus *M. anisopliae* (DAT-F001, Chafeguard™), were selected for their known scarab activity to compare against a *B. brongniartii* (F636) isolated from cadavers of *Pyronota* larvae collected from Cape Foulwind pastures in May 2007. The worst performing of these isolates, F227, was replaced in the final bioassay with a strain of *B. brongniartii* (F156) known to have caused an epizootic in grass grub larvae in the Waikato in 1993.

The fungal strains were tested in a series of bioassays against both species of manuka beetle larvae to determine infectivity and time to death among the treated larvae over a range of experimental conditions. For Bioassays 1, 2 and 3, suspensions of the fungi were prepared by suspending conidia from 14 day old PDA cultures in 0.01% Triton X-100. The spore concentration was estimated using a haemocytometer (improved Neubauer, 0.1 mm depth) and adjusted to a final concentration of 5×10^7 conidia/ml. For Bioassay 4, conidia were produced via solid substrate production (rice) to

achieve a loading of approximately 10^8 conidia/g of rice. All four bioassays were kept in the dark in controlled temperature cabinets at 15°C for the duration of the trials.

Bioassay 1: Preliminary testing

Twelve healthy feeding 3rd instar larvae were each treated with a dose of 10 µl of fungal suspension containing 5×10^7 viable conidia for each treatment (DAT-F001, F227, F636 and F605). The inoculum was applied topically on the underside of the larva using a pipette. The control larvae were treated with a 10 µl droplet of tap water. Treated larvae were kept individually in tissue culture trays for 24 h then placed into 35 ml tubes containing 22–25 g of sieved Wakanui silt loam soil at 17–20% soil moisture. Each tube had a 5 mm cube of carrot placed on the soil surface as a food source for the manuka beetle larva. The carrot cubes were replaced at each assessment. Assessments began 3 weeks post-treatment when each larva was removed from its tube and assessed as healthy, moribund or dead then replaced in the same tube. Weekly assessments continued until 7 weeks post-treatment.

Bioassay 2: Stage of development

The same fungal treatments and procedures were used as in Bioassay 1 and applied to twelve healthy feeding 3rd instar and twelve 'mature' (fully fed) 3rd instar larvae of both species. The control larvae were treated with a 10 µl droplet of tap water. The same assessment regime was followed.

Bioassay 3: Spore dosage

In this assay, twelve healthy feeding 3rd instar larvae of each *Pyronota* species were treated with topically applied 10 µl doses containing 0, 5×10^3 , 5×10^5 and 5×10^7 conidia of *B. brongniartii* (F636). The control larvae were treated with a 10 µl droplet of tap water. Again the same procedures and assessment regime was followed.

Bioassay 4: In-soil test

Twelve healthy, feeding 3rd instar *P. festiva* and *P. setosa* larvae were placed individually into 120

ml clear plastic tubes containing 75 g of either the grey sand soil typical of most damage-prone pastures on Cape Foulwind or Wakanui silt loam typical of a grass grub-prone area in Canterbury. Both soils were at approximately 20% soil moisture. For each treatment one conidia-covered rice grain was placed in each tube, equivalent to approximately 7.2×10^{14} spores/ha. Tubes in the control treatment had one non-fungus-infected rice grain placed in the soil. Other procedures were the same as used in previous assays except this assay was assessed 4, 6 and 8 weeks post-treatment.

Statistical analysis

The full percentage mortality data set from all four bioassays was statistically analysed using the Log-Rank test. The Log-Rank test compares the time-varying mortalities of any two treatment groups over the trial period and assesses the level of significance of the difference between the groups.

RESULTS

The tables below record the percentage mortality to 3rd instar larvae of *P. festiva* and *P. setosa* caused by the fungal isolate treatments applied directly (topical application) or indirectly (as conidia on rice into soil). Analysis of the full data set (not presented) showed that, in general, both species and both stages of 3rd instar larvae exhibited equal susceptibility to the strains of fungi used in the bioassays. Thus, in the tables below, mean mortalities for the two manuka beetle species and for different stages of 3rd instar larvae, where tested, are reported in an overall analysis for each bioassay.

When fungal conidia were applied topically to larvae in Bioassay 1 the fastest and highest mortality rates were caused by treatment with *B. brongniartii* F636, which killed all larvae within 5 weeks of treatment and caused significantly ($P < 0.05$) higher mortality to larvae than all other fungal treatments in Bioassay 1 (Table 1).

In Bioassay 2 mortality due to the various fungi treatments did not differ significantly ($P < 0.05$) between the two manuka beetle

species, and therefore the data were pooled. Overall mortalities were lower than in the first bioassay but the highest mortalities followed treatment with *B. brongniartii* F636 ($P < 0.05$) (Table 2). This strain again caused significant early mortality, reaching more than 50% by 6 weeks from treatment. Two exceptions (data not shown) to this overall result were feeding stage *P. setosa* larvae treated with F605 and mature stage *P. setosa* treated with F227, which both had mortality rates not significantly different to the same *P. setosa* life stages treated with F636.

In Bioassay 3 (Table 3) mortality due to dose rate did not differ significantly ($P < 0.05$) between the two manuka beetle species, and therefore the data were pooled. When larvae were treated with varying doses of *B. brongniartii* F636, the highest dose of 10^5 conidia/larva produced mortality similar to that seen in the previous experiments reaching 83% after 7 weeks. This was significantly ($P < 0.01$) greater than the mortality caused by treatment with 10^3 conidia per larva, which only reached 38% by 7 weeks post-treatment. Mortality due to both the higher rates was significantly ($P < 0.01$) greater than the 10^1 rate and control treatment mortalities, which were not significantly different from each other.

Excessively high levels of *Pyronota* larval mortality in the Wakanui soil control treatments in Bioassay 4 meant that no valid comparisons could be made between the various fungal isolates tested in this soil. Table 4 shows control mortality was higher in the grey sand soil than in previous experiments but, once again, *B. brongniartii* F636 caused significantly ($P < 0.05$) faster and higher mortality rates than the other fungal treatments or control reaching 100% death by 12 weeks post-treatment.

DISCUSSION

The common manuka beetle *P. festiva* has often been reported as a minor pasture pest but the uncommon *P. setosa* has been either unrecognised or unrecorded as causing even minor pasture damage. As *P. setosa* comprise up to half of the manuka beetle numbers in some of the most damaged pastures on Cape Foulwind it

Table 1 Mortality (%) of manuka beetle larvae (mean for both species combined) caused by topical application of four strains of selected entomopathogenic fungi in Bioassay 1.

Treatments		3 weeks	4 weeks	5 weeks	6 weeks	7 weeks
Control	a ¹	8	8	13	13	13
DAT-F001	bc	25	33	42	42	46
F227	ab	21	21	21	29	29
F605	c	25	34	54	63	67
F636	d	67	96	100	100	100

¹Treatments with no letter in common are significantly different ($P < 0.05$).

Table 2 Mortality (%) of manuka beetle larvae (mean for species and life stages combined) caused by topical application of four strains of selected entomopathogenic fungi in Bioassay 2.

Treatments		3 weeks	4 weeks	5 weeks	6 weeks	7 weeks
Control	ab ¹	2	2	4	9	11
DAT-F001	acd	0	0	7	15	25
F227	bce	4	4	11	17	19
F605	de	9	11	11	17	36
F636	f	32	42	48	58	71

¹Treatments with no letter in common are significantly different ($P < 0.05$).

Table 3 Mortality (%) of manuka beetle larvae (mean for species combined) treated with four rates of topically applied *B. brongniartii* F636 in Bioassay 3.

Treatments		3 weeks	4 weeks	5 weeks	6 weeks	7 weeks
Control	a ¹	0	0	0	0	0
10 ¹ spores	a	4	4	4	4	4
10 ³ spores	b	4	4	13	25	38
10 ⁵ spores	c	42	58	63	75	83

¹Treatments with no letter in common are significantly different ($P < 0.05$).

is important that any potential biocontrol agent is equally effective against both species. The bioassays reported in this paper have revealed that both species are susceptible to infection by a range of entomopathogenic fungi with similar effects on both species. Interestingly, strains of *Metarhizium* and *Beauveria* isolated from scarab hosts collected elsewhere in New Zealand or overseas did not have as much effect as the local strain, *B. brongniartii* F636, isolated from *Pyronota* larvae collected from Cape Foulwind pastures.

The results suggest that species specificity may be more important than wider adaptation to the Family Scarabaeidae. This is all the more interesting as few naturally fungus-infected larvae have been recovered from the *Pyronota* infested pastures (R.J. Townsend, personal observations).

Beauveria brongniartii F636 is clearly the best biocontrol candidate, as it consistently caused the fastest mortality and the highest total mortality in all bioassays. It also appeared to be effective against feeding and late stage 3rd instar larvae.

Table 4 Mortality (%) of manuka beetle larvae (mean for species combined) in grey sand soil caused by four fungal isolates applied as conidia grown on rice in Bioassay 4.

Treatments		4 weeks	6 weeks	8 weeks
Control	abc ¹	13	29	46
DAT-F001	ade	0	29	54
F156	bdf	17	33	54
F605	cef	13	33	58
F636	g	38	83	100

¹Treatments with no letter in common are significantly different ($P < 0.05$).

This is of practical importance as observations in the field have shown that development of manuka beetle larvae can be very variable with some becoming fully fed or 'mature' by early May while others do not complete their growth until as late as October. The 'mature' 3rd instar larvae have fully developed fat bodies and are not as active as feeding 3rd instar larvae. Both the feeding and mature 3rd instar larvae were shown to be equally susceptible to topically applied *B. brongniartii* F636. This is an important finding as it indicates that *B. brongniartii* F636 could be effective in both autumn, when most larvae are still in the feeding phase, and spring, when soil temperatures rise and a large proportion of the manuka beetle larvae will already be 'mature'.

Larval dose rate testing with F636 showed a significant increase in the speed with which mortality occurred and in the total percentage mortality as the dose rate increased. This suggests that a granular formulation, which applies concentrated doses of conidia, may be an effective field application strategy to establish fungal disease within a manuka beetle population. The results of Bioassay 4 support this hypothesis by showing that *B. brongniartii* F636, applied as conidia on rice, caused extremely high mortalities of manuka beetle larvae in the grey sand 'flipped' soil type commonly found under badly damaged pastures on Cape Foulwind. A field trial using two different formulations of *B.*

brongniartii F636 has been established in autumn 2010 to test the efficacy of *B. brongniartii* F636 under field conditions.

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