# *Beauveria pseudobassiana* and the Flax weevil from Mana Island

Travis Glare and Jenny Brookes

*Bio-Protection Research Centre, Lincoln University*

November 2017

[Photo, Dr John Marris]


**Bio-Protection Research Centre**
PO Box 85084
Lincoln University
Lincoln 7647, New Zealand
**P** + 64 3 423 0932
**F** + 64 3 325 3864
**www.bioprotection.org.nz**

## Summary

Flax weevil larvae and adults, and soil samples were collected from Mana Island to assist the investigation into the role of *Beauveria pseudobassiana* in population regulation of the weevil. Over 80 larvae were received, including 3 already infected with the fungus. Sixty larvae died within 2 days, before any bioassay was conducted. The remaining 29 larvae were exposed to *B. pseudobassiana* by dipping in spore suspensions. Eventually all but one larva died, mainly from the fungus, whether treated or not, showing the fungus is already in this population of weevils on Mana Island. The collected adults were also bioassayed using the fungus, with one dying within 3 days and showing *Beauveria* infection. This was likely to have been already infected before collection. In the highest dose, 90% of adults died by 20 days after inoculation, some showing signs of *Beauveria* infections. Soil samples from the island were assessed for levels of *Beauveria*. Soil that came with the larvae was heavily infested with *Beauveria*, at a rate of approximately $7.25 \times 10^4$ conidia/ml, while most other the other samples from the "A" site contained similar levels of *Beauveria*. The "B" site soil had low or no detectable *Beauveria*.

These results were unexpected as they suggest *Beauveria* is well-established in this population of flax weevil and should be limiting the population.

## Background

The flax weevil, *Anagotus fairburni,* was introduced to Mana Island in 2004 and 2006, as part of a restoration project (Fig. 1). These flightless weevils are present in only small populations on other rodent-free islands and a few mountain tops. They are considered 'At Risk – Relict' and are protected under the Wildlife Act.

Unlike other islands, the weevil has thrived on Mana Island to the point of killing some extensive flax stands. As the flax is important for maintaining other endangered species, the cause of the difference in population behaviour between Mana Island and other localities is being investigated. One possibility is that microbial insect pathogens are limiting the populations on some islands but not Mana. A search for diseased weevils on Maud Island, the source of the original population introduced to Mana Island, found only one weevil larvae (Colin Miskelly, pers. comm.). That larvae appeared unhealthy, so was sent to Lincoln University for examination. The larvae eventually died of a fungal infection, with the causative agent identified as *Beauveria pseudobassiana*.

A meeting between Te Papa, DoC, friends of Mana Island and Iwi in October 2017 determined that a small exploratory project should examine the distribution and virulence of *B. pseudobassiana* to flax weevil on Maud and Mana Islands. Initially the project had three objectives:

1. Determine the distribution of *Beauveria* in soil on both islands
2. Determine the virulence of *B. pseudobassiana* to flax weevil larvae and adults
3. Identify any disease in recovered weevils.

This report is a preliminary report on the results from sampling of Mana Island in October-November 2017.

## Materials and methods

### Samples received

All weevils were collected by Colin Miskelly and Dale Shirtliff on 23 Oct, on the plateau at south-west of Mana Island, all within about 50 m radius of the point shown. The release site (2004 & 2006) was beside the track just south-east of the marked area.


Figure 1: A. Collection site on Mana Island (yellow dot). B. Damage of flax by weevil adults. C. Adult weevils on Mana Island. D. Dead flax on Mana Island (Photos and map courtesy of Dr Colin Miskelly).

The samples were received at Lincoln University 25th October 2017. The soil samples A were from 2 clusters of 5 plants each all under the yellow dot in Figure 1A (within the heavily flax weevil infested zone, with many dead and dying flaxes). The B samples were from 2 clusters of 5 plants each all under the red dot shown here, approximately 50 metres beyond the spreading wave of flax weevils.

Larvae were collected from flax roots mainly from the woody stem of the plants. One hundred and twenty larvae were found but younger larvae discarded at the site. Among the larvae, three were obviously fungal infected and kept separate. The fungus on each of these three larvae was isolated into pure culture (Potato Dextrose Agar) and grown at 25°C. The species was confirmed through morphological examination as *B. pseudobassiana.*

Originally there were 89 larvae were in the shipment to Lincoln University (Fig. 2). The adult weevils were processed first so it was 2 days after arrival by the time the larvae were processed (Fig. 2). They were kept in a controlled temperature incubator at 15°C. There were 29 alive larvae for testing with 60 dead.

All adults that arrived at Lincoln were alive (Fig. 3).


Figure 2: Mana Island Flax weevil larvae a) on the day of arrival at Lincoln University; B) dead larvae prior to treatment and C) 29 alive for used in bioassays.

Figure 3: Flax weevils in pasture grass, as received.

**Larval bioassay**

To inoculate with *B. pseudobassiana*, conidia of the fungus was produced by growing the originally recovered strain from Maud Island on PDA at 25$^{o}$C. Conidia were harvested directly from the PDA cultures into 0.01% triton X100. Conidial solutions were adjusted to concentrations of 1.4 x $10^{9}$ and 1.4 x $10^{8}$ conidia/ml. Larvae were dipped in a conidial solution or 0.01 % triton X (control), then placed in tubes with 5 ml of sterile 3-4 month old potting mix (Fig. 4). An air hole was made in the centre of the plastic lid. All tubes were placed on a tray with a moistened paper towel on the tray in a large plastic bag and incubated at 25°C. Larvae were checked daily for mortality. Dead insects had a moist paper towel added into the tube to aid any fungal sporulation.

Figure 4: Tubes used to contain treated flax weevil larvae

**Adult bioassay**

Each weevil adult was placed in a deep Petri dish on filter paper with a lid with mesh holes (Fig. 5). The treatments were of *Beauveria pseudobassiana* at conidial concentrations of $10^9$ conidia/ml through to $10^5$ conidia/ml with 2 controls; water and 0.01% triton X. Each weevil was sprayed with 300 µl of a treatment (Fig. 5B). There were 10 adults treated in each treatment making a total of 70 weevils used in this bioassay. The weevils were kept on trays with moist paper towels, enclosed in plastic bags, in a control incubator at 25°C, 18/hr light-8 hr/dark.

Eleven weevils were not used which have been kept in the CT rooms. One has since died from drowning and has been given to John Marris (Entomology Collection, Lincoln University).

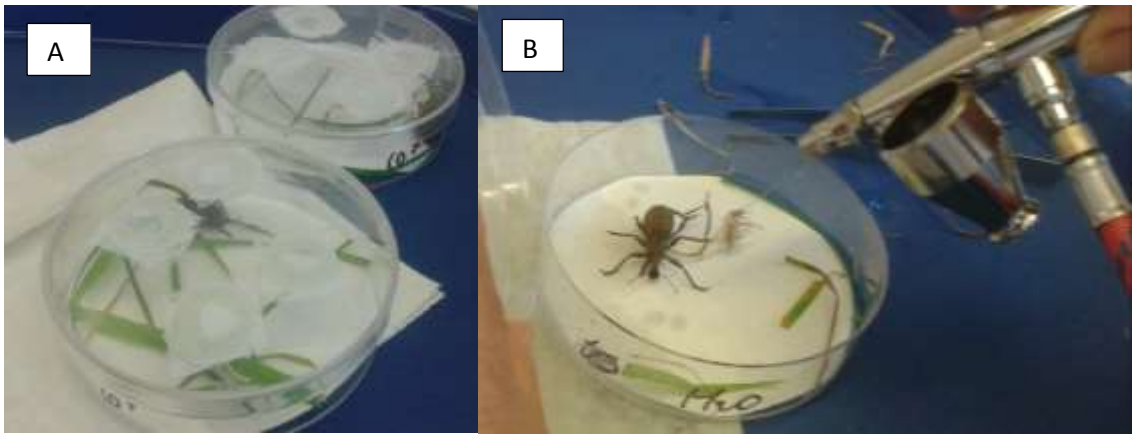


Figure 5: A; Flax weevil in Petri dish with mesh covered holes on lids with a small amount of grass that came with the insects. B; spraying the weevil

Flax collected from the Lincoln University gardens was used as food after a few days on just the grass as no droppings on the filter paper suggested none were eating. The flax leaves are changed every 2-3 days and 500 µl of sterile water was added to filter paper (Fig 6). The filter paper was changed as needed.


Figure 6: Petri dish indicating stage needed cleaning and changing food.

**Soil**

*Larval soil*

Soil dilutions of the material the larvae arrived in was processed for presence of *B. pseudobassiana*. Five grams of soil was added to 45 ml of sterile 0.01% triton x ($10^{-1}$ dilution) and agitated @ 300 rpm for 30 minutes. Serial dilutions of $10^{-2}$ and $10^{-3}$ were each plated with 2 reps of all making a total of 6 plates.

*Soil samples*

Twenty soil samples were received. Five g of each sample was diluted in 45 ml of 0.01% Triton X-100 and shaken at 300-400 rpm for 30 mins (= first dilution, $10^{-1}$). Serial dilutions of $10^{-2}$ and $10^{-3}$ were prepared with 100 µl of each dilution spread over the surface of an agar plate and incubated in the dark at 25°C. Two replicates of each serial dilutions were plated. Each soil sample had a total of 24 plates over 4 dilutions. The growth medium used was *Beauveria* selective medium (BSM) consisting of PDA (Difco, NJ) amended with 1.5% (3.5ml/L) of Streptomycin sulphate (Sigma) and 10% (3.33 ml/L) of Tetracycline hydrochloride (Sigma) and 1.56 (8ml/L) of Cyclohexamide.

Plates were checked and counted for *Beauveria* CFU's once sporulation occurred, approximately 7-10 days after inoculation. Identification of *Beauveria* was by morphology once sporulation occurred, using the distinct 'cotton ball' formation of spores and rachis.

## Results

### Larval soil samples

The soil that larvae arrived in was tested for the density of *Beauveria*. *Beauveria* was found at a density of approximately 7.6 x $10^4$ colony forming units/gm of soil and plant material. This is quite high for naturally occurring *Beauveria* from environmental samples.

### Larval bioassay

The bioassay of *B. pseudobassiana* against larvae of the flax weevil was compromised by the high background infection of the field collected larvae. Only 29 larvae were alive at the time of testing. While the majority of larvae eventually died of *Beauveria* infection (only one control larvae remained at the end of the seven day bioassay), mortality was more rapid where *B. pseudobassiana* has been applied (Figure 7). Almost every dead larvae eventually showed external symptoms of *Beauveria* infection, including control (triton x100 treated) larvae (Fig. 8).

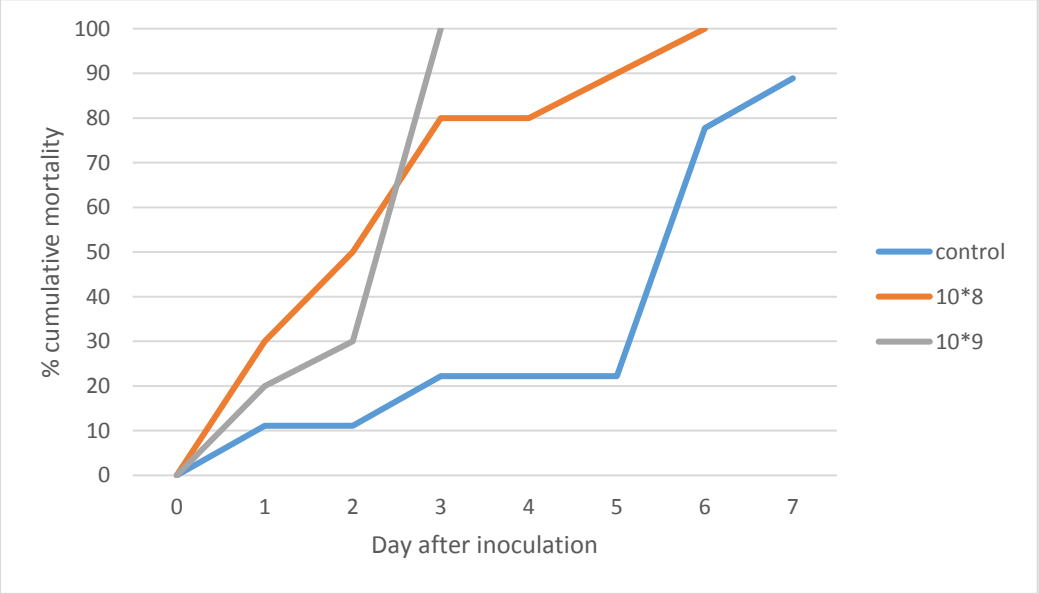


Figure 7: Mortality of larvae after treatment with two inoculum rates of *Beauveria pseudobassiana*

Figure 8: Larvae treated with 0.01% triton X all died showing presence of *B. pseudobassiana*

The last live larva was given to John Marris for the BPRC/LU entomology collection on 2/7/17.

**Adults**

One adult died in the bioassay of *B. pseudobassiana*, in the treatment sprayed with a conidial concentration of $10^8$ conidia/ml after 3 days (Fig. 9). It is likely this indiviudal was already infected with *Beauveria* as the majority of mortality due to spraying with *B. pseudobassiana* did not occur until after 15 days. By day 20, 90% of adults had died in the highest doses (Figure 10), but only 10-20% in the controls. Evidence of *Beauveria* infection was seen on most cadavers from the $10^9$ conidia/ml treatment, and a few other dead also supported *Beauveria* sporulation

Figure 9: Adult weevil infected with *Beauveria* growing out of exoskeleton.

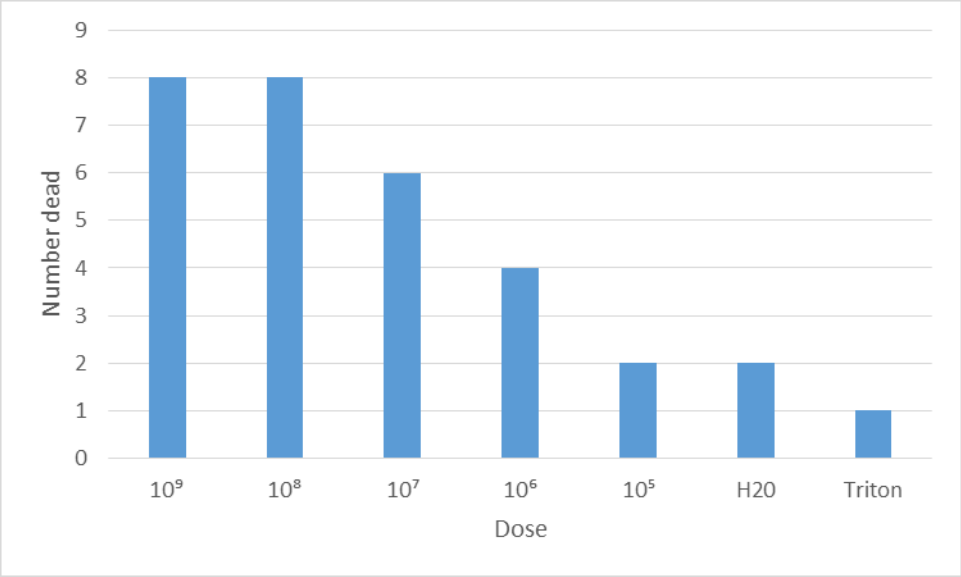


Figure 10: Mortality of adult flax weevil 20 days after inoculation with *Beauveria pseudobassiana*. Dose is in conidia/ml of spray, controls were water and 0.01% triton X-100. (n=10).

**Recovery of *Beauveria* from soil**

In addition to the soil and plant material that the larvae came in, another twenty soil samples were supplied. *Beauveria* colonies were recovered from only six soils, with high numbers in all but B04 (Table 1). Naturally occurring cfu's of over $10^4$ /gm of soil are generally consider to be able to exert control on susceptible insects. Two of the samples had over $10^4$ cfu/gm and the original larval containing material was around 7.25 x $10^4$ cfu/gm and there was significant infection of the larvae.

Table 1. Recovery of *Beauveria* colony-forming units on semi-selective medium from Mana Island soil samples.

| Soil samples number | *Beauveria* cfu/gm |
|---|---|
| Mana A5 | 6,000 |
| Mana A6 | 11,000 |
| Mana A9 | 5,000 |
| Mana A10 | 18,000 |
| Mana BO4 | 1,000 |
| Larval Flax weevil soils | 7,250 |

## Discussion

This report covers research aimed at determining the presence of *Beauveria* on Mana Island and susceptibility of flax weevil larvae and adults to the previously recovered strain of *B pseudobassiana*. The working hypothesis was that *B. pseudobassiana* was not present on Mana Island, and this lack of a control agent could have led to the outbreak levels of the weevil. However, significant levels of *Beauveria* were recovered from some of the soil/plant material, as well as a major cause of mortality amongst the larvae collected from Mana Island. Weevils were collected from a single site on the island where high levels of *Beauveria* were found in soil (samples A). The site which was beyond the leading edge of weevil infestation, samples B, had little *Beauveria* in the soil. This suggests the weevils introduced to the island had *Beauveria* in the population.

It is difficult to reconcile with the high populations of the weevil at one site on the island and the high infection levels of the collected flax weevils. The bioassays demonstrated that larvae are highly susceptible to the fungus, but adults are more resistant. This suggests that if the fungus is present in the soil and plant root zones, the larvae would have high mortality, but movement to new areas through the adults may be slow. It will be important to check other areas of Mana Island for presence of the fungus. It raises the question how long and how isolated is this occurrence of *Beauveria*? If it was a recent introduction, this may be the start of a population collapse, or it could be an isolated occurrence and the fungus is not spreading very quickly. Conversely, the fungus may not be a factor in the population density on other islands, although given the level of infection found this is unlikely.

### Future research suggestions

It is now important to understand if the *Beauveria* is widespread on Mana Island and if it is exerting any control on the weevils. Our soil results suggest that where the larvae were collected was particularly high in *Beauveria* in the soil, but other samples had none. We are still waiting on soil and larvae collections from Maud Island, which will give a background level of *Beauveria* on an island with a low population density for comparison.

### Acknowledgements

We thank Dr Colin Miskelly and Friends of mana Island for collection of samples. This project was funded by Friends of Mana Island, gratefully acknowledged.