The Flax Weevils of Mana Island

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William David Brockelsby

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Abstract

In 2004 and 2006 flightless endemic flax weevils (*Anagotus fairburni*) were deliberately translocated to Mana Island, New Zealand, as part of the island's restoration plan. The flax weevil population grew exponentially and by 2018 the weevils were dispersing and had destroyed large areas of their host plant, flax (*Phormium* spp.), on which threatened bird and lizard species on Mana Island rely for both habitat and food. Little is documented about flax weevil ecology, so it wasn't immediately obvious why the flax weevil population was growing beyond the ability of the flax to sustain them.

Investigations by Colin Miskelly into possible natural enemies of the flax weevil led to the discovery of a strain of the entomopathogenic fungi *Beauveria pseudobassiana*, naturally occurring on the island that was highly virulent to flax weevil larvae. This native pathogen was prevalent at the flax weevil release site, but uncommon further down the island, which led to the hypothesis that the flax weevil was able to disperse ahead of its natural enemy on the island. We decided to investigate the feasibility of deliberately spreading *B. pseudobassiana* ahead of the flax weevils, to protect flax plants from collapse and death.

In August 2018 a trial site was established in the centre of the island well ahead of known flax weevil sites and 80 mature flax plants were individually marked. A fauna monitoring programme was put in place to understand the flax plant animal community before and after any experimental changes at the site. An irrigation system was designed and installed to deliver supplementary water to some flax plants, to test whether access to water during the crucial dry summer months was a critical variable for survival of flax plants. In March 2020 marked flax plants were treated with a solution of the virulent *B. pseudobassiana* strain, or a control mixture, both delivered via water the base of the plant. These plants were then stressed by the deliberate introduction of 1600 flax weevils.

Numerous novel observations on flax weevil ecology and behaviour were made throughout the duration of the flax plant trial. Flax weevils were found to go through 6 to 7 instars as larvae in the soil, were mobile at the trial site as adults, and could walk long distances from their flax release sites over time. An alternative feeding plant was observed for adult weevils, which were found to feed on the leaves of *Muehlenbeckia complexa*.

The trial release of *B. pseudobassiana* failed to protect most flax plants at the experimental site, with 35 out of 43 marked plants now (2022) in a state of heavy collapse or death. We attribute this failure to an insufficient dosage rate, although it is possible that release timing or environmental conditions might also have led to an unsuccessful release. Possible future ecological scenarios for flax weevil on Mana Island are discussed.

Acknowledgements

Inevitably in projects as large as this there are many people involved in making it happen, and try as I might, I won't be able to name everyone. I had many interesting conversations with smart and dedicated Friends of Mana Island volunteers, and without their labour and company this would have been an impossible task. Dale Shirtliff was a real champion not only with the logistics, but with the championing the project in his executive work at FOMI. Andrea Wilson's inordinate fondness for flax weevils must be mentioned, her steady hands during the flax weevil marking and sharp eyes at spotting weevils was a huge help during some of the trickier Mana Island visits.

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Brooke Singer has a sharp eye for detail and draws a good map, both useful qualities to have around.

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Chapter 1 Introduction

The story of this thesis begins with the translocation of the flax weevil, *Anagotus fairburni* (Brookes, 1932) (Curculionidae: Aterpini) (Fig 1.) to Te Mana o Kupe ki Aotearoa (Mana Island), an action recommended as part of the Mana Island Restoration Plan (Miskelly, 1999). The translocated weevils were gathered from Te Pākeka/Maud Island and were moved in two batches, the first in 2004 (80 adult weevils) and the second in 2006 (a further 70 adult weevils). In both 2004 and 2006, weevils were released at the same location near the southern end of Mana Island (Department of Conservation, 2006). This was the third known translocation of flax weevils, which had previously been translocated in Fiordland (from Wairaki Island to Breaksea Island in 1991) and the Marlborough Sounds (from Te Pākeka/Maud Island to Titi Island in 2001).

Figure 1. A flax weevil (*Anagotus fairburni*) on flax, Pororangi/Mt Stokes, Picton, 2018. Photo: W. Brockelsby CC-BY.

Mana Island had ideal habitat for the weevils; less than 10 years later they had proliferated beyond what had been seen before in any other translocated population, and greatly outnumbered their original source population on Te Pākeka/Maud Island. Mature flax plants near the weevil release site on Mana Island were collapsing by 2013, and the site changed from a dense flax sward to rank grass with scattered patches of dead flax leaves (Miskelly, 2013). Tens of thousands of weevils were estimated to be on the island and had spread north of the original release site, reaching the centre of the island by 2018. While this would be considered a successful translocation under many measures of translocation success, concerns were raised about the rapid spread of the weevil and its possible ecological impact on other species that were reliant or partially reliant on the flax plants. Native birds on Mana Island such as tui, *Prosthemadera novaeseelandiae* (Gmelin, 1788), korimako *Anthornis melanura* (Sparrman, 1786) and yellow-crowned kakariki, *Cyanoramphus auriceps* (Kuhl, 1820) are all consumers of the abundant but irregular resource created by flax mast flowering, consuming its nectar or seeds (Wehi, Biological flora of New Zealand 10. Phormium tenax, harakeke, New Zealand flax, 2007). Raukawa gecko, *Woodworthia maculata* (Gray, 1845) are avid consumers of flax nectar. Even the Cook Strait giant wētā, *Deinicrida rugosa* Buller, 1871 can be observed climbing flax stalks, perched precariously, consuming the resources that the flax flowers provide. Of greatest concern was the goldstripe gecko, *Woodworthia chrysosiretica* (Robb, 1980), as a significant proportion of the national population is currently found on Mana Island (Flannagan, 2000). This gecko on Mana Island is primarily found in flax, seeming to prefer it over other refugia though it is not necessarily entirely reliant on flax (T. Bell, pers. comm., 2020). There is a concern that widespread loss of flax habitat on Mana Island might seriously impact the species abundance of goldstripe geckos on the island, and possibly lead to a deterioration in their threat status.

Very little is documented about the ecology of the flax weevil. Populations exist only on predator free islands or in alpine areas, which can be remote and difficult to access for research purposes. What little is known, comes from captive weevils raised in the 1930's when the species was first discovered (Gourlay, 1931). When the weevil population on Mana Island began to expand, there were very few documented accounts or published information to explain why this unusually rapid population growth rate may be occurring. Follow up monitoring of other translocated populations had not seen similar dispersal or rate of population growth (Keall, Gruber, & Miller, 2007; Anderson, 2017; C. Miskelly, pers. comm., Oct 2020).

Various hypotheses were suggested as to the cause of the weevil's rapidly increasing population; one was that the hybrid flax on Mana Island had lost resistance to weevil browse, and a trial was set-up to investigate this on the island, comparing flaxes grown from seed sourced from Te Pākeka/Maud Island (i.e., the same source as the flax weevils) compared to locally grown flaxes. This trial is still in progress as of the time of writing, although preliminary results show no apparent effect of plant provenance (C. Miskelly, pers. comm., March 2020).

Another hypothesis was that the large numbers of flax weevil larvae (the most damaging life stage) browsing on the root structures and burrowing into the ground are potentially limiting the plant's ability to gain access to water. There was backed up by observational evidence that flax plants in gullies appeared to survive better, perhaps by having better access to water or by reduced larvae survival via periodic drowning. This formed the first major research question of the thesis:

- Does variable access to water or differing water levels affect flax plant survival over time when under sustained weevil browse?

Our final hypothesis was that the weevils were undergoing a form of ecological release (otherwise known as enemy release), where an important predator or limiting factor was left behind on Te Pākeka/Maud Island after the translocation of the weevils to Mana Island. At the suggestion of an overseas weevil expert Dr Richard Bull, Colin Miskelly (Museum of New Zealand Te Papa Tongarewa, Wellington) began to investigate the possibility that for weevils, pathogens in the soil were key limiting factors. To investigate this, he visited the original source population on Te Pākeka/Maud Island to search for diseased weevils. He found one sickly larva which was sent to Lincoln University, where it subsequently died of a fungal infection. This fungus was identified as a previously unknown strain of *Beauveria pseudobassiana*, an entomopathogenic fungus.

A follow up investigation was organised on Mana Island to ascertain whether the *Beauveria* fungus had been inadvertently transferred with the translocation of the adult weevils. Larvae, adults, and soil were sampled on Mana Island and, surprisingly, these samples confirmed that the fungus was present in high levels on the island near the release site and that it was undetectable further up the island. The fungal strain was also found to be highly lethal to larvae, with adults only slightly more resistant (Glare, 2017) (Fig. 2).

Figure 2. A deceased flax weevil (*Anagotus fairburni*) infected with the fungus *Beauveria pseudobassiana,* Mana Island, 2019. Photo: W. Brockelsby CC-BY.

After the discovery of the *Beauveria* fungus being on the island already, it was hypothesised that adult weevils were asynchronously escaping the fungus by spreading to pristine areas of flax (with low or no fungal spores present) and were reproducing in large numbers before the fungus could build up in the soil and become a limiting factor on the next generation of larvae. The dominance of flax plants on the plateau of Mana Island and therefore the huge amount of food and habitat available led credence to this theory. It was decided to set up a trial to see whether this asynchrony could be addressed by manually applying *Beauveria pseudobassiana* fungal spores at the edge of the advancing weevil population as a proactive management strategy. This trial forms the second major research topic of this thesis:

- Can the manual application of spore of the fungus *Beauveria pseudobassiana* limit the population growth of flax weevils below a crucial threshold, enabling the survival of flax plants that would otherwise collapse?

Alongside these primary research questions, I aimed to fill in some important gaps on the ecology of the weevil itself and the ecology of animal communities associated with flax, in particular the invertebrates and herpetofauna. The secondary research questions were:

- Basic weevil ecology, how much do they move around in various flax plant patches? How many instars do they go through in development to adulthood?
- What impact do the weevils have on the Mana Island flax animal community?

Chapter 2 Literature Review

Weevil biology

The Flax Weevil (*Anagotus fairburni*) is a large flightless weevil, which is dependent on flax plants (*Phormium* spp) for its survival during all stages of its lifecycle. Measuring just over 2 cm long, it is one of the many extant members of the weevil "megafauna" of New Zealand.

Flax weevils first became known to western science when found by a member of Ernest (Ernie) Fairburn's party on the northern side of Rangitoto ki te Tonga/D'Urville island in 1931 (Gourlay, 1931). Originally described as *Phaeophanus fairburni*, this beetle was found on a track by Miss R. F. B. Huffam of Richmond, Nelson in January 1931. Two days later Ernie Fairburn (an amateur entomologist from Whangarei) figured out the host association, finding the weevil on a flax plant, and together with Edwin Gourlay they collected several specimens which formed the basis for the original description (Brookes, 1932).

Gourlay's original account of the discovery of the weevil in 1931 is also one of the only detailed published records of the life history the weevil. In his work Gourlay (1931) describes the "injurious effects" of weevil on the flax of Rangitoto ki te Tonga/D'Urville island and documents his observations of the 100+ captive weevils he took back to the Cawthorn Institute in Nelson. He describes their nocturnal feeding habits, gives a description of the egg (Fig. 3), larvae (Fig. 4), pupae, and includes observations on their preferred oviposition sites. Most importantly for the topic of this thesis, Gourlay (1931) describes an experiment designed to measure the reproductive potential of the weevil. He placed 100 weevils on a fresh flax plant in February and left them contained there for twenty-four hours. After removing the weevils, he recorded 546 eggs, all of which were laid during this time. Unfortunately, Gourlay (1931) did this without sexing the weevils, and so the true female fecundity is not knowable, but if one assumes a rough 1 to 1 ratio of male to females, this equals to over 10 eggs per female, all of which were laid in a very short window of time.

Figure 3. A flax weevil (*Anagotus fairburni*) egg photographed in October 2019, Mana Island. Photo: C. Stephens CC-BY.

Usefully, Gourlay (1931) also followed the development of these eggs, describing a hatch rate of 86% and average duration of egg stage of 21-25 days, with most eggs hatching in late March. He also states that "egg laying ceased in March", working backwards from this we can assume that peak egg production happened in mid-February. Finally, Gourlay (1931) wrote that his study weevils began to die in April and by 11th July "only a few insects" remained. This is an interesting point as it seems contrary to observations of other large-bodied weevils such as *Lyperobius huttoni* Pascoe, 1876, *Karocolens pittospori* Kuschel, 1987*, Hadramphus spinipennis* Broun, 1911*, Hadramphus tuberculatus* (Pascoe, 1877) and *Anagotus stephenensis* Kuschel, 1982, most of which describe adult life cycles of at least a year, and in some circumstances up to several years (Bull, 1967; Bennett, 1987; Schops, 1998; Fountain, et al., 2016; M. Anderson, pers. comm, 2019). Gourlay makes no mention of how old he believed that the weevils he collected were.

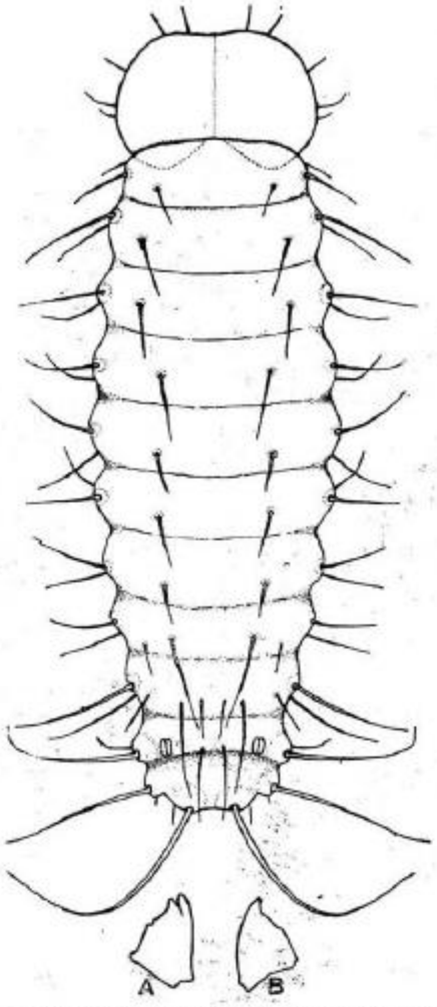


FIG. 6.—First-instar larvæ, with large dorsal spiracles on last segment. A, left mandible, ventral view; B, left mandible, dorsal view. Original.

Figure 4. Gourlay's original drawing of the first instar larva of a flax weevil (*Anagotus fairburni*). Reproduced from Gourlay (1931).

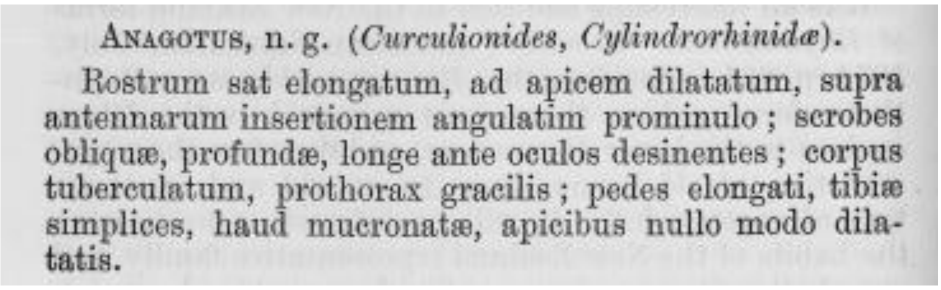
After Gourlay (1931), there is very little published about the weevil lifecycle, work seems to shift to discovering new localities. A wave of exploration took place across Aotearoa, with thorough searches of predator-free islands and alpine zones (Table 1, Fig. 6).

During this time flax weevils and other large-bodied weevils began to be recorded all over the country. After one of these trips, a report was published describing the beetle fauna of the Poor Knights Islands in which many genera of weevils in the Aterpini were synonymised by Guillermo Kuschel (Kuschel, 1982). Kuschel examined the flax weevil and credited Sharp with providing the first proper description of the generic characters under the genus name *Anagotus*, written in the Latin which was the standard at the time (Fig. 5) (Sharp, 1882).

Table 1. A non-definitive list of localities recorded by the NZAC, iNaturalist, and published literature, designed to show the broad geographic spread of the flax weevil (*Anagotus fairburni*) in New Zealand.

OBSERVER	**COLLECTION DATE**	**LOCALITY**
E.S. Gourlay	13/01/1931	Rangitoto ke te Tonga D'Urville Island, Marlborough Sounds
no collector given	15/12/1960	Te Hauturu-o-Toi Little Barrier Island, Hauraki Gulf
P.M. Johns	11/12/1963	Moekawa South-West Island, Three Kings Islands
J. Campbell	7/08/1965	Kuru Pongi Trio Islands, Marlborough Sounds
T.J. Smit	13/10/1967	Francis Creek, Tararua Ranges
G. Kuschel	14/11/1968	Taukihepa Big South Cape Island, Titi Islands
J.I. Townsend	28/05/1969	Pororangi Mt Stokes, Picton
J.I. Townsend	14/10/1969	Dun Mountain, Mount Richmond Forest Park
G. Kuschel	1/11/1970	Great Island, Three Kings Islands[1]
J.G.R. McBurney	1/01/1971	Takapourewa Stephens Island, Marlborough Sounds
G.W. Ramsay	13/11/1972	Ruamahuanui Island, Ruamaahu Alderman Islands
R.C. Craw	29/11/1972	Taratahi Mt Holdsworth, Tararua Ranges
P.R. Notman	3/09/1980	Te Pākeka/Maud Island, Marlborough Sounds
G. Kuschel	1/11/1980	Tawhiti Rahi, Poor Knight Islands
J.S. Dugdale	11/11/1981	Aorangi Island, Poor Knight Islands
R.C. Craw	2/12/1984	Dundas Hut, Tararua Ranges
C.M. Miskelly	15/11/2016	Seal Islets, Dusky Sound, Fiordland
C.M. Miskelly	17/11/2016	Anchor Island Harbour Islet, Dusky Sound, Fiordland
C.M. Miskelly	19/11/2016	Shag Islands, Dusky Sound, Fiordland
C.M. Miskelly	22/11/2017	Round Island, Preservation Inlet, Fiordland
C.M. Miskelly	24/11/2017	Small Craft Harbour Island, Chalky Inlet, Fiordland
C.M Miskelly	28/02/2020	Coal Island, Preservation Inlet, Fiordland

[1] May be another unnamed similar *Anagotus*, as this specimen was collected from *Cyperus ustulatus*.



ANAGOTUS, n. g. (*Curculionides, Cylindrorhinidæ*).

Rostrum sat elongatum, ad apicem dilatatum, supra antennarum insertionem angulatim prominulo; scrobes obliquæ, profundæ, longe ante oculos desinentes; corpus tuberculatum, prothorax gracilis; pedes elongati, tibiæ simplices, haud mucronatæ, apicibus nullo modo dilatatis.

Figure 5. The original description of the genus *Anagotus*, as written in Latin by Sharp (1882).

Flax weevils are no longer considered to be present on Rangitoto ki te Tonga/D'Urville (the type locality). Kiore, mice, stoats, hedgehogs, pigs, cats, dogs and weka are all present on Rangitoto ki te Tonga/D'Urville, which may explain why the flax weevils persist in undetectable numbers or are potentially extinct on the island (M. Anderson, pers. comm, March 2021). In more recent work it has been shown that the weevil is relatively well distributed (though cryptic to find) on small rat free islets scattered throughout Fiordland (Miskelly, Tennyson, & Bishop, 2018).

This wide geographic spread across rat free islands and alpine zones (Fig. 6), and evidence from work on predator free islands has led to the widely held belief that flax weevils are unable to coexist with rats (Towns, 2009; Gibbs, 2009). There is circumstantial evidence pointing to rats (*Rattus sp.*) being the drivers of this relict distribution, with one of the more infamous examples being the extinction of the flax weevil and the knobbled weevil (*Hadramphus stilbocarpae*) from Taukihepa/Big South Cape Island after the irruption of ship rats on the island in 1967 (Bell, 1978). This event is well known in ecological circles due to the extinctions of the greater short-tailed bat *Mystacina robusta* Dwyer, 1962, bush wren *Xenicus longipes* (Gmelin, 1789) and South Island snipe *Coenocorypha iredalei* Rothschild, 1921 and the dramatic rescue of the South Island saddleback *Philesturnus carunculatus* (Gmelin, 1789), but the total loss of large-bodied terrestrial invertebrates on the island is often overlooked (Miskelly, Tennyson, & Bishop, 2018).

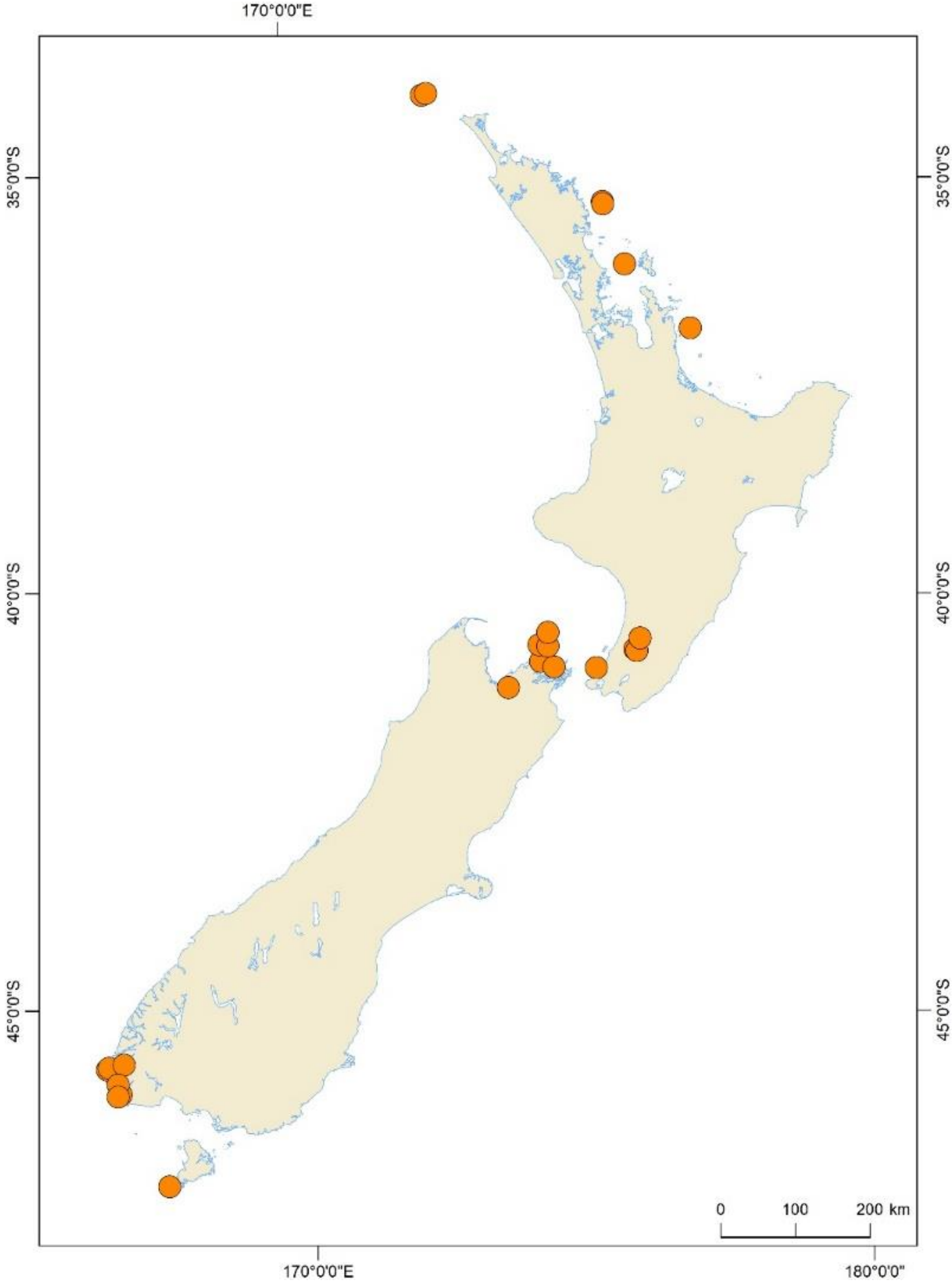


Figure 6. Known localities for the flax weevil (*Anagotus fairburni*). Note the large gaps in the weevil's distribution with hotspots in the southern South Island, Cook Strait region and offshore islands in northern North Island. Map: G. Moore (Department of Conservation) CC-BY.

Further evidence for the theory that these flightless large-bodied weevils cannot coexist with rats comes from the world of paleoecology, where specimens of the related weevil *Anagotus stephenensis* were abundant in late Quaternary laughing owl *Ninox albifacies* (G. R. Gray, 1845) deposits located in North Canterbury (Worthy & Holdaway, 1996). At present, *Anagotus stephenensis* is considered 'Nationally Critical' by the NZ Department of Conservation, having only ever been found naturally on Takapourewa/Stephens Island. This subfossil pattern of range restriction and apparent population extinction is repeated across the country with other *Anagotus* species (Worthy, 1984; Clarkson, 1988; Watts C. H., 2019).

It is also worth noting that there is currently no known name for the flax weevil in Te Reo Maori, nor is there a currently known name for the distinctive damage that it causes to *Phormium*. It is therefore quite possible that the kiore *Rattus exulans* (Peale, 1848) had already had a severe impact on this species prior to the arrival of European rat species (C. Miskelly, pers. comm, September 2020). A mouse incursion in 2013 on Te Pākeka/Maud Island caused a major drop on flax weevil numbers on the island. The mice were eradicated on the island in 2016, but flax weevils still haven't recovered to numbers seen prior to the incursion (M. Anderson, pers. comm, June 2022). The impact of mice on ground dwelling invertebrates has also been reported from ecosanctuaries in New Zealand (Watts, et al., 2022).

Due to the flax weevil's disjunct distribution and its susceptibility to rat predation, this species is one of very few invertebrates fully protected under an amendment made to the NZ Wildlife Act in 1980 (Miskelly, 2014), which gives them the highest species level protection currently available under New Zealand law. The weevil is 'At Risk' with the qualifier of 'relict' under the New Zealand Threat Classification System (Leschen R. A., 2012).

History of weevil translocations

Species translocations are a commonly employed method in restoration ecology and conservation biology. There are plenty of examples in the literature of species being moved from one site to another, either to improve the threat status of the species in question, or to restore an important ecological function to an area where a species formerly occurred. Translocations are traditionally considered high risk, high effort endeavours, often with a low rate of success (Fischer, 2000), but despite this have achieved some remarkable successes for the conservation of species in New Zealand (Butler, 1992; Stringer, 2014). In New Zealand, translocations overwhelmingly relate to birds, with over 1100 conservation translocations recorded up to 2012 (Miskelly & Powlesland, 2013). This contrasts to just 183 non-avian translocations (Sherley, 2010). Out of these 183 non-avian translocations, 85 were invertebrates, the vast majority of which were members of the

Mollusca (44) and Orthoptera (27). Just six translocations in this publication were large-bodied weevils, half of which involved the flax weevil (Sherley, 2010). Since the publication of the Sherley review, another species of *Anagotus* (*Anagotus stephenensis*) has been translocated, and another translocation of the flax weevil has taken place.

The first recorded weevil translocation in New Zealand was to Breaksea Island in March 1991 after the eradication of Norway rats three years earlier. This was recommended as part of the restoration plan for the island after early research mapping the density of invertebrates in the Breaksea Sound found a strong relationship between the presence of introduced mammals and low numbers of large-bodied invertebrates when compared to similar islands without mammal presence (Bremner, The density of indigenous invertebrates on three islands in Breaksea Sound, Fiordland, in relation to the distribution of introduced mammals, 1984). The Breaksea Island translocation involved the moving of two different species, the flax weevil and knobbled weevil (*Hadramphus stilbocarpae*). Twenty flax weevils were collected from a source population on Wairaki Island in Fiordland, and 40 knobbled weevils were collected from a small island stack named “Outer Gilbert 3” (OG3). All the weevils were released onto Breaksea Island near the hut on the northern end of the island. The translocation appears to have worked with flax weevil sign last seen on the northeast side of the island as recently as 2019 (C. Miskelly Pers. Comm. Oct 2020).

The next weevil translocation was to Titi Island in 2001, from which Norway rats had been eradicated in the mid-70s. This translocation involved just the flax weevil; 82 individuals were taken from nearby Te Pākeka/Maud Island. The Titi Island translocation was considered successful, with weevil feeding signs found across the island in 2007 during follow up tuatara monitoring (Keall, Gruber, & Miller, 2007). Another small translocation took place in 2006 in Northland, where *Anagotus turbotti* (Spiller, 1942) was moved from Muriwhenua Island in the Hen and Chickens Island group a small distance away to Lady Alice Island (also in the Hen and Chickens Island group). Thirty weevils were moved, and the translocation outcome is currently not known, though it is thought to have failed (Bellis, Bourke, Williams, & Dalrymple, 2019).

The next flax weevil translocation was the largest and forms the focus for this thesis. The weevils were taken from a source population on Te Pākeka/Maud Island and translocation was done in two waves, the first wave was 80 weevils in 2004 and the second 70 weevils in 2006. Both times weevils were released at the same location near the southern end of Mana Island (Department of Conservation, 2006; L. Adams, pers. comms, Oct 2020). At a similar time in 2006, forty-one Wellington speargrass weevils (*Lyperobius huttoni)* were taken from the Wellington south coast and transferred to Mana Island (Sherley, 2010). One of the more urgent translocations involved the movement of thirty critically endangered ngaio weevils (*Anagotus stephenensis*) from Takapourewa/Stephens Island to Te Kakaho/Outer Chetwode Island in 2012 (Anderson, 2012). Large numbers of this weevil have not yet been reported in follow-up trips, but feeding sign indicates they are still present (Anderson, 2016).

The latest translocation was of flax weevils taken off Te Pākeka/Maud Island and moved to the newly rat-free Oruawairua/Blumine island. These weevils were released at two separate places on the island and follow-up monitoring undertaken by Mark Anderson in 2017 showed that the weevils were numerous at the eastern end of the island. This was followed up by citizen science group Untouched World Blumine Island Whanau in 2018, who found them at the north-western side of the island (Anderson, 2017; M. Anderson, pers. comm., April 2021). All these translocations were either driven by ecological restoration purposes or species conservation outcomes or both. A summary of the translocations is given in Table 2 and Fig. 7.

Table 2. A summary of weevil translocations undertaken in New Zealand as of March 2021.

Weevil species	**Source Population**	**Destination**	**Reference**	**Date**	**Number**
A. fairburni	Wairaki Island	Breaksea Island	(Thomas, 1992)	1991	20
H. stilbocarpae	Outer Gilbert 3	Breaksea Island	(Thomas, 1992)	1991	40
A. fairburni	Te Pākeka/Maud Island	Titi Island	(Sherley, 2010)	2001	82
A. fairburni	Te Pākeka/Maud Island	Mana Island	(Sherley, 2010)	2004	80
A. fairburni	Te Pākeka/Maud Island	Mana Island	(Sherley, 2010)	2006	70
L. huttoni	Wellington South Coast	Mana Island	(Sherley, 2010)	2006/7	41
A. stephenensis	Takapourewa/Stephens Island	Te Kakaho/Outer Chetwode	(Anderson, 2012)	2012	30
A. fairburni	Te Pākeka/Maud Island	Blumine Island	(Anderson, 2017)	2012	74

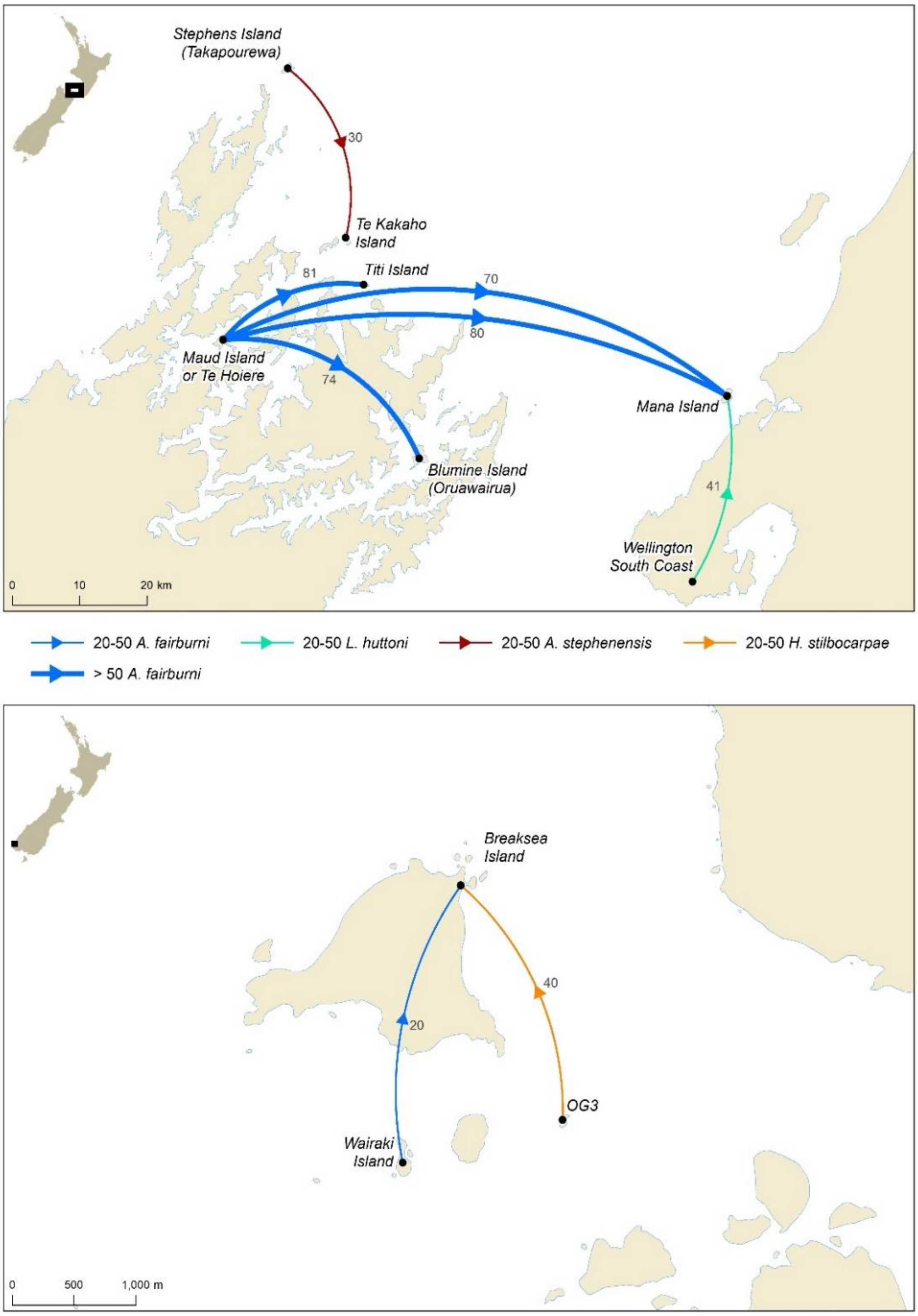


Figure 7. Maps of some of the known weevil translocations performed to date in the New Zealand region. Figure 7a shows the Cook Strait region of New Zealand where the bulk of translocations have taken place, and Figure 7b shows the first recorded New Zealand weevil translocation, in Fiordland. Map by G. Moore (NZ Department of Conservation) CC-BY.

Enemy release hypothesis

One of the hypotheses put forward to explain the flax weevil population increase was based around the idea that a natural limiting factor such as a predator, parasite, or competitor had been left behind after the translocation had taken place. Known as the “enemy release hypothesis” (Keane & Crawley, 2002), this hypothesis is typically used to explain the rapid proliferation of introduced invasive species in new host ranges, rather than extirpated species that have been reintroduced to their presumed native range.

Enemy release hypothesis has its roots in invasive plant ecology, but there are many examples from the animal kingdom that use this theory to explain observed population dynamics. From North America there is evidence that spatial enemy release was the driving dynamic of a reported western tussock moth *Orgyia vetusta* (Boisduval, 1852) outbreak in California (Maron, Harrison, & Greaves, 2001). The western tussock moth feeds on tree lupin (*Lupinus arboreus*) and the female adults are flightless which limits their dispersal ability. Existing moth populations were largely static and confined to one area over a long period and were kept in check by a range of predators and parasites. When an opportunistic outbreak of the tussock moth occurred in an area of tree lupin previously unrecorded, it allowed researchers from the University of California to perform field tests and limited in situ experimental manipulation. They found that parasitism rates were much higher in the older population and that moth parasites, and in particular flies in the family Tachnidae, were the key factor between the differing dynamics in the outbreak and source moth populations. Brown argus butterfly *Aricia agestis* (Denis & Schiffermüller, 1775) is another invertebrate which has experienced a range shift over time in primarily due to climate change (Menendez, Gonzalez-Megias, Lewis, Shaw, & Thomas, 2008). Researchers in Britain followed the argus butterflies’ northward expansion over 30 years and monitored parasite mortality across multiple populations over time. They found that populations in the newly colonised areas suffered lower mortality than the areas with long-standing populations. Interestingly this occurred despite the fact many of the parasitoid species associated with the species existed in the newly colonised areas.

A meta-analysis across 26 different taxonomic groups of animals also found similar results: parasite loads were significantly smaller in invasive populations when compared to their native populations (Torchin, 2003). It was suggested that the complex life cycle requirements of parasites might not survive founding events, that parasites may be lost during the founding process, or go through a population bottleneck which breaks the chain of transmission. There are varying degrees of evidence for enemy release being a factor in the spread of the invasive Harlequin ladybird *Harmonia axyridis*

(Pallas, 1773) in the United States as well (Firlej, Girard, Brehelin, Coderre, & Boivin, 2012; Haelewaters, et al., 2020).

While the outbreak of the flax weevil on Mana Island led us to believe that this may be an example of enemy release hypothesis, it was uncertain which taxa or guild of enemies should be focused on. The only enemies known from published literature for the *Anagotus* genus were introduced mammals and the extinct laughing owl (*Ninox albifacies*) (Worthy & Holdaway, 1996), neither of which are plausible explanations for the situation on Mana Island. It was the suggestion of a weevil expert, Richard Bull, who recommended that pathogens in the soil may be a key limiting factor in weevil populations.

Beauveria as an insect control agent

The suggestion that flax weevils may be controlled in the ground led to an initial search of their original home on Te Pākeka/Maud Island by Colin Miskelly, and the discovery of lethargic larvae found on Te Pākeka/Maud Island. A follow up search of Mana Island using similar methods found similar larvae near the release site. The cause of the sickness (and subsequent death) of the larvae was identified by Travis Glare and Jenny Brookes of the Bio Protection Research Centre (Lincoln, NZ) as a strain of the anamorphic, entomopathogenic fungus *Beauveria pseudobassiana* (Cordycipitaceae) (Glare, 2017).

Beauveria species are pathogens of invertebrates, causing a characteristic white “icing sugar” outbreak and death of the host insect. A simplified version of a typical *Beauveria* infection is summarised below in Fig. 8.

Beauveria species will attack almost all insects, as well as ticks and mites, and many species have a world-wide distribution (Mascarin & Jaronski, 2016). While *Beauveria* species host ranges can be quite broad, within each species individual strains of *Beauveria* tend to be much more selective about their hosts (Rohrlich, 2018). Traditionally, the *Beauveria* group consisted of just a couple of species with *Beauveria bassiana* and *Beauveria brongniartii* being the most common, but more recent taxonomic work greatly expanded the number of species in this group to well over twenty species, and found linkages to another fungal parasitic group the sexually reproducing *Cordyceps* (Rehner & Buckley, 2005; Rehner, et al., 2011; Bustamente, et al., 2019). The number of *Beauveria* species continues to grow, with most of the new *Beauveria* species almost impossible to distinguish from each other using just morphological techniques. As such genetic sequencing is now the primary method used to identify the various species of *Beauveria* (Bustamente, et al., 2019).

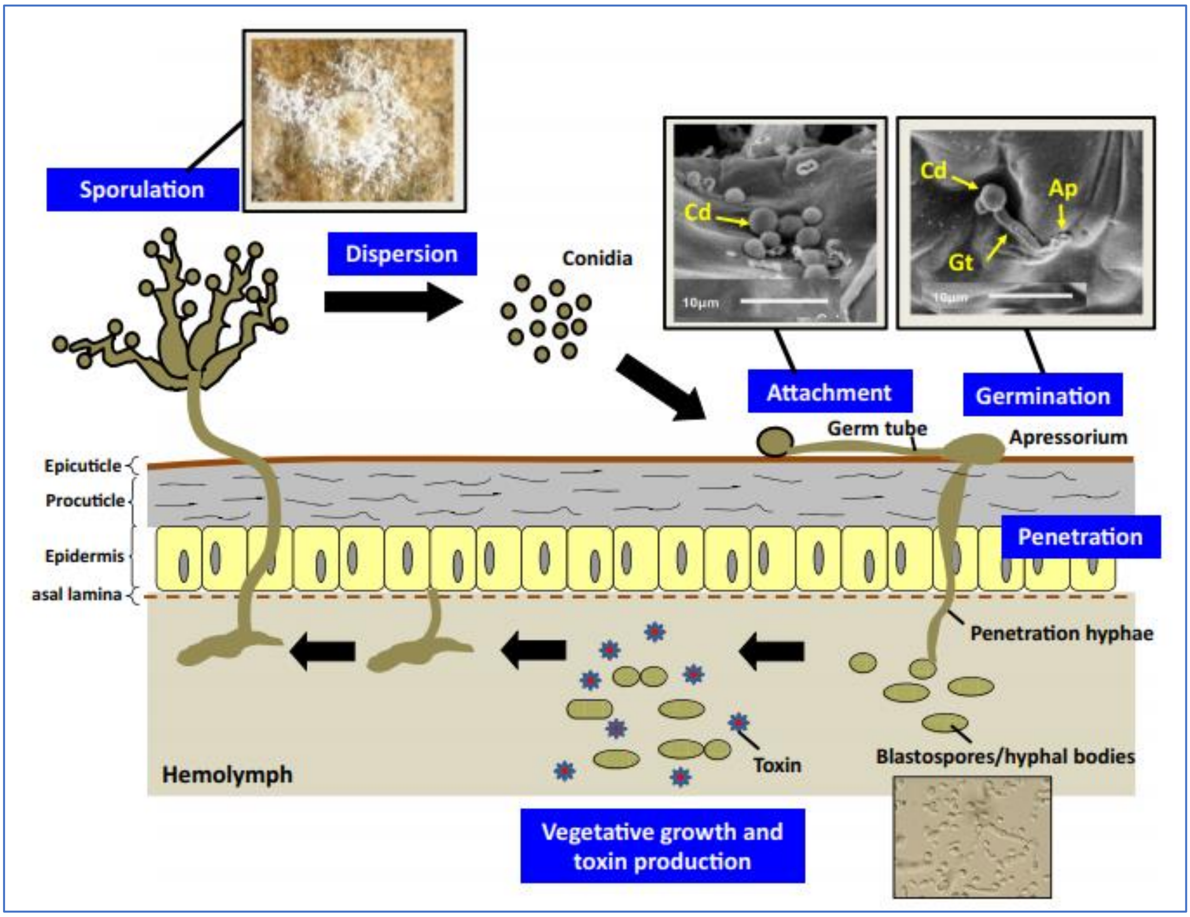


Figure 8. An overview of the basic infection cycle of an entomopathogenic fungus *Beauveria bassiana*. Reproduced from Mascarin & Jaronski (2016) with permission from SpringerNature.

Beauveria has a long and storied history in the literature as a biocontrol agent, starting not long after 1835 when entomologist Agostino Bassi discovered its effect on Italy's silkworms (Bassi, 1836). *Beauveria* is used in countless biocontrol programs, helping to control some of the world's most notorious pests such as the diamond back moth *Plutella xylostella* (Linnaeus, 1758), the silverleaf whitefly *Bemisia tabaci* (Gennadius, 1889), the Russian wheat aphid *Diuraphis noxia* (Kurdjumov, 1913), the Colorado potato beetle *Leptinotarsa decemlineata* Say, 1824, the European corn borer *Ostrinia nubilalis* (Hübner, 1796) and Western flower thrips *Frankliniella occidentalis* (Pergande, 1895) (Vandeberg, Shelton, Wilsey, & Ramos, 1998). Closer to home, *Beauveria* has also been investigated as a potential biocontrol agent against pinhole borers (Curculionidae: Platypodinae) in native New Zealand beech forests, though its efficacy did not meet the threshold needed for commercialisation (Reay, Hachet, Nelson, Brownbridge, & Glare, 2007). *Beauveria* diversity has been studied in New Zealand native forest ecosystems more broadly and was found to be an abundant pathogen of a wide array of invertebrate orders, the most common being Hymenopterans (including the introduced genus *Vespula*), the Coleoptera, and the Hemiptera (particularly the Cicadidae) (Cummings, 2009).

Beauveria-based myco-insecticides are produced via several different formulations and are available for sale under many different brand names around the world, including in New Zealand (Mascarin & Jaronski, 2016). A search performed in 2021 of the EPA managed HSNO application register database for "*Beauveria*" gave 17 unique results (Environmental Protection Authority, 2021).

Beauveria literature is full of many thousands of bioassays and "field trials" (15,700 examples via a Google Scholar search for *"Beauveria* bioassay", 14,800 for "*Beauveria* field trial*"*), confirming the lethality of *Beauveria* to a vast array of different insect species. Closer investigation show that these field trials are typically done in a glasshouse or agricultural setting in a field near a lab or University, often in a monoculture crop system. Less typical are longitudinal studies examining lots of sites with variable biotic and abiotic conditions. One such long-term study involving *Beauveria brongniartii* application across a large variety of sites took place in Switzerland (Enkerli, Widmer, & Keller, 2004). The study not only showed that the introduction of *B. brongniartii* brought the levels of the introduced pest, the chafer beetle *Melolontha melolontha* (Linnaeus, 1758) below the damage threshold for commercial crops, but also used genetic microsatellite makers to show that even 14 years later the applied *B. brongniartii* was still present in these sites (often alongside indigenous varieties), and that the chafer beetle and fungus now "coexist in a balanced host pathogen relationship" across the sites.

Even though *Beauveria* can infect a broad range of hosts of arthropods, long-term community level effects on non-target species in the field have not been widely reported, with published sources reporting little or no effects of *Beauveria* application on the community composition (Brinkman, 1999; Devotto, 2007; Garrido-Jurado, 2011; Goettel, 2021).

Chapter 3 Flax irrigation and *Beauveria* trial

Introduction

With flax weevils proliferating beyond anything originally expected after their introduction to Mana Island, it was decided by Friends of Mana Island and the Department of Conservation to setup a trial on Mana Island *in situ* to ascertain the potential use of *Beauveria pseudobassiana* as a biocontrol agent. The trial was designed to investigate if the fungus could be manually spread ahead of the flightless weevils, and whether it would then be effective at protecting flax plants from total collapse and death. The goal was not to eliminate the nationally protected flax weevil from parts of Mana Island, but to reduce the weevil population to a level where the flax plants could cope with the effects of weevil browse without collapsing. At the same time, I wanted to understand the effect of soil moisture on flax plant survival while under weevil attack, as anecdotal evidence suggested that plants in damp gullies survived better.

To run this trial, I needed a patch of undamaged flax on Mana Island which was ahead of the advancing flax weevils, could be experimentally manipulated, and was representative of the habitats across the island. A site was selected near the centre of the Mana Island (Fig. 10), where the trial was set up in August 2018.

When planning the irrigation and *Beauveria* trial, I considered it important to understand the effects of experimental treatments may have on the wider flax ecological community, especially the impact on the soil fauna. As discussed in Chapter 2, fungi in the genus *Beauveria* are not always species-specific with their hosts and can infect a large range of invertebrates, though individual strains tend to be more targeted. If the *Beauveria* treatment was to be utilised as a management option for the island, we need to be confident that it will not have a detrimental effect on flax ecological community above and beyond the negative effects of the weevils themselves. Therefore, flax community monitoring had to be set up alongside the plant health monitoring, to help evaluate the entire flax plant community's response to irrigation, deliberate introduction of *B. psuedobassiana*, and the interaction between both treatments.

Materials and Methods

Flax trial site

Mana Island is located off the coast of the city of Porirua, in the lower North Island of New Zealand (Fig. 9). The island has a long history of Māori and European occupation (Jones, Early gardening on Mana Island, Cook Strait, New Zealand, 1987) and a good summary of the general history is available from (Day, 1987). Despite being a functional farm for over 100 years and its proximity to the mainland, the island never had any introduced mammalian predators except for mice *Mus musculus* Linnaeus, 1758. Mana Island's lack of the typical suite of mammalian predators enabled the survival of a unique island fauna, with threatened species persisting on island, such as the Cook Strait giant wētā *Deinicrida rugosa*, the goldstripe gecko (*Woodworthia chrysosiretica)* and McGregor's skink *Oligosoma macgregori* (Robb, 1975). The island was also a natural nesting site of the sooty shearwater *Ardenna grisea* (Gmelin, 1789). The presence of this relict fauna was a strong factor in the decision to vest the island as a scientific reserve in 1987 once the island rendered itself unsuitable to agricultural practice after the outbreak of scrapie in 1978 (Timmins, Atkinson, & Ogle, 1987). Most of these rare species were heavily impacted by a "mouse plague" (mice population explosion) in the late 1980s, caused by the removal of mammalian grazers and the subsequent proliferation of grass seed availability on the island, but the numbers of unique protected species bounced back to healthier populations after the eradication of mice on Mana Island in 1991 (Newman, 1994).

The island itself is an old marine terrace and has a distinct "flat" look; its highest point at the northern end sits at only 121 metres above sea level. This leaves the island exposed to strong winds from all sides, and this wind shapes the floral components on the top of the island quite literally, with most plants and low trees having some degree of tolerance for strong winds. Originally the island was blanketed in mature podocarp forest, and a massive planting restoration effort was started by Forest & Bird in 1987 and continued through to the eventual formation of Friends of Mana Island around 2000. To date more than 500,000 plants have been planted on the island as part of this restoration programme. A more detailed description of the site is given by Timmins et al. (1987).

The trial site was selected near the top of the terrace on Mana Island, close to the centre west of the island (Fig. 10). The soil is a sandy loam, and the water table depth varies across the site. When the trial was established in August 2018 it was believed that the expansion of flax weevils had not yet reached the site, which did not show weevil browse signs[2]. The trial site vegetation comprised mostly of rank pasture grass and many large mature flaxes which provide shelter from all but the strongest of winds (Fig. 12). The flax plants in the trial site are generally considered to be hybrids of harakeke (*Phormium tenax*) and wharariki (*Phormium cookianum*), with wharariki naturally occurring on the island coastal cliffs on Mana Island, whereas harakeke was planted as a windbreak during the Ministry of Agriculture period of farming on the island in the 1970s (Miskelly C. M., 2017).

There are many other native plants present amongst the flax community, including larger shrubs/small trees such as manuka (*Leptospermum* sp.), *Coprosma robusta, Coprosma repens, Coprosma propinqua,* ngaio (*Myoporum laetum*), and smaller shrubs and vines such as *Clematis forsteri, Haloragis erecta*, *Muehlenbeckia complexa* and *Muehlenbeckia australis*. Raupo (*Typha orientalis*) grows in a small cluster near the damper lower area of the site. Many of these plant species will have been deliberately replanted at the site during extensive revegetation and replanting of Mana Island in the 1990s and early 2000s, while others will be there from the natural regeneration after the removal of mammalian browsers.

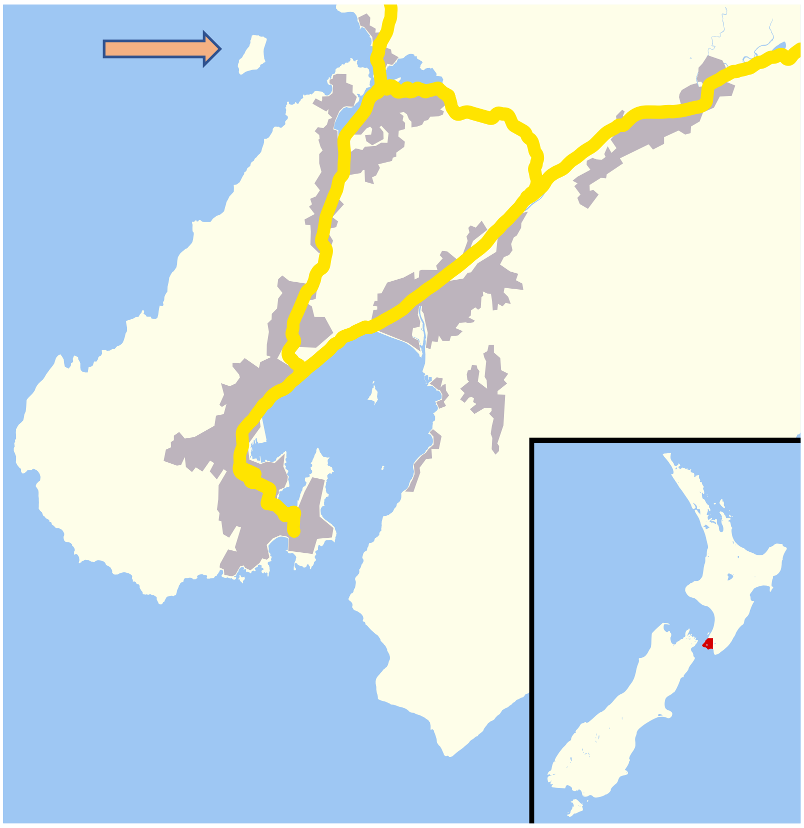


Figure 9. Map with arrow showing the location of Mana Island, located in the lower North Island of New Zealand. Modified from map created by user Benchill, CC BY-SA 2.0 via Wikimedia Commons

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[2] Weevils were, in fact, already there in small numbers, see Results.

Figure 10. Aerial photograph of Mana Island (New Zealand) taken in 2016/2017, with white polygon showing the location of the trial site established in 2018. Photo: LINZ Wellington 0.3m Rural Aerial Photos Index Tiles (2016-2017) CC-BY.

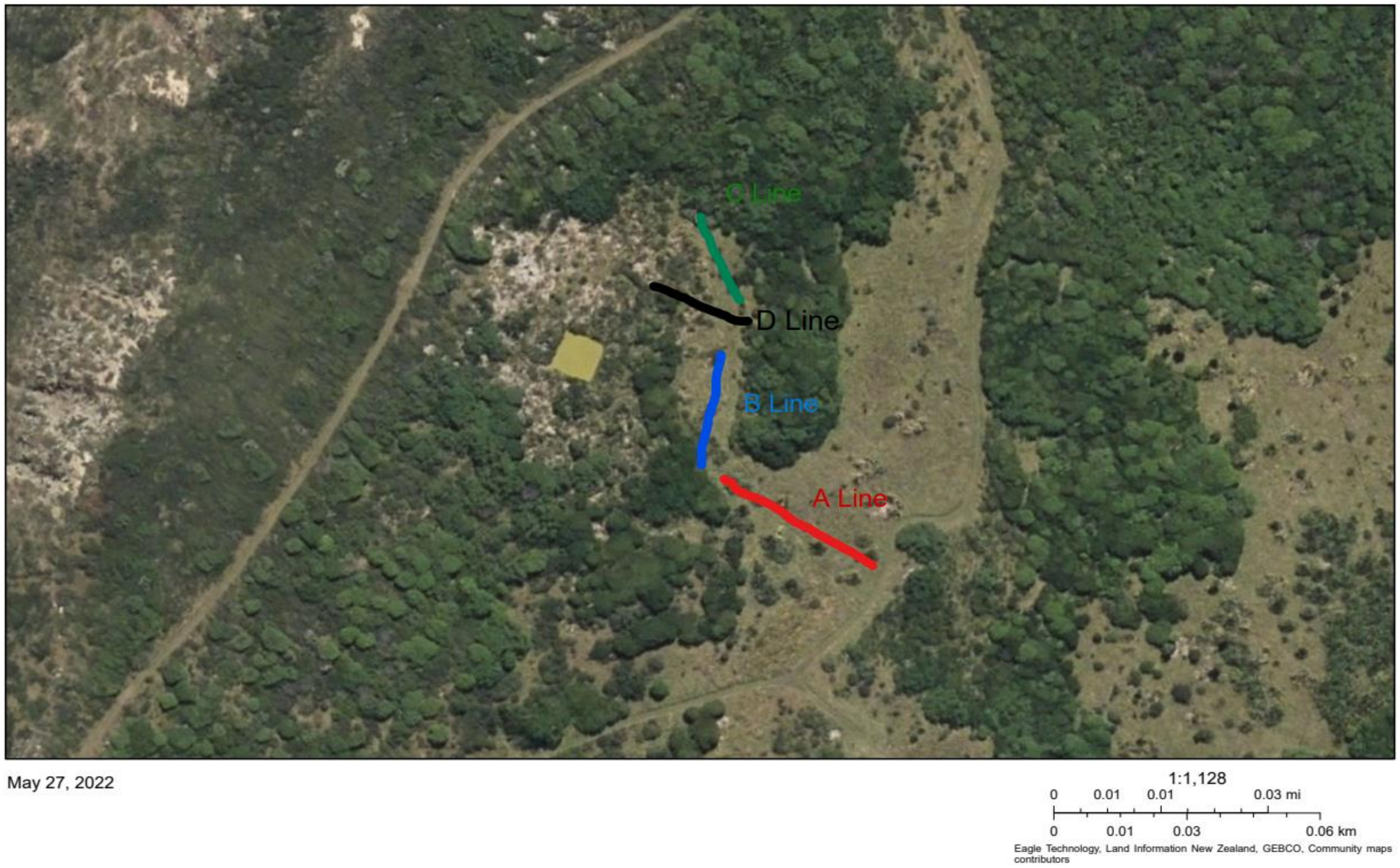


Figure 11. The flax trial site showing the approximate location of transect lines mentioned in the text. The olive-green square is a fire pond, the extensive pale brown patches near this are dead flax plants. Generated with DOC GIS software, https://www.doc.govt.nz/map/index.html.

Figure 12. The flax plant trial site with large healthy plants in December 2018, pre weevil and fungus release. Colin Miskelly in the foreground. Photo: W. Brockelsby, CC-BY.

Trial site setup

Once the site was selected, 80 mature flax plants were tagged and divided into four lines of 20 plants each. The lines were labelled as A, B, C and D, and within each line plants were numbered one to twenty. As mentioned above, the site was selected originally because it was believed to be slightly ahead of the spreading weevil population on the Mana Island and exhibited little, if any, weevil browse sign. The setup and tagging of the plants took place in August 2018 (Fig. 13).

After tagging of plants, an irrigation system was designed and built at the site in the spring of 2019. The purpose of this irrigation system was to address the hypotheses that plants with better access to water were better able to survive weevil damage, or that flooding with water reduced weevil larvae survival in the ground via drowning, a deliberate management technique used during early flax cultivation in New Zealand (Wehi, 2007; Miskelly C. M., 2017).

Figure 13. Colin Miskelly marking a flax on Mana Island with a cattle tag (number D16) during the setup of the monitoring plot in August 2018. Each plant was tagged with the line letter (A, B, C or D) and a number between 1-20. Photo: Friends of Mana Island, 2018.

The irrigation system consisted of a 4000 litre tank connected to a pre-existing island irrigation pipeline. The island's water bore is solar-powered and the flax tank was periodically filled and discharged by the island rangers. From the water storage tank, we ran two lines of PVC garden pipes behind the flax of the A line and the B line. Behind each plant three adjustable taps (Fig. 14) were inserted to the pipe which were tightened or loosened depending on the position in the system. The water supply was gravity-fed from the tank which was positioned at the uphill end of the Line B (with Line A further downhill). After the setup of the system, we tested the flow rates to each plant by placing ice-cream containers under each outlet and running the system. Outlet taps were then adjusted based on the results to achieve relatively even flows to each plant. This test was done once in spring 2019 and once again in spring 2020. In the end it was only possible to consistently deliver even amounts of water to 19 experimental plants in the A & B lines. Each of these 19 plants received approximately an equal amount of water from the 4000-litre irrigation system each time it was run. A full list of the plants selected and dates when the tank was emptied is available in Appendix 1. Lines C and D were the non-irrigated control, with plants provided with no supplementary water.

Figure 14 An outlet tap from the irrigation system which fed supplementary water to selected flax plants at the trial site. The taps were adjusted for flow to ensure all plants got an even distribution of water from the gravity-run irrigation system. Photo: W. Brockelsby, March 2021, CC-BY.

Flax plant monitoring

Soil moisture

One of the questions I was interested in was the effect of soil moisture on flax plant survival, particularly during the summer months when the island can experience significant dry periods. To track the changes in soil moisture on the island over time I measured the water content of the soil below each of 80 experiment plants over two years, three times a year, at roughly similar times depending on work trip availability. Measurements were taken in March, April, and September 2019 and in March, June, and October 2020.

Soil moisture content was measured close to the base of each flax plant, between the dug-in pitfall traps using a portable TDR 300 Soil Moisture Probe (Spectrum Technologies Inc., Aurora, IL, USA). Soil moisture (% volumetric content) was measured three times at each sampling point, and the average of the three readings was recorded. The measurements were taken using the medium length probes (5 cm) from the TDR 300 Soil Moisture Probe kit.

Plant browse score and plant collapse score

To a measure weevil activity on study plants that was independent of actual flax weevil sightings, I used their distinctive feeding marks (Fig. 15) as a proxy for their presence. To create a quantitative measuring system, I modified the version of browse scoring system used by Mike Meads during possum monitoring in the Orongorongo valley (Meads, 1976).

The scoring system works by quantifying flax plant damage on a scale from 1 to 5 (Table 3) based on the amount of weevil notch marks on the plant. This scoring system was selected due to its simplicity, as volunteers would often be collecting and recording data over the life span of the experiment, and it was important to have the scoring system that was intuitive and quickly understood. Half points (e.g., 2.5, 3.5) were used when volunteers or the author were unable to form consensus. This browse score was introduced from August 2018, just after the setup of the trial site, and continues to date.

Figure 15. Flax leaves heavily chewed by flax weevils (*Anagotus fairburni*) on Mana Island, NZ, November 2013. This plant would score a 5 for browse (all leaves showing signs of weevil damage) and 3 for collapse (more than two thirds collapsed but multiple crowns remaining). Photo: J. Hall, NZ Department of Conservation, CC-BY.

A similar plant collapse scoring system (Table 4) was added in October 2020 when many of the plants were heavily browsed by weevils and some were collapsing. The flax collapse score was designed to focus on the number of fans that had collapsed, rather than the degree of browse on the leaves. Again, this score had half units of 0.5 between them when consensus could not be reached. This scoring system was used as a surrogate for overall plant health.

Table 3 The flax plant browse score system used on Mana Island, NZ, as a proxy for flax weevil (*Anagotus fairburni*) activity on flax (*Phormium sp*.), as adapted from Meads (1976).

Plant Browse Score	**Description of browse**
0	No browse
1	Only a few leaves browsed
2	Light (up to one third browsed)
3	Medium (up to two-thirds browsed)
4	Heavy (more than two-thirds browsed)
5	Dead

Table 4 Flax plant (*Phormium sp*.) scoring system for the number of collapsed fans of flax, used as a proxy for overall plant health, Mana Island, NZ. Adapted from the plant browse scoring system used by M. Meads (1976).

Plant Collapse Score	**Description of plant**
0	No collapse of fans, just natural leaf attrition.
1	One, or maybe two collapsed fans in a large plant.
2	A few fans collapsed, but less than half the plant.
3	Heavily collapsed fans, clearly more than half the plant.
4	Only a few fans half poking up through mostly dead plant.
5	Nothing green left, just a pile of dead leaves.

Flax community monitoring

A multifaceted fauna monitoring program was designed that ran from the initial tagging of the plants in August 2018, to the fungus trial release in 2020, all the way until October 2021. The two primary methods used to monitor animal community were pitfall traps and night spotlighting.

Pitfall trapping

Under each of the 80 tagged flax plants, two different sized pitfall traps were installed. The first trap was a large non-lethal pitfall trap made from a dug in 4L bucket, with a wooden lid raised up 1cm from the ground via nails resting over the top of it as shown in Fig. 16. The bucket had a small drainage hole in the base to allow water to run out of the trap. These large pitfall traps were non-lethal traps primarily designed to sample the abundance of skinks, geckos, and large mobile arthropods such as carabid beetles or vagrant spiders (*Uiliodon sp.)*. While left unarmed and inactive these traps were filled up with sticks and pasture grass to enable easy access in and out of the trap for anything that wanted to enter.

Less than a metre away from the large pitfall, also under the flax plants, a small pitfall trap was installed. The small pitfalls consisted of a piece of standard PVC pipe with similar wooden lid resting on raised up nails (Fig. 16). These small pitfalls were armed by inserting small plastic disposable water cups with a centimetre depth of 70% ethanol into the pipes (Fig. 17). The cups were covered with 1 cm wire mesh to minimise by-kill of non-target species such as small skinks and geckos. The smaller pitfalls were primarily designed to sample smaller soil invertebrates, that might be affected by the release of *Beauveria*.

The arming of all pitfall traps happened on the Saturday morning of the Friends of Mana Island (FOMI) work trip weekends and was done by the author and a couple of FOMI volunteers, approximately every two months (see Appendix 3 for monitoring dates). Both pitfall traps were then left armed for 24 hours before being cleared and disarmed on the Sunday morning. During the arming process, many animals were often evicted from residence within the dormant pitfall traps.

Identification of lizards was done to species level, and invertebrates were usually identified to recognisable taxonomic units, but larger abundant invertebrate species were taken to the lab for further identification.

Figure 16. A large pitfall trap set on Mana Island at the *Beauveria* trial site. Photo: W. Brockelsby, October 2021, CC-BY.

Figure 17. A small pitfall trap disassembled, showing the downpipe into which a small cup of ethanol is placed to complete the arming process. This was then covered by the wooden lid shown in the bottom right. Photo: W. Brockelsby, Mana Island, October 2021, CC-BY.

Figure 18. The plastic water cup with ethanol covered by wire mesh, which was inserted into the pipe shown in Fig. 17 to complete the arming of small pitfall trap. The mesh was used to minimise bycatch of young skinks and geckos commonly encountered at the site. Photo: W. Brockelsby, October 2021, Mana Island, CC-BY.

Night spotlighting

All the 80 marked flax plants were monitored by night-time spotlighting with the support of FOMI volunteers each FOMI work trip weekend, approximately every two months (see Appendix 3 for monitoring dates), typically on the first night of a FOMI weekend work trip, unless weather conditions were poor. Night-time spotlighting commenced after sundown, once true dark had come in. The exact hour of the monitoring itself varied depending on the season.

The spotlighting was structured around four transects at the flax weevil site (lines A-D). One group of approximately five FOMI volunteers would walk line A, and then after A was completed, would walk line B. In parallel, another group of five or six FOMI volunteers would monitor C and D, starting from line C. Each group would spend one minute per plant (timed) recording animals found on the flax. Animals were recorded on a best effort basis to the nearest recognisable taxonomic unit, and photography was encouraged if people were not sure on what animal they had seen. Searchers would typically be encouraged to examine the outer leaves of the plant first and then move in all the way down to the base of the plant, all within the designated one minute of search time.

The main targets for this monitoring were the flax weevils themselves, which are nocturnal, but we were also interested in monitoring other nocturnal fauna that was unlikely to show up in pitfall traps, including arboreal species such as the Wellington tree wētā *Hemideina crassidens* (Blanchard, 1851), the two gecko species (*Woodworthia chrysosiretica* and *Woodworthia maculata*), as well as the giant wētā *Deinacrida rugosa* Buller, 1871. Metadata was recorded for each night monitoring trip, including weather during the trip, as well as the times the line monitoring began and finished.

Beauveria treatment

Trial design

To test the efficacy of *Beauveria* as a biocontrol for flax weevil, we selected random treatment flax plants, and applied the fungus in a standardised and replicable way to each plant. The goal was to make the fungus application relatively simple to administer, so that if it were successful, we would have a realistic chance of using this method to treat a good portion of the 217 ha Mana Island.

First, we had to ensure that there was true independence between experimental plants, to avoid contamination by the fungus of the soil or root systems of neighbouring plants at the trial site. On advice from Travis Glare (Bio Protection Research) a minimum distance of 3 m was used as a necessary gap between flax plants in the *Beauveria* trial (T. Glare, pers. comm. 2019). All flax plants were mapped, and the distances between bases of plants were measured. As a result, 43 flax plants of the total 80 tagged were identified as independent (at least 3 m apart from all other plants) and were used as replicates for the *Beauveria* fungus trial. Of these plants, 19 were on irrigated lines (A and B) and 24 on non-irrigated lines (C and D), creating a slightly unbalanced design. Each plant within irrigated and non-irrigated lines was randomly assigned to either receive the fungal treatment (suspension with *Beauveria*), or a control solution (suspension without *Beauveria*). The full details of the trial plants with their irrigation status and their *Beauveria* treatment (treated or control) are shown in Table 5.

Weevil translocation

Prior to the application of the *Beauveria* treatment in March 2020 and the deliberate translocation of weevils into the site, any flax weevils found during the night spotlighting were caught and removed from the experiment site. To stress the plants and to enable the fungus to come into immediate contact with its weevil host, 20 adult flax weevils were released onto each of the 80 tagged flax plants in the two nights before the *Beauveria* application took place. This meant we needed to catch 1,600 weevils and move them into the trial site over a period of two nights.

A subset of the weevils that were released onto the 43 experimental plants were individually tagged, to attempt to understand their movement at the site, and to gain information on their longevity. Ten weevils were marked per experimental plant, meaning that a total of 430 weevils were tagged over the two nights. The tagging method and results are discussed in greater detail in Chapter 4.

Table 5. The 43 tagged experimental plants used in the *Beauveria* trial, Mana Island, NZ, 2018-2021. Control plants received a triton-water solution without *Beauveria* fungus, while treatment plants received the same solution but with the *Beauveria* fungus. Letters A-D represent the four plant lines (transects).

Irrigated plants	Control/Treatment	Non-irrigated plants	Control/Treatment
A4	Control	C1	Control
A7	Treatment	C3	Control
A8	Control	C5	Treatment
A11	Treatment	C6	Control
A14	Treatment	C8	Control
A19	Treatment	C10	Treatment
A20	Control	C12	Treatment
B2	Treatment	C13	Treatment
B4	Control	C15	Treatment
B5	Treatment	C17	Treatment
B7	Control	C19	Control
B8	Treatment	C20	Control
B9	Control	D1	Control
B11	Control	D4	Treatment
B10	Control	D5	Treatment
B13	Treatment	D6	Control
B15	Control	D7	Treatment
B18	Treatment	D9	Treatment
B20	Treatment	D11	Treatment
		D12	Control
		D13	Control
		D15	Treatment
		D18	Control
		D20	Control

On the 28th of February 2020, the first action undertaken by the FOMI work party was to perform a night search of the trial site to remove all the weevils that had moved onto the plants since the last search in November 2019. By this stage weevils have been steadily invading the trial site from the south of the island, and 508 weevils were caught and removed from the trial site flax plants. These 508 weevils were supplemented by another 292 caught by searching flax plants nearby on the island on the same night. These 800 weevils were separated into groups of 20, each group was assigned to a tagged flax plant and released onto that plant on the same night.

The following night another 800 flax weevils were collected from the centre of Mana Island by volunteers and assigned to the remaining plants. The net result was that over two nights, all 80 tagged flax plants at the trial site had 20 weevils release onto each plant, with a total count of 1,600 weevils moved over the weekend. Both nights were warm, and relatively windless, and it took only a few hours for a group of 6-7 volunteers to find 800 adult weevils on each night. More could have easily been collected from the island if required.

After the experimental fungus application, weevils on each plant were caught by volunteers at each visit, to check for marked weevils and to accurately count all weevils seen. Once counted and checked for marks, all weevils were placed back onto the plant from which they were removed.

Fungus application

The application of *Beauveria* to the flax plants took place on the 3-day weekend of 28/02/2020 – 01/03/2020. A group of volunteers funded by the Friends of Mana Island (FOMI) travelled to Mana Island to assist with the release alongside Colin Miskelly (Te Papa Tongarewa), Jenny Brookes (Bio Protection Research Institute), Dale Shirtliff (FOMI executive committee) and the author.

The *Beauveria* spore solution was mixed on the island by Jenny Brookes (Fig. 19) using the methods described in Appendix 2. Once mixed, the active fungal mixture was transported to the experiment site, where it was further diluted with water and Triton wetting agent (0.05%). The 4L of liquid applied to each treatment plant, contained *Beauveria* spores at a rate of 8.4×10^7 /ml. As described earlier (Table 5), 22 plants were treated with an active mixture containing *Beauveria*, and 21 plants were treated with control mixture (4L of water with Triton added).

The control and treatment solutions were delivered to the trial plants using watering cans with the help of FOMI volunteers. Plants were watered by placing the water can part way down the base of fans of the flax plants and letting the solution run down to the base of plant where it was less likely to dry out quickly (Fig. 20). Each watering can was assigned to only deliver control or treatment solutions, to avoid any possible sources of cross-contamination between them.

Figure 19. Jenny Brookes from the Bio Protection Research Centre diluting the *Beauveria* fungal spore solution used to inoculate the treatment plants. In the background is the water tank from the irrigation system. Photo taken on Mana Island at the Flax Weevil Trial site, 01/03/2020. Photo: D. Shirtliff.

Figure 20. A Friends of Mana Island volunteer (Adrian Jull) applying the *Beauveria* solution to a trial flax plant using the "watering can" method on Mana Island during the fungus release in 01/03/2020. Photo: D. Shirtliff.

Testing soil for *Beauveria*

To test for *Beauveria* and other fungi in the soil under experimental plants, soil samples were taken from each of the 43 experiment plants prior to the application of the *Beauveria* at the site, and again after the *Beauveria* release in March 2020.

Soil cores were taken using a 5 x 5 x 5 cm soil corer. Four cores were collected randomly around the base of each flax plant (Fig. 21). Leaf litter and dead flax leaves were removed, and the corer placed 10-20 cm from the roots. The four cores were then pooled together into a single sample for each plant. Samples were kept dry and cool in sealed Ziplock bags and were sent to the Bio-Protection Research Centre in Lincoln University for analysis.

Both sets of samples (pre-release and post-release of *Beauveria*) were analysed by plating them out using a semi-selective medium at different concentrations following the methods described in (Brownbridge, Reay, Nelson, & Glare, 2012), and the number of colony-forming units (cfu's) was recorded. Two replicate plates were assessed for each soil sample at three different dilution strengths: x 10^{-1}, x 10^{-3} and x 10^{-4}. Averages were taken across each strength of dilution. Any *Beauveria* fungus found was also genotyped to confirm the identification of the strain and species of fungus detected.

Figure 21. The author (right) taking a pre-treatment soil core sample using a foot operated soil borer from a flax plant on Mana Island, Feb 2020. On the left is FOMI volunteer Jaz Hamilton. Photo: D. Shirtliff.

Data Analysis

All statistical data analysis was done using R Studio 3.6.1. The main packages used were base R, vegan, ggplot2, and car.

Irrigation and soil moisture

To test the effectiveness of our irrigation system at delivering water to each plant at the site, we used the soil moisture measurements taken in 2019 and 2020, and analysed them using ANOVA with the following variable structure:

- Soil Moisture (% moisture volumetric, continuous dependent variable).
- Irrigation Status (Irrigated or Not Irrigated, independent factor).
- Line (location of plant in the A, B, C or D lines, random factor).
- Year (date of measurement 2019 or 2020, independent factor).

Interactions between these variables were tested and post-hoc Tukey HSD tests were performed to interpret differences between any factor levels.

Plant collapse

To understand what might be driving the differences in plant collapse scores at the study site, an ANCOVA (Type III sums of squares) model was constructed with the following variable structure:

- Plant Collapse Score (0-5 health score, continuous dependent variable).
- EPF treatment (*Beauveria* fungus solution or control solution, independent factor).
- Line (location of plant in the A, B, C or D lines, entered as independent factor here, as line was significant for soil moisture in the Irrigation and Soil Moisture analysis).
- Soil Moisture (volumetric soil moisture averages across all dates as a covariate).

Interactions between these variables were also tested.

Flax animal community data

Flax animal community data (night spotlighting data and pitfall traps data) were analysed using non-metric multidimensional scaling (NMDS) to investigate the treatment effects on community structure. Night spotlighting data and pitfall traps data were analysed separately. Taxa with low counts (≤5 over entire sampling period) were removed, and some taxonomic aggregation of count data was performed to enable stronger conclusions. The grouping variables used in NMDS were:

- Year of sampling
- EPF treatment (*Beauveria* added or Control)
- Irrigation Status (Irrigated or Not Irrigated)

- Line (A, B, C or D lines)

Following NMDS, ANOVA was undertaken to test for impact of these variables on individual species or taxa considered to be good indicators for the flax animal communities.

Results

Flax plant monitoring

Soil moisture

Soil moisture varied markedly between seasons at the trial site, dropping by more than 10 % (volumetric soil moisture) over summer periods in both years (Fig. 22). The D line was generally observed to be the driest overall, many plants on this line are slightly more elevated above sea level than plants on other lines. Irrigated lines A and B in 2019 and 2020 did not have significantly increased soil moisture compared to non-irrigated lines, but which 'Line' as a variable had a significant effect on impact soil moisture (Table 6). Soil moisture in line D was lower than lines C and B by 3.64 % and 3.23 %, respectively (Fig. 23).

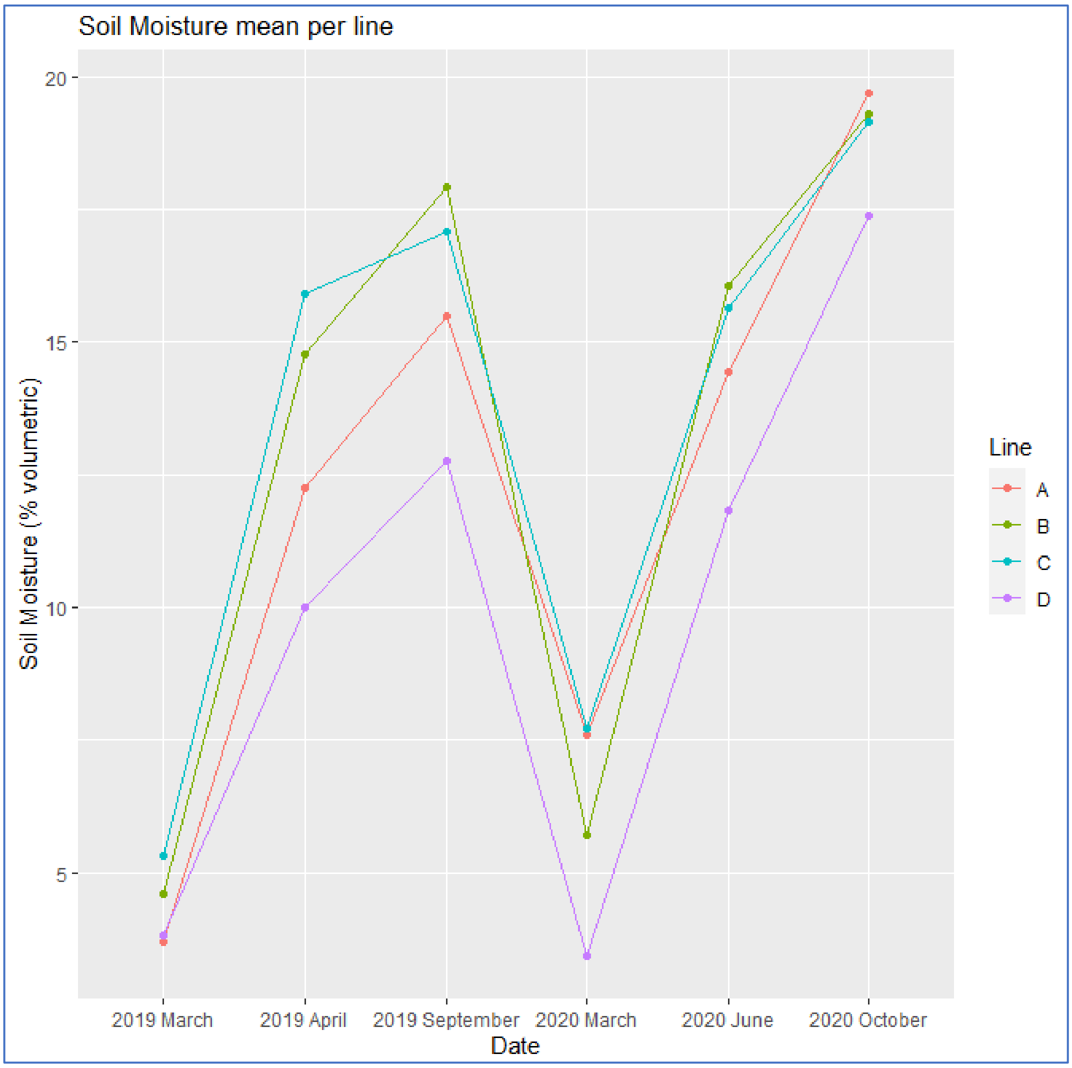


Figure 22. Mean soil moisture at experimental lines (averages of all experiment plants soil measurements on respective lines), showing the drop in soil moisture over summer in both 2019 and 2020.

Table 6. ANOVA Type III sums of squares table showing test statistics for soil moisture in the flax trial site on Mana Island

Factor	**df**	**Sum Sq**	**F Value**	**P value**
Line (A, B, C or D)	3	566	3.8808	<0.01
Year (2019 vs. 2020)	1	232	2.4792	0.12
Irrigation (Irrigated vs. Not Irrigated)	1	1	0.032	0.858
Residuals	251	11271		

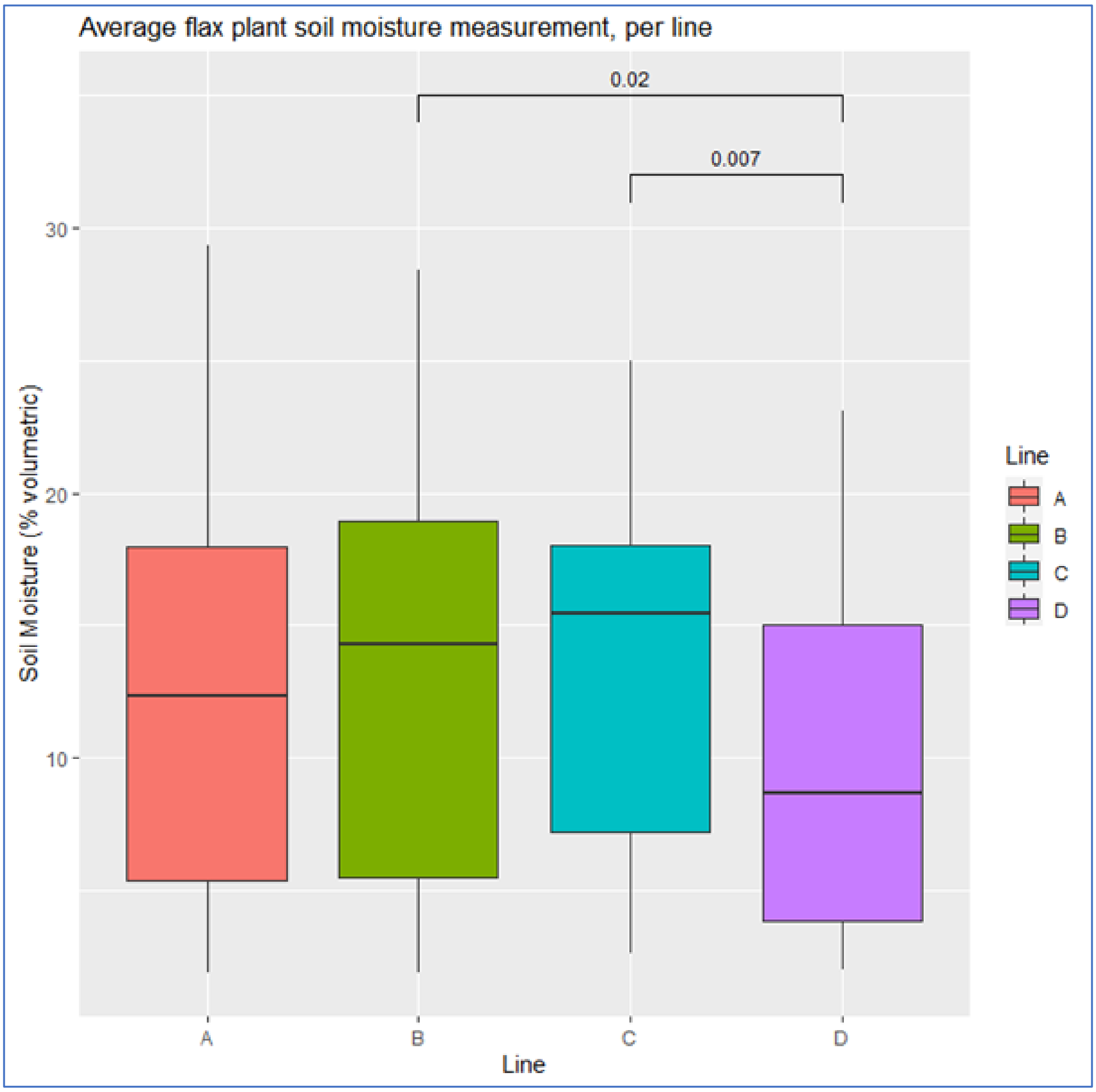


Figure 23. Boxplots showing the average soil measurements for all flax plants located on a line at the Mana Island trial site across in both 2019 and 2020. The D line was significantly drier than both B and C by 3% (volumetric), with Tukey HSD p values shown for significant differences between the lines.

These results suggest that the irrigation system we designed wasn't having a strong effect on the overall soil moisture, at least as far as could be measured by soil moisture sampling. This meant that irrigation status of any plant may not be a useful factor in explaining plant survival. However, these results did show that there was variability in the soil moisture at the site, across plants, lines, and years. This variability could help to understand the effect of weevil feeding on plant collapse, therefore I decided to use the soil moisture measurements as a covariate in the analysis of plant collapse at the trial site.

Plant browse score and plant collapse score

Flax weevil browse score proved early on to be a non-sensitive index: it was not a great indicator for weevil activity when compared to night spotlighting counts and it didn't directly correlate with the health of the plant, as plants could be heavily browsed but still relatively healthy. The flax weevil browse score was discarded in further analysis, instead being replaced by night counts for weevil activity and the plant collapse score for plant health. A full list of flax plant collapse scores for each plant is available in Appendix 4.

The health of the flax declined rapidly after flax weevil introduction. Plant collapse had begun in February 2021 and was well advanced by October 2021, with large swathes of experimental flaxes collapsing and many dead. Seventeen of the 80 flax plants marked in 2018 were completely dead in October 2021, and a further 37 were barely alive, with just one or two green fans showing (see the foreground of Fig. 24). The northern-most A line plants were generally the healthiest at the time of writing, but I suspect this will not last, as the weevils appear to be arriving from the south and then spreading to the north of the site after flax collapse. When looking at the 43 plants that were given either a *Beauveria* solution or a control solution in 2020, by October 2021 ten plants had completely died and 22 were severely damaged (Table 7).

One-way ANCOVA results revealed that soil moisture had no significant effect on plant collapse score. *Beauveria* treatment was significant only at 10% significance level ($p = 0.059$), and 'Line' the plant was in was highly significant (Table 8). The plant collapse score for line A was significantly lower than for the B line and C lines (both $p < 0.05$, Tukey's HSD test) (Fig. 25). A line scores were on average one plant collapse score level lower than C and B. Other lines were not significantly different. Fewer plants collapsed under *Beauveria* treatment than non-treated plants when counted, there are four treatment plants that were still relatively healthy at the time of writing. This is shown by the long distribution of plant collapse scores for treatment plants in Fig. 26. The possible causes behind this are discussed in greater detail in Chapter 5.

Table 7. Plant collapse scores for the 43 experimental plants in the *Beauveria* trial on Mana Island, October 2021. No plants were healthier than score 2 at the site.

Plant collapse score criteria	**Number of plants with each score, October 2021**	
	Control plants (no *Beauveria*)	**Plants treated with *Beauveria***
2 (few fans collapsed, but less than half the plant)	0	4
3 (heavily collapse of fans, clearly more than half the plant)	2	5
4 (plant mostly dead, but few fans still half-standing)	12	5
4.5 (some green left but close to dead leaves only)	2	3
5 (nothing green left, just a pile of dead leaves)	5	5
Totals	**21**	**22**

Table 8. ANCOVA results table (Type III sums of squares) with the important plant collapse score factors and covariates

	Sum Sq	**DF**	**F value**	**P value**
Mean soil moisture (%)	0.465	1	5.1462	0.390
Line (A, B, C, D)	8.683	3	6.4053	0.007
Beauveria (Treatment vs. Control)	2.332	1	3.5299	0.059
Residuals	22.824	37		

Figure 24. The experiment site on Mana Island photographed from the same photopoint 3 years apart, in December 2018 (left) and in October 2021 (right), 3 years after the study began; C. Miskelly in both photos, in 2021 he is no longer surrounded by tall healthy flax. Photo: W. Brockelsby, CC-BY.

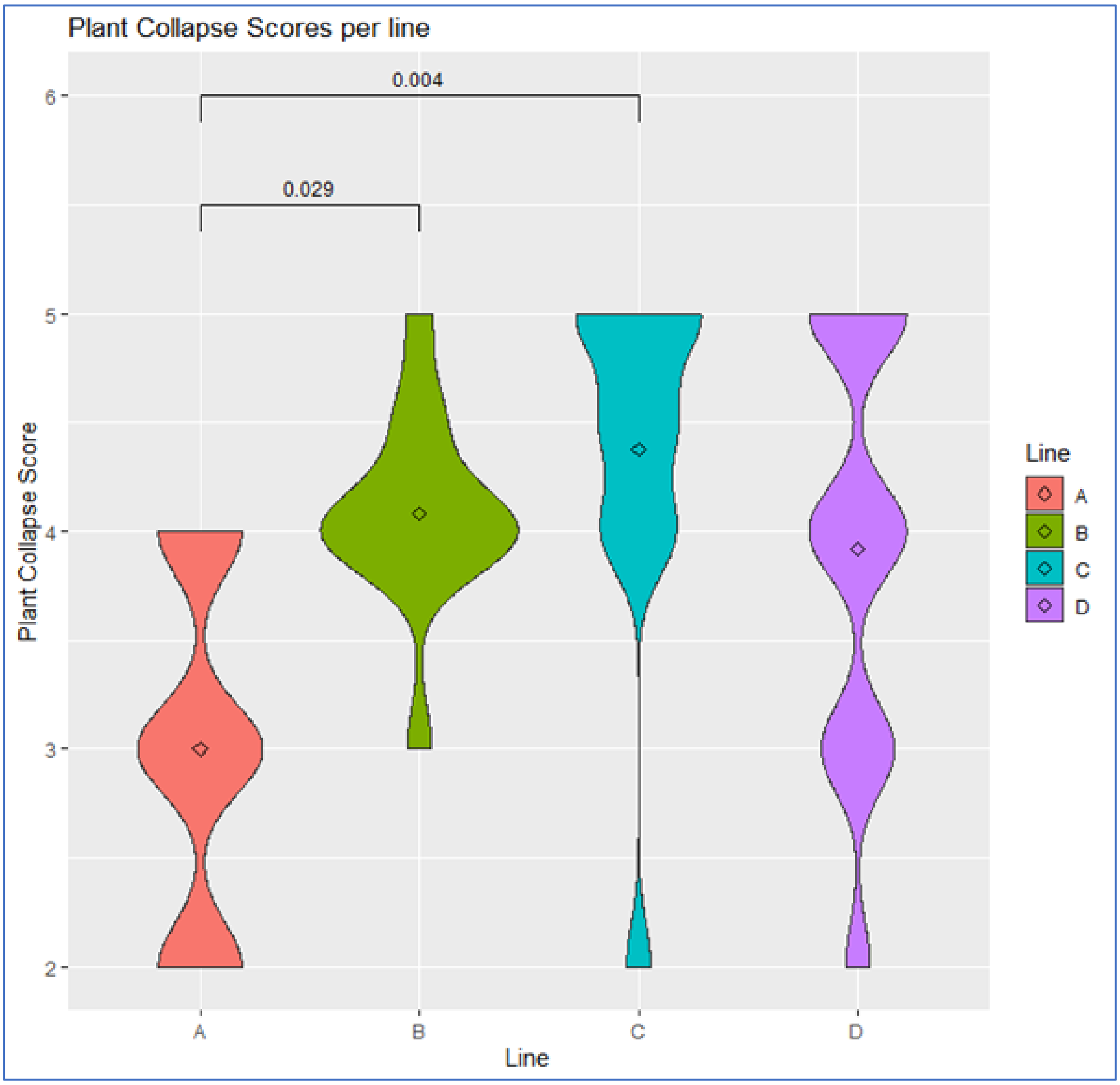


Figure 25. Violin plots for flax plant collapse scores, Mana Island, October 2021. Plots show the plant collapse score distribution for flax across the transect lines. The wider plots have more numbers of flax plants at a particular level. Note the A line which still has mostly healthy flax, probably due to being the last line that weevils were arriving at naturally at the site. Tukey's HSD p-values are displayed for lines that show significant differences. Also of interest are the long plots for C and D, which show a couple of outlying plants that were still relatively healthy as of October 2021.

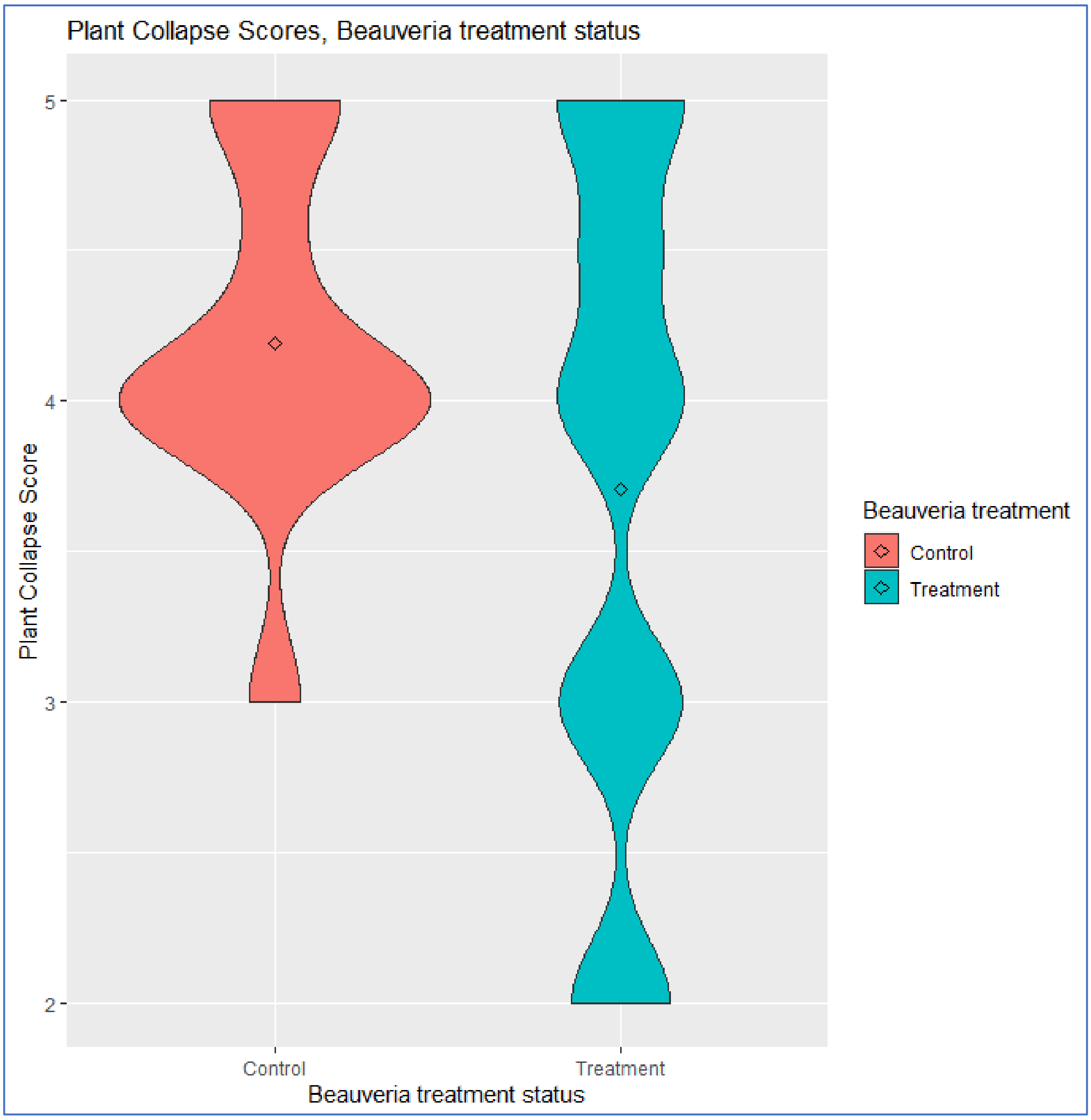


Figure 26. Flax plant collapse scores at the Mana Island in October 2021 trial site sorted by their treatment status. Note the stretched distribution of scores in the treatment plants, showing a few plants still healthy at the time of writing.

Beauveria in soil cores

There were no detectable levels of *Beauveria* in any of the soil samples collected prior to the *Beauveria* release in March 2020. When the soil testing was repeated in November 2020, after fungus release, *Beauveria* was detected in samples from several plants across 3 different lines, confirming its presence in the site. Genotyping of the *Beauveria* found at the trial site confirmed it to be the strain released by this trial (J. Brookes, pers. comm. 2020).

Soil samples were plated out for all plants, but *Beauveria* was detected only for the plants listed in Table 9. *Beauveria* was found in the soil under both control and treatment plants. By October 2021 a few of these plants had died, and many had collapse scores of 4 or higher. Interestingly, the *Beauveria*-treated plant D5 had the highest CFU result and was the healthiest plant left remaining in the D line in October 2021.

During the entire monitoring period no *Beauveria* infected weevils were found, either as adults or larvae. Adults were informally searched for during pitfall trapping, but sampling for larvae among flax roots was too destructive, something I wasn’t prepared to do while the trial was running.

Table 9. Mana Island results for soil cores taken after experimental release of *Beauveria* in March 2021, plated results and counts of colony forming unit's (cfu's) per gram soil. Each dilution was plated out twice to maximise the likelihood of detection of fungus using methodology in Brownbridge *et. al.* (2012).

	cfu's per dilution (10^{-1})		cfu's per dilution (10^{-3})		cfu's per dilution (10^{-4})					
Plant number	**Replicate 1**	**Replicate 2**	**Replicate 1**	**Replicate 2**	**Replicate 1**	**Replicate 2**	**Average 10^{-3}**	**Average 10^{-4}**	***Beauveria* treatment status**	**Plant Collapse Score (October 2021)**
D1				2			10000		Control	3
D4	++		1				10000		Treatment	3
D5			12	4	9	5	140000	1150000	Treatment	2
D7	++		13	3			145000		Treatment	3
D9			2	1	1	1	25000	150000	Treatment	5
D11				1			5000		Treatment	4
D12			1	1	1		15000	100000	Control	4
D13			33	6			360000		Control	4
D15	++			2	2		10000	200000	Treatment	5
D18	++								Control	4
D20			1				10000		Control	5
C19				3			15000		Control	5
C20			1				10000		Control	4
B13				1			5000		Treatment	4.5

++ *Beauveria* present but unable to quantify cfu's due to the number of other colonies growing.

Flax community monitoring

Night spotlighting results

Overall, the main change to the flax fauna community detected via night spotlighting was the drastic increase of flax weevil numbers. Even on the very first night of monitoring in August 2018, just after the setup of the plot, our assumption that no weevils were present in the site was proven incorrect when two weevils were found on the D line. This trend continued, as the D line was constantly on the forefront of the flightless weevil's dispersal northwards from their release point down the end of the island.

Total weevil counts peaked in February 2021 where weevil counts exceeded 2000 on the 80 tagged plants. Numbers had begun to eventually trend downwards by the time of the final monitoring trip in October 2021, with 1,163 weevils found (Fig. 27). Over half of these were from the A line, as by this time the tagged plants in the B, C and D lines had died and forced the dispersal of adult weevils away from these lines (see results for Chapter 4 for more discussion on this). Full flax weevil count data is available in Appendix 6.

The NMDS analysis of night spotlighting counts showed clear community structuring (stress value =0.03) by year of sampling, with a decrease in the numbers of geckos (*Woodworthia* sp.) and leaf-veined slug species (Athoracophoridae) observations at site, as the numbers of flax weevils increased (Fig. 28). No strong associations with year were evident for other fauna, including the Cook Strait giant wētā (*Deinacrida rugosa*). There was no night spotlighting community structuring by line (A-D), irrigation status or *Beauveria* treatment. The *Woodworthia* species included the more abundant Raukawa gecko *(Woodworthia maculata*) (n=742) and the much less common goldstripe gecko *(Woodworthia chrysosiretica*) (n=74). The Athoracophoridae found at the site were tentatively identified by volunteers as morphotypes *Pseudaneitea* spp*.* and *Athoracophorus* spp, but these determinations have not been confirmed by experts. Both morphotypes were commonly encountered on flax at night in sufficiently damp conditions.

ANOVA analysis confirmed that *Woodworthia* gecko sightings declined over time at the site $F(3,64) = 7.479$, $p < 0.01$, with average counts per line in 2020 and 2021 significantly lower than in 2018 and 2019 counts. Athoracophoridae sightings were significantly higher in 2018 $F(3,64) = 25.22$, $p < 0.01$, before staying much lower from 2019 onwards (Fig. 29).

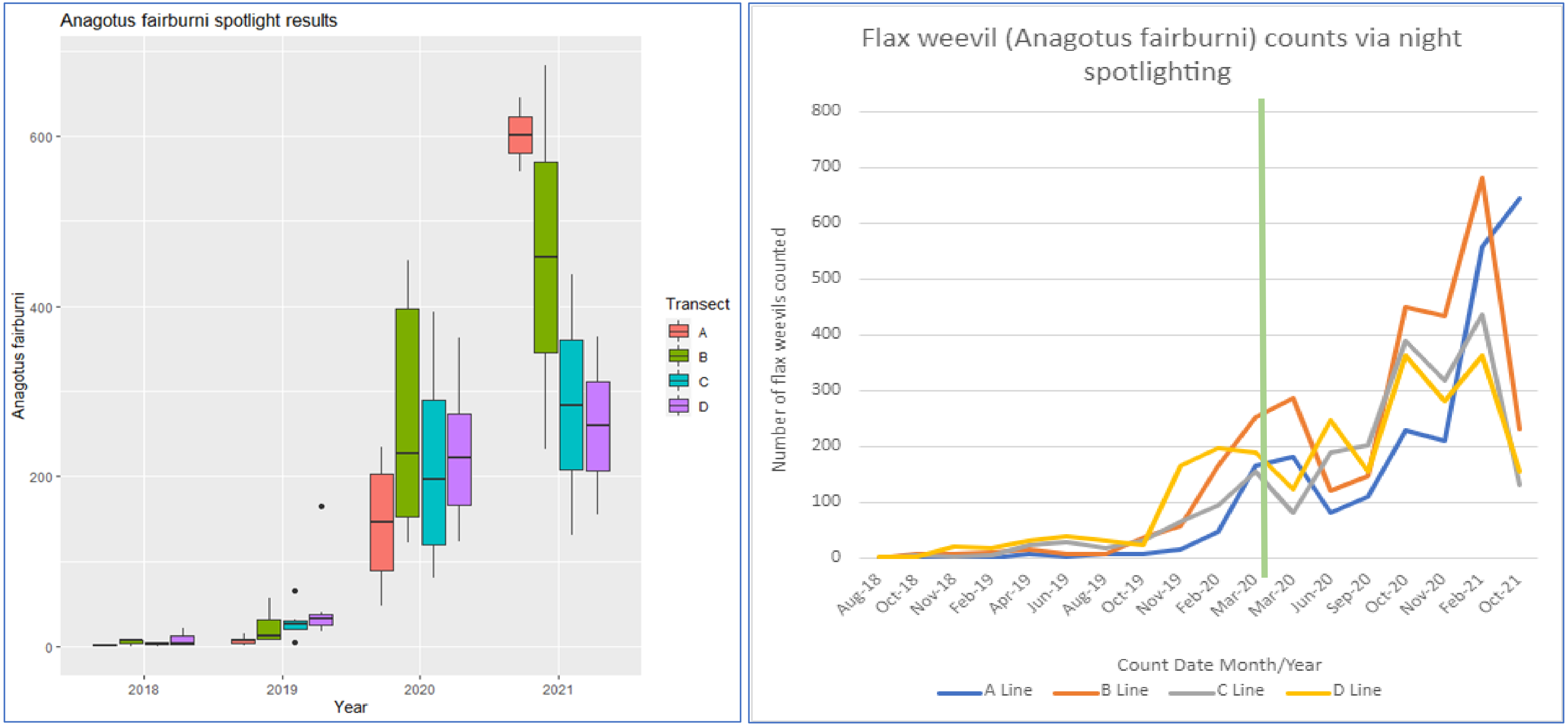


Figure 27 Flax weevil counts on experimental site on Mana Island over the duration of the monitoring (from August 2018 to October 2021): A – mean ± SE per transect, B – timeline for total counts; 1600 (400 per line) weevils were added in March 2020 as part of *Beauvaria* trial (shown by the green line on graph B). The only line with significantly increasing numbers of weevils by throughout the study time period was the northern-most line A, while in lines B, C and D many flax plants had died by October 2021 and weevil populations crashed. The sampling dates on the x axis in graph B are non-regular, as monitoring happened when it could around external factors, such as boat availability and Covid-19 disruptions.

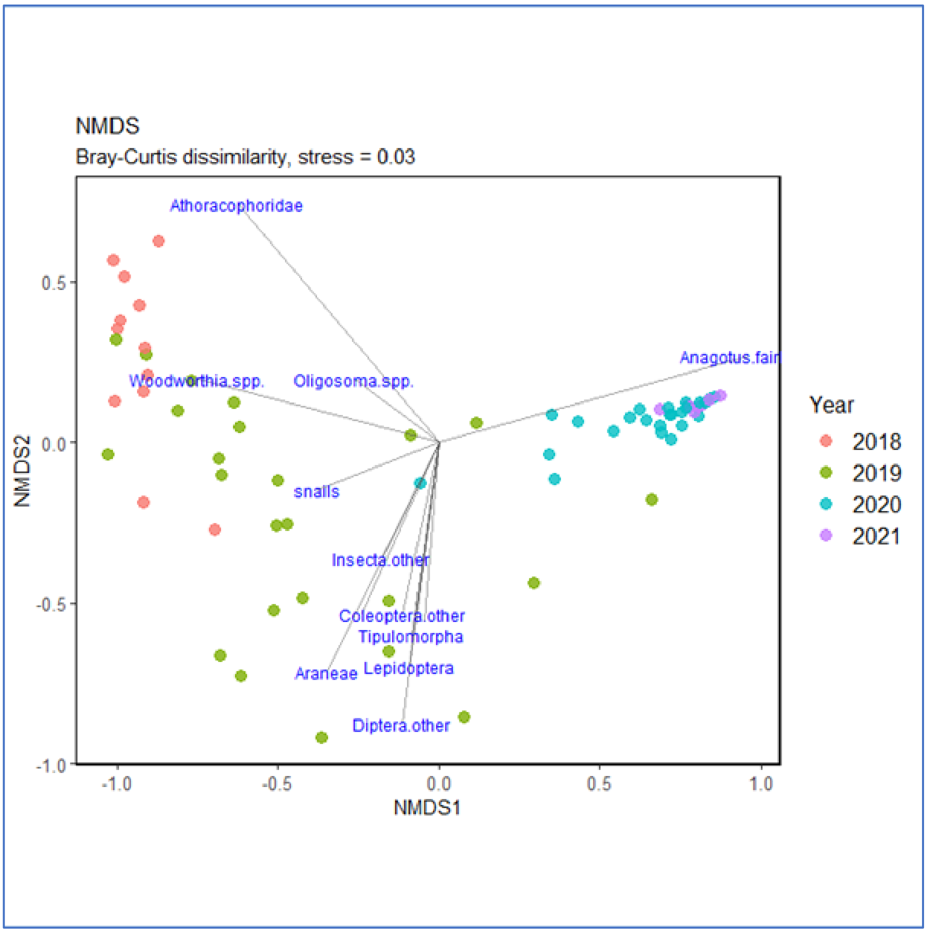


Figure 28. Non-metric multidimensional scaling ordination for night spotlighting data from study site on Mana Island, showing community trends across years.

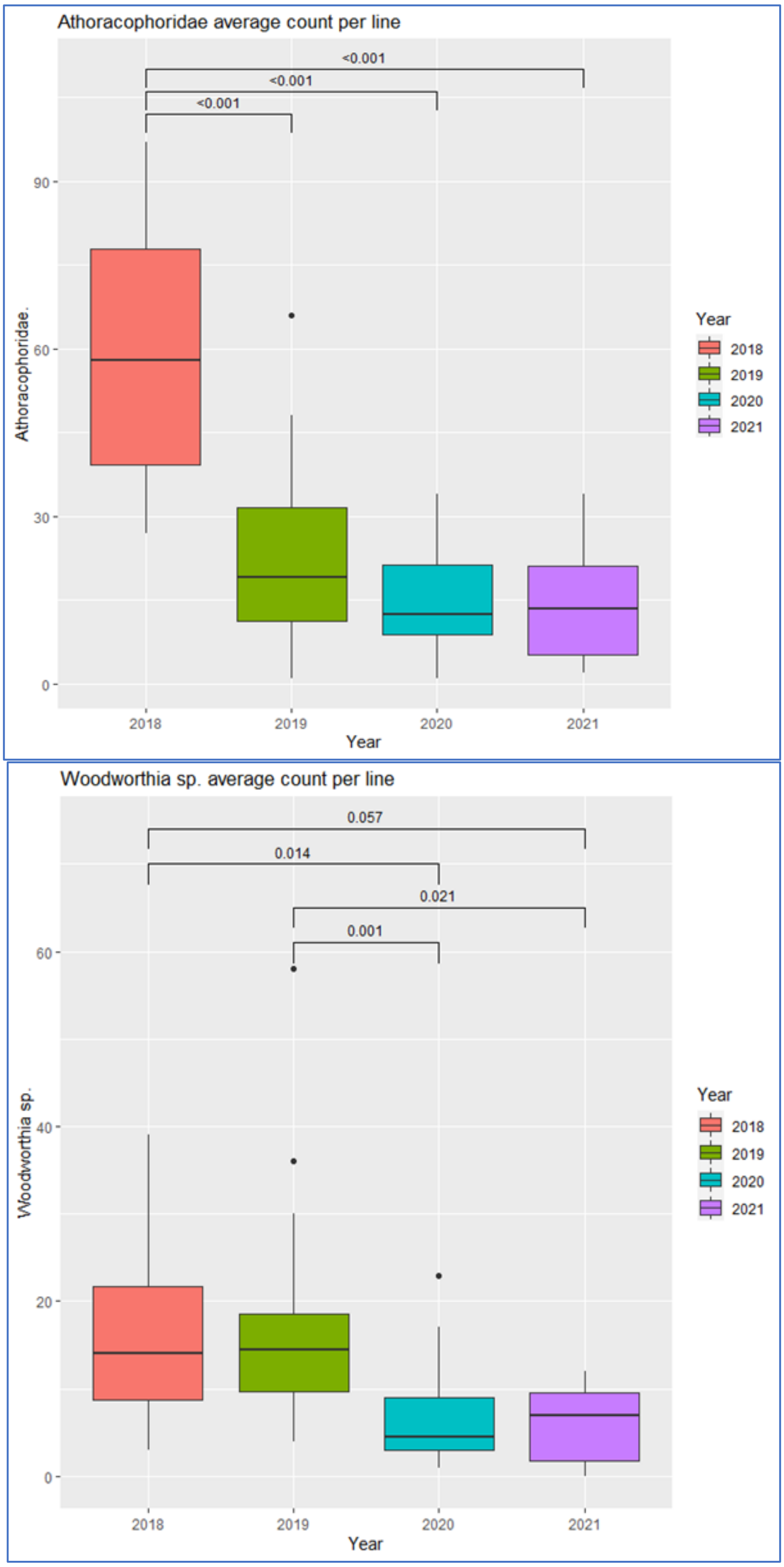


Figure 29. Night spotlighting data (box plots) for Athoracophoridae leaf-veined slugs and *Woodworthia* geckos, two taxa that showed significant decline over the monitoring period from 2018 to 2021. Each year's plot shows the range and mean counts of animals found across all transects in any given monitoring session in a year. Significant p-values for Tukey's HSD tests are shown for changes across years.

Pitfall trapping results

Some of the abundant species found in the large pitfalls at the flax trial site were the copper skink *Oligosoma aenum* (Gerard, 1857) (n=922) and the common skink *Oligosoma polychroma* (Patterson & Daugherty, 1990) (n=374). Among the Arthropoda, carabid beetles *Mecodema oblongum* (Broun, 1882)(n=236) and *Megadromus capito* (White, 1846) (n=230) were very commonly encountered in both large and small pitfalls, and members of the Amphipoda (n=349) and Isopoda (n=366) were regularly picked up in the small pitfalls. NMDS ordination for all 80 pitfall traps showed very little structuring of the pitfall trap community data (stress value = 0.15) by year, line or irrigation status (Fig. 30).

When NMDS was run for pitfall data using just the 43 *Beauveria* trial flax plants, results were similar with no obvious structuring of the pitfall trap community data (stress value = 0.12) by treatment status (Fig. 31). When the species most common in pitfall traps (those labelled in Fig. 31) were visually reviewed for differences across years, and ANOVA tests run for several taxa, most taxa showed minimal differences or slight changes between years. None showed large decline after the *Beauveria* fungus release and the gradual death of the flax plants (Fig. 32).

Pitfall trapping data could not be collected on all visits to the island. Trapping was disrupted in November 2020, when Mana Island saw unprecedented heavy rain (33 mm of rainfall was recorded within 30 minutes in nearby Plimmerton). This washed out most of the pitfall traps, heavily affecting the results of that session. Most of 2021 was also impacted by external factors, including prolonged power supply problems (preventing overnight stats until the power supply system was replaced) followed by the loss of the boat that ran regular trips to the island, as well as the second nationwide COVID-19 lockdown. These all meant that in 2021 only one pitfall session was completed (in October 2021), reducing the strength of some of these data and following conclusions. As of March 2022, monitoring trips have still not resumed. Pitfall trapping will continue at the site in the long term, with the aim of understanding what effect the loss of the flax plants will have on invertebrate and reptile communities over the long term. This is further discussed in Chapter 5.

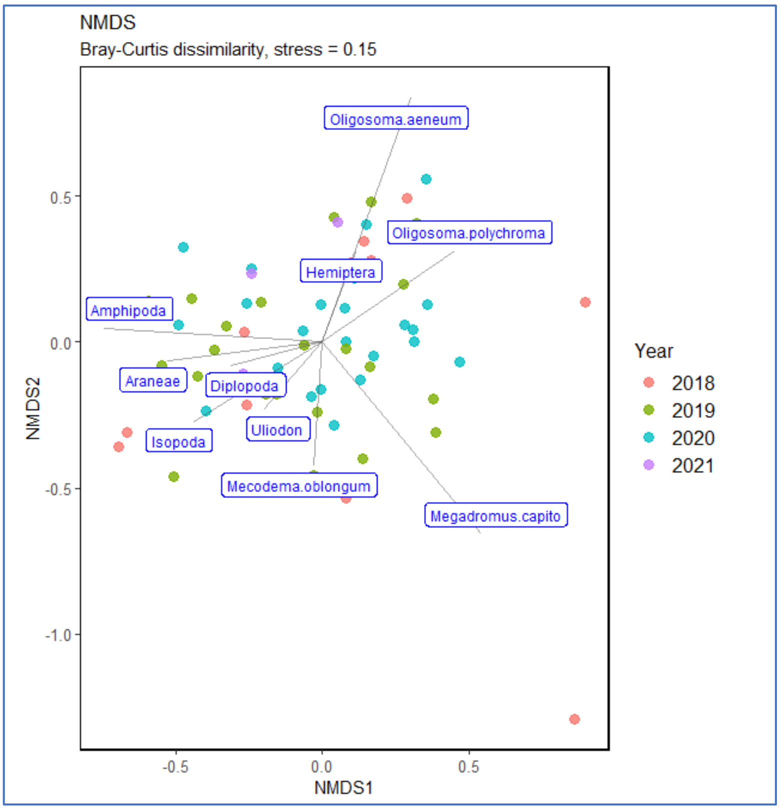


Figure 30. NMDS ordination for all pitfall trap communities at the study site on Mana Island, with species having significant correlation to axes labelled.

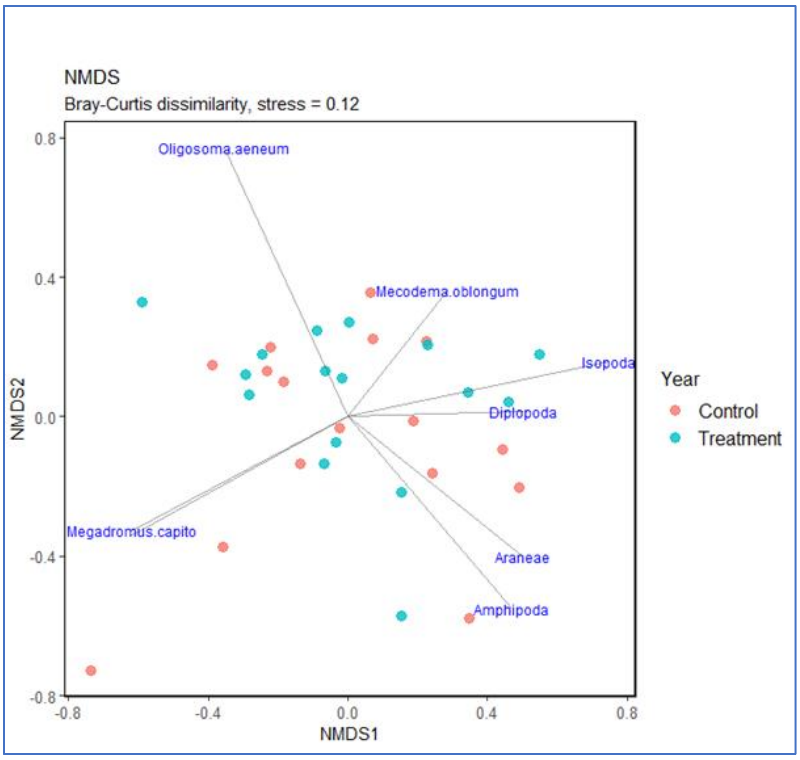


Figure 31. NMDS ordination for pitfall trap communities under 43 flax plants in *Beauveria* treatment trial on Mana Island, with species having significant correlation to axes labelled. “Treatment” – flax treated with *Beauveria* , “Control” – untreated (control) flax plants

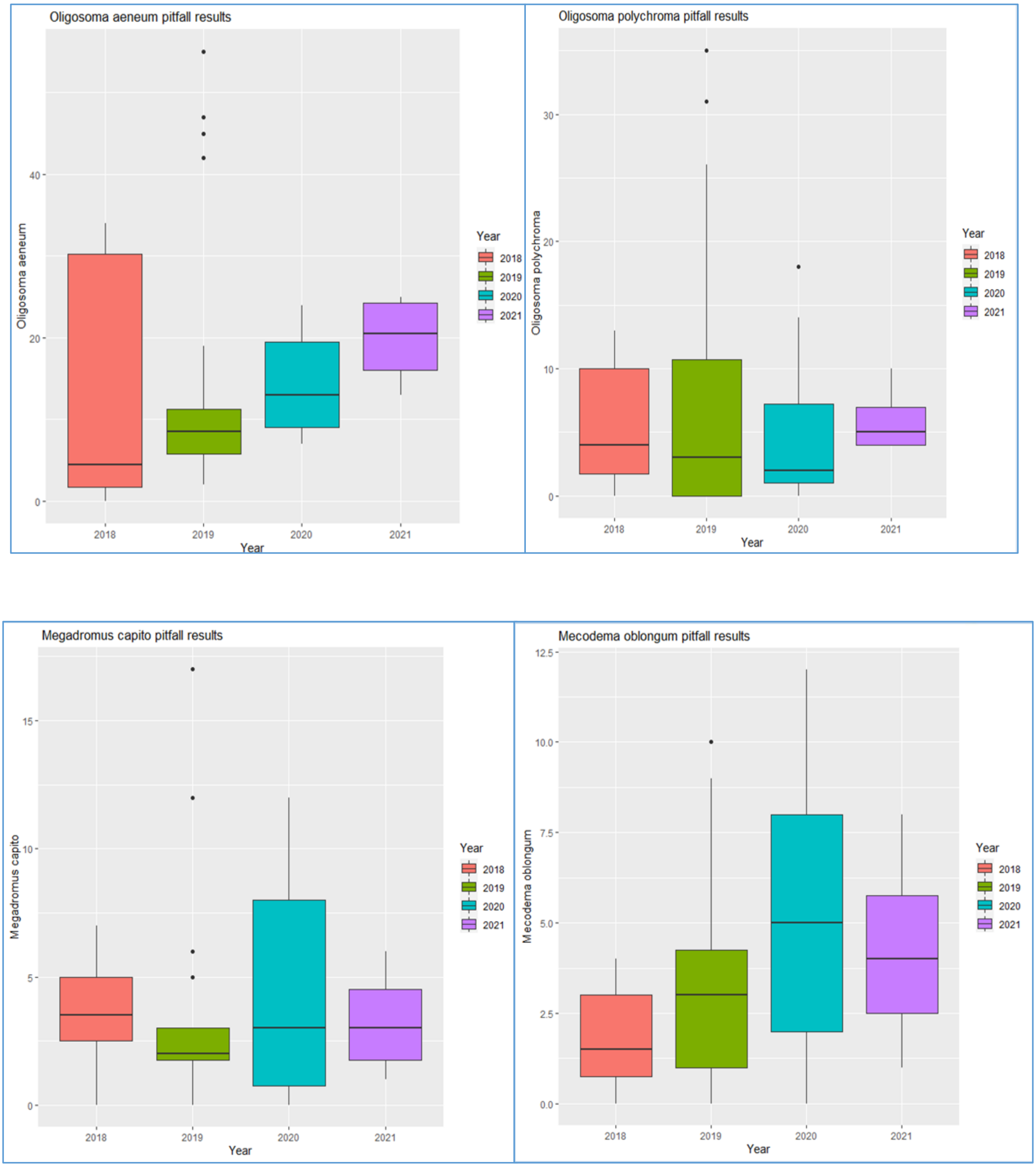


Figure 32. Box plots for some of the most common species in pitfall traps at the Mana Island flax trial site. Counts are averaged per pitfall session across the whole site. The outliers for *Oligosoma* skinks were caused by summer sampling sessions, where skinks were much more active and would turn up in pitfalls in large numbers, relative to other times of the year. No species sampled via pitfalls showed significant trends across time, just minor variation between years.

Chapter 4 Flax weevil ecology

As discussed in the literature review, very little is known about flax weevils, including basic biological information such as the time the larvae spend in the soil, the lifespans of adults, and their behaviour. Over the course of the *Beauveria* trial I opportunistically and deliberately aimed to address these knowledge gaps by performing a few classical studies on habits and lifecycle of these weevils when this could be fitted in around the primary purpose of the *Beauveria* trial.

Larval Instars

Introduction

One of the questions of most interest was the lifecycle of the flax weevil while it was in the ground, especially how many instars the weevil goes through before becoming an adult. To investigate this, I excavated larvae from the base of flax plants and measured their head capsule widths. By measuring the head capsule widths and grouping the measurements I hoped to determine the number of instars by using the Brooks-Dyar rule, also known as Dyar's rule (Crosby, 1973), which states that the sclerotised parts of an arthropod's development follow a linear geometric growth pattern (Brooks, 1886; Dyar, 1890). There is a wealth of literature generated across a range of insect taxa that have used this rule to develop a deeper understanding of their life history (Hawking & New, 1996; Ahmad & Nabi, 2012; Ramaiah, 2018; Ferreira-Keppler, Rafael, & Martins, 2019; Kondur & Şimşek, 2021). This rule is particularly useful when data is difficult to gather or incomplete, as the theory enables a scientist to make inferences from imperfect data (Skuhrovec, 2019). Not all species conform perfectly to the Brooks-Dyar rule (Elmes, 2001; Lackmann, 2018), but it is still widely used in entomology and other related sciences.

While knowing the number of instars doesn't necessarily address all core ecological questions for the weevil's lifecycle phenology (e.g., its total development time), having the ability to accurately assign an instar number to any individual larva specimen collected would enable us to make more accurate investigations into the flax weevil on Mana Island in the future.

Methods

Head capsule measurements

The sampling was done in September 2019. Larvae were extracted from flax plants on Mana Island by hand pulling out whole rosettes of heavily damaged flax and methodically searching the roots and leaves for larvae within their burrows. To do this I would first uproot a rosette, search the root, and then moving on to each flax fan. Leaves would be removed from the fan one leaf at a time and methodically searched for larvae burrowing into the soft tissue. Heavily damaged flaxes were targeted primarily for convenience, as flax heavily damaged by weevils is severely weakened and simple to excavate compared to healthy plants.

Identification of the larvae was done using the description of the newly emerged larvae provided in (Gourlay, 1931). The number of larvae found on each flax plant was recorded and then head capsule widths were measured. For large larvae, head capsules were measured in the field using electronic field callipers accurate to the nearest 0.1mm (Fig. 33). Three head capsule widths measurements were taken using the callipers for each "large" larva and an average measurement generated from those three measurements. This average then formed the data point used in analyses.

The smallest larvae found were transported in ethanol to the lab at Massey University Palmerston North. They were then measured in the lab using a dissecting microscope microscopic scale in the lab using the Olympus CellSens software and recorded accurately to the nearest 0.01mm.

Figure 33. A flax weevil (*Anagotus fairburni*) larva freshly excavated on Mana Island in 2019 having its head capsule measured by the author using electronic callipers. Photo: B. Singer, CC-BY.

Data analysis

Data analysis followed the methods described by Lydia Sukovata (Sukovata, 2019), in her paper comparing the three methods of separating larval instars of *Dendrolimus pini* based on their head capsule widths. All analysis was done in R Studio 3.6.1, using the following packages: stats, ggplot2 and nlstools.

Following Sukovata's (2019) methodology, I used the head capsule data I had collected to generate histograms and visually review the data to hypothesise expected peaks of the various instar sizes. After reviewing the histogram, I then followed approach 2 in Sukovata (2019), which uses the data to generate kernel density estimates (KDE) (Sukovata, 2019, p.3). While a variety of kernel functions exist, here we used the normal (Gaussian) distribution function, in which case the KDE peaks correspond to the means (of head capsule width in instars). Multiple bandwidth values were compared, with final values chosen for goodness of fit. KDE was done using the density function from the *stats* package.

After fitting the KDE with appropriate bin widths, the KDE peaks were used to generate the mean head capsule size of each larval instar. This mean value was then used as a starting value to fit a non-linear squares (NLS) model to head data using the *nlstools* package. The NLS fits a Gaussian curve to each instar KDE peak, producing estimates of mean and standard deviation for the head sizes in all instars. Finally, the resulting estimates of mean head width for each instar were plotted, with linear and ln regression fitted to test for the overall adherence to the Brooks-Dyar rule.

The head capsule width dataset is presented in Appendix 5.

Results and Discussion

In total, 86 larvae were collected and measured, off 12 different flax plants. A range of larvae were found in most plants, e.g., Fig. 34. Visual inspection of the data plotted as a frequency histogram suggested four instar groupings (Fig. 35), although the separation between 2nd and 3rd groupings is less clear. As shown in Fig. 36 (circled), one of the instar groups contained a large range of values.

Figure 34. A range of *Anagotus fairburni* larvae instars collected from the base of a single flax plant on Mana Island in 2019. The numbers on the side refer to the order in which they were collected. Photo: B. Singer, CC-BY.

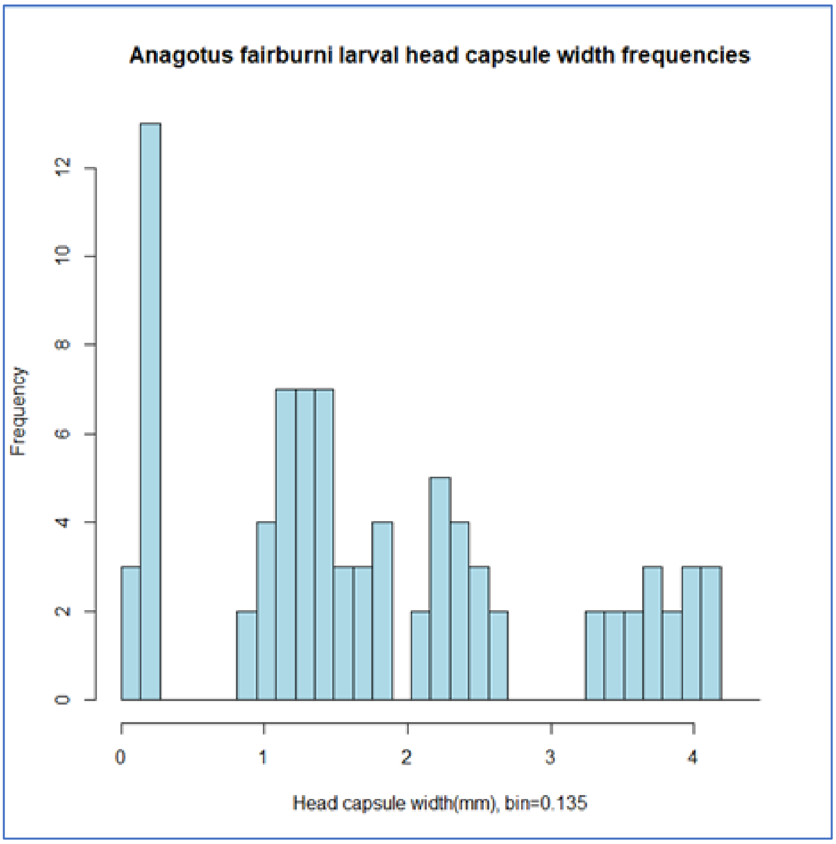


Figure 35. Frequency histogram of all *Anagotus fairburni* larvae head capsule widths, used to visually review and assign appropriate bandwidth (bin size).

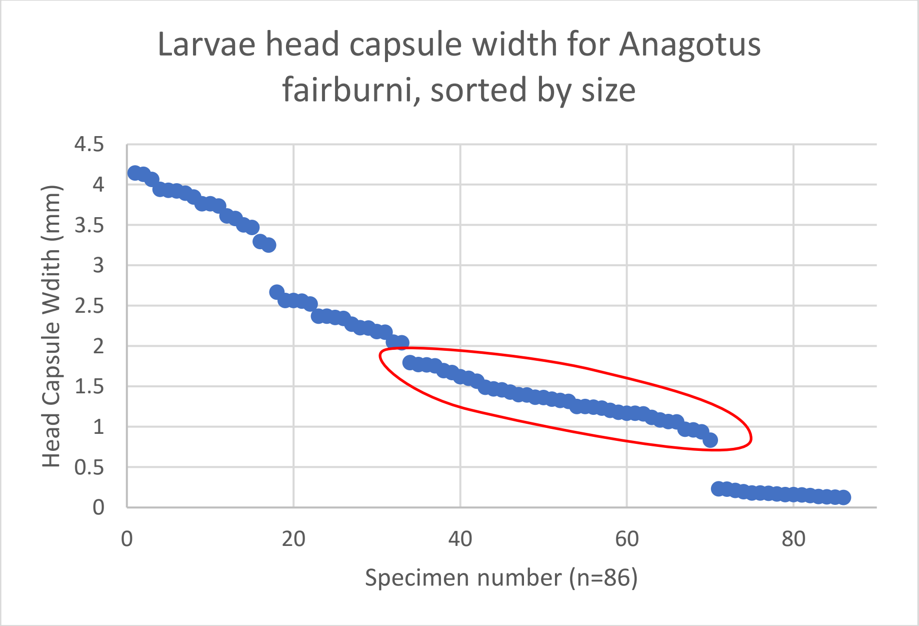


Figure 36. Plotting all 86 head capsule width measurements done for *A. fairburni* larvae collected on Mana Island in 2019. Circled in red is the largest range of head capsule width measurements that represent a broad range of head capsule width measurements.

When run through Sukovata's KDE methods, four main peaks were identified (Fig. 37). These peaks then were used to generate values for the most likely head capsule width for each of the four instars. In a Gaussian model, the maximum probability coincides with the mean value. As expected, when plotted in the Gaussian non-linear squares model there is some overlap between size ranges of instars. However, peak two and three (green and red) would not converge in the NLS models, so were manually fitted with sigma values and the means derived from the KDE function, and then plotted as shown in Fig. 38.

Finally, the estimated means for each of the four instars were plotted and fitted with OLS linear regression, showing strong linear relationship (Fig. 39).

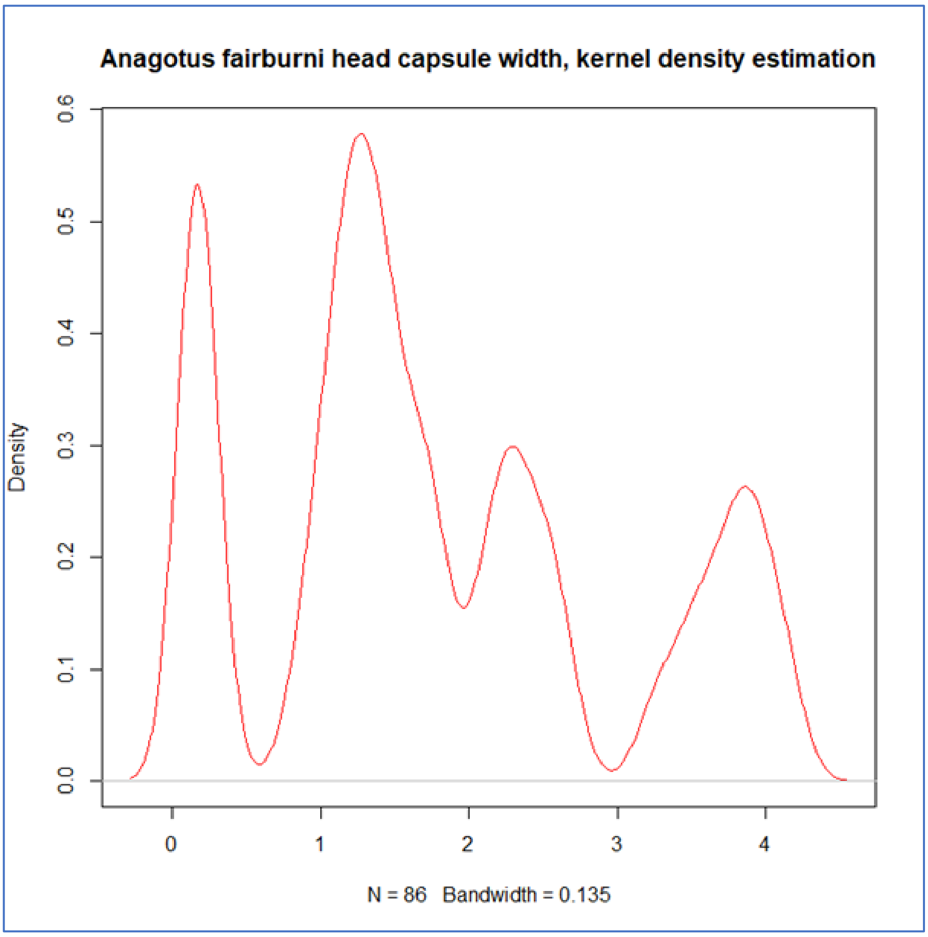


Figure 37. Kernel density estimates (KDE) of *Anagotus fairburni* larvae head capsule widths, Mana Island data, n=86, bandwidth (bin set at 0.135) measured in mm.

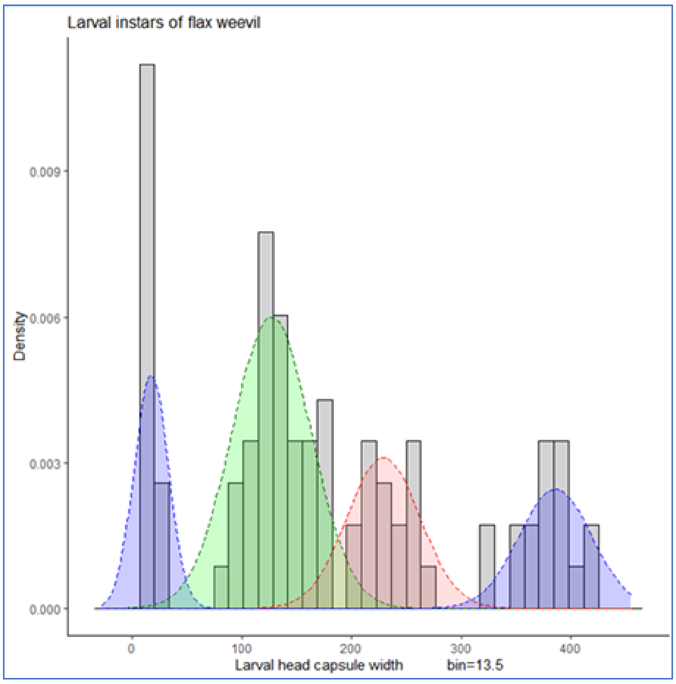


Figure 38. The *Anagotus fairburni* larvae head capsule width peaks plotted using a Gaussian non-linear squares (NLS) model, showing the probabilities that each measurement would fall within an observed range. X axis is set to $1x10^{-5}$m on the metric scale.

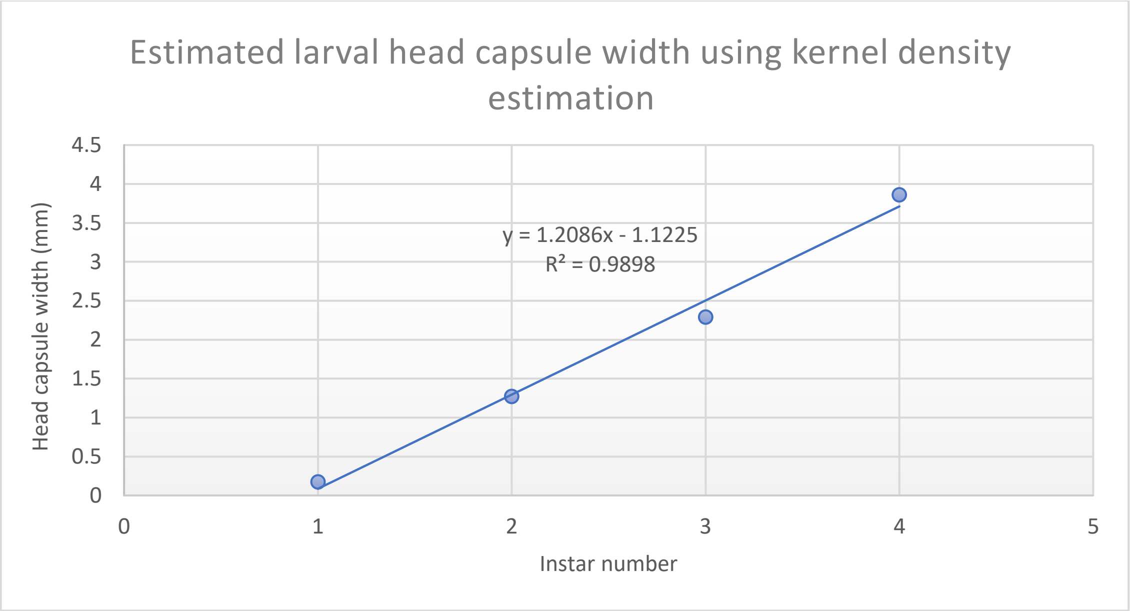
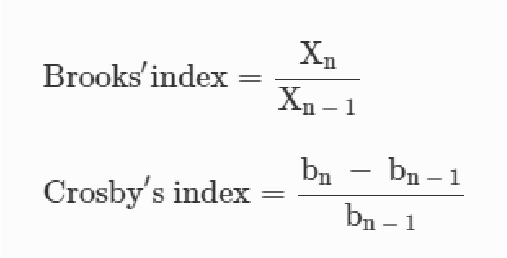


Figure 39. Four *Anagotus fairburni* instars and their estimated mean head capsule widths, plotted and tested for adherence to Dyar's rule (OLS regression).

From the data collected on Mana Island, there appears to be at least four larval instars in *Anagotus fairburni*. Prior to beginning this work, my hypothesis was that there would be five or six instars, as is seen in other large weevils such as *Hadramphus spinipennis* (Schops, 1998) and the co-generic weevil *Anagotus helmsi,* which was thought to have "probably five instars" (McBurney, 1976). Finding only four instars didn't match these expectations, so I then looked to confirm the accuracy of my four-instar results by calculating the Brooks-Dyar ratios and corresponding Crosby growth ratios for each instar (Brooks, 1886; Dyar, 1890; Crosby, 1977). I used the following equations to generate these ratios (reproduced from Chen 2021).



$$\text{Brooks}'\text{index} = \frac{X_n}{X_{n-1}}$$

$$\text{Crosby}'\text{s index} = \frac{b_n - b_{n-1}}{b_{n-1}}$$

Where Xn is the mean measurement for (n instar) larvae, Xn − 1 is the mean measurement for (n − 1 instar) larvae, bn denotes Brooks' index of (n instar) larvae, and bn − 1 denotes Brooks' index of (n − 1 instar) larvae (Chen, 2021). The resulting data are presented in Table 10.

Table 10. Estimated mean head capsule widths of *Anagotus fairburni* larval instars based on KDE peaks, and corresponding Brooks-Dyar and Crosby growth ratios; Mana Island weevils, n=86.

Larval Instar #	**Most likely head capsule width (mm)**	**Brooks-Dyar ratio**	**Crosby growth ratio**
1	0.173		
2	1.270	7.32	
3	2.292	1.80	-0.75
4	3.861	1.68	-0.06

A Brooks-Dyar ratio is expected to be consistent between instars (Sukovata, 2019). The results for instars 2/3 and 3/4 in Table 10 are consistent with this expectation. However, for instars 1/2 the Brooks-Dyar ratio of 7.32 suggests it is highly likely that at least one instar was missed. The same shows up very clearly with the Crosby's growth ratio of -0.75 – when the absolute value of Crosby's growth ratio is greater than 0.1, it indicates that the instar groupings do not match the Brooks-Dyar rule and that the instar values hypothesised are not correct.

Sukovata (2019) recommends plotting the log natural values of your instars and checking the linear regression equations, stating that the ln-transformed mean of the head capsule widths should be as close to a straight line as possible (i.e., R-squared >0.99). When this was done for this data for the four instars, the equation line was not within these bounds. However, when the first instar was removed, the regression for the remaining 2, 3 and 4 conforms to this rule (Fig. 40).

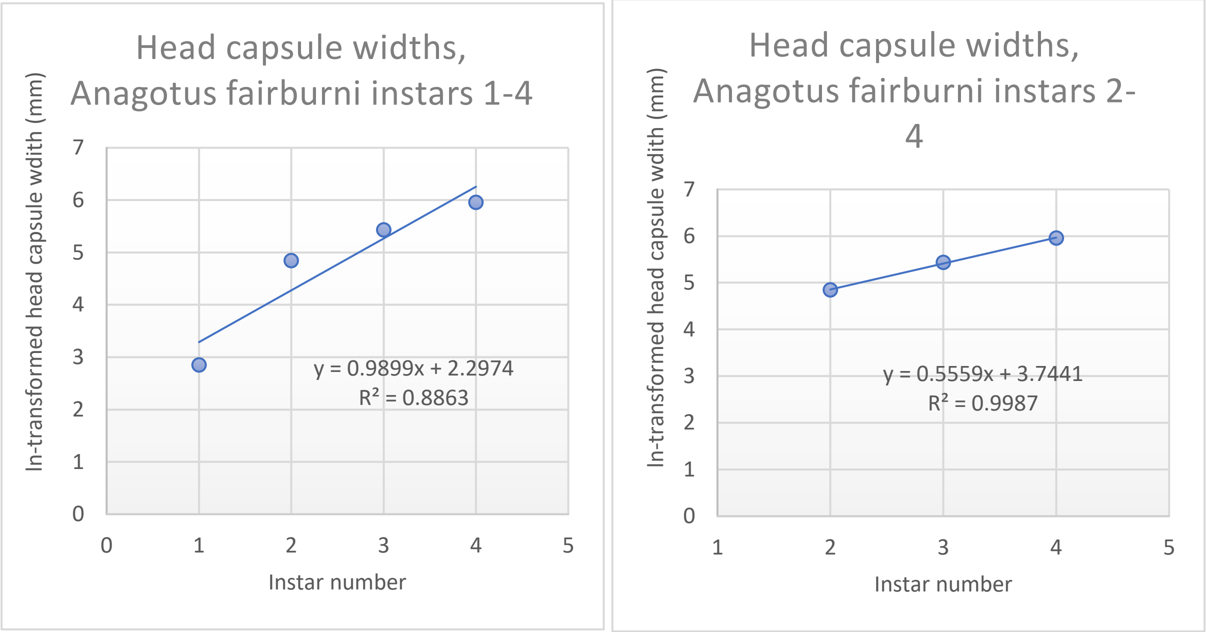


Figure 40. The ln-transformed head capsule width measurements and their linear regression equations for flax weevils (*Anagotus fairburni*) found on Mana Island in 2019.

Given that the data for larger instar sizes (instars 2, 3 and 4) showed conformity with the Brooks-Dyar and Crosby's ratios and met the assumptions of linearity when ln-transformed, I felt confident that they are likely to be correct estimates for head capsule width of older flax weevil larvae on Mana Island. Armed with this data, I then tried to predict the likely number of smaller instars and their head width values, by starting from the largest head capsule width and calculating instars and their possible head capsule width sizes using the Brooks-Dyar ratio of 1.72 (average of the two values from Table 10). Using an estimated Brooks-Dyar ratio of 1.72 suggests existence of six or seven instars, before coming within size range of our smallest observed larvae (Table 11).

Table 11. Estimated instars for the flax weevil (*Anagotus fairburni)* projected from largest to smallest based on the observed mean head capsule width for the largest instar and the mean observed Brooks-Dyar's ratio of 1.72.

Larval Instar #	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Head capsule width (mm) estimated	0.149	0.256	0.441	0.758	1.305	2.245	3.861
Head capsule width (mm) observed	0.173	-	-	-	1.270	2.292	3.861

As mentioned previously, I had hypothesised 5 to 6 instars in *A. fairburni* based on previous work on New Zealand weevils (McBurney, 1976; Schops, 1998), but much larger number of instars is possible in weevils from the same tribe as *Anagotus* (Aterpini). For example, the raspberry weevil *Aegorhinus superciliosus* (Guérin-Méneville, 1830) has received a lot of scientific attention due to its destructive feeding on economically important berry crops in southern Chile, so there is detailed information available on its lifecycle (Aguilera & Rebolledo, 2001). When raised in a lab on alternate host plants (*Daucus carota*) this weevil went through 14 instars with very low Brooks-Dyar ratios (<1.2 on average) (Aguilera, 2001). Such large number of instars could be the artefact of lab environment and diet, but it does show that a much wider range of instar stages is possible in this tribe of weevils.

There are a couple of other important factors which may be influencing our results, such as intraspecific variation in weevil larvae size. Goldson et. al. (2008) found that larvae of the Argentine stem weevil *Listronotus bonariensis* (Kuschel, 1955) sorted into different size groupings reflecting different generations and seasons, something the authors hypothesised may have to do with differing environmental conditions. Goldson et. al. (2008) also found that within each generation and season the larval instars would mostly conform to Brooks-Dyar rule (with ratios close to 1.4) and concluded that four instars are most likely to be correct for this weevil. Interestingly, it has also been shown that sexual dimorphism in weevils can affect larval head capsule widths, particularly in later instars, as shown in the pea leaf weevil *Sitona lineatus* (Linnaeus, 1758) (see Hamon, 1984). This may be a factor in *Anagotus fairburni,* as there is some evidence that females are slightly larger than males (Unpublished data, W D Brockelsby, 2022).

To conclude, based on data presented here, it is likely that the flax weevil (*Anagotus fairburni*) has six or seven instars. Ultimately, a stronger sample size, especially for smaller larvae, and more accurate measurements using lab equipment would help to confirm our hypothesised number of instars. Further work is being pursued in this area on Mana Island now, where a ‘captive rearing’ experiment is being undertaken in-situ on the island.

Weevil Marking

Introduction

Flax weevils are nocturnal, flightless insects that now only persist in remote alpine areas or islands, often at quite low densities. Very little is known about the longevity, movement, or behaviour of these weevils. On Mana Island, flax weevils in large numbers are advancing steadily across the island, with clear evidence of their presence shown by their characteristic feeding damage appearing on plants spreading outwards from the release site[3]. When Mana Island's weevil situation was raised with invertebrate ecologist Eric Edwards (Department of Conservation), he commented that these flightless weevils can live a long time and walk considerable distances (E. Edwards, pers. comm., 2019).

Given a unique opportunity to experimentally manipulate flax weevils on a large scale as part of the *Beauveria* trial release, we decided to make the most of it by developing a marking program. The purpose of this marking program was to help understand if flax weevils also shared similar longevity and movement characteristics as those reported in the genus *Hadramphus*, while also understanding how flax weevil behaviour may influence any potential management prescriptions for the island's flax weevil population.

Methods

Marking

A total of 1600 flax weevils was moved as part of the *Beauveria* trial experiment, as described in Chapter 3. Of these 1600, 430 weevils were individually marked prior to their release in March 2020. The weevil marking process took place over two nights, during the *Beauveria* release weekend on February 29 and March 1, 2020. On both nights, 215 adult weevils were captured by volunteers via night-time hand searching and were brought into a weevil processing room setup in an office on Mana Island.

In the weevil processing room, weevils were individually weighed using field micro scales with accuracy of 0.01 grams. They were then marked using a unique plastic bee tag bought from the New Zealand commercial bee company Ecrotek. The bee tags were numbered 0 to 99 and came in a range of five colours (red, yellow, white, green, and blue). Each individual weevil was marked with a

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[3] Weevil dispersal across the Mana Island is not completely uniform and there is a distinct possibility that they are inadvertently being moved around the island by hitchhiking on people and vehicles. This happened to the author and the Mana Island ranger on multiple occasions.

single tag and its colour-number combination was recorded; alongside the marked flax plant the weevil was released on.

Most weevils were marked on the upper to mid elytra, though a few of the earliest weevils were marked on the thorax instead (Fig. 41). In the actual marking procedure, each weevil was gently restrained by an assistant who would then add a small drop of Selley’s superglue to the elytra. I would then use tweezers to gently place the bee marker onto the glue, and firmly press down on the marker to ensure its proper adhesion. Acetone was available to remove any excess glue from the weevil’s surfaces. Once marked, weevils were placed into ice cream containers for several minutes to give the glue time to dry. Marked weevils were then transported across the island and released onto the experiment plants on the same night, by being placed carefully into the base of their designated flax plant.

Figure 41. A group of flax weevils found during a night monitoring session on Mana Island in March 2020. Note that 'Green 3' had a marker placed on the thorax, while 'Yellow 76' and 'Blue 3' have their markers placed on the upper-mid elytra. Also note the weevil’s characteristic habit of gripping onto each other when handled. Photo: W. Brockelsby, CC-BY.

Follow-up monitoring and data analysis

Subsequent monitoring of marked weevils followed the methodology outlined in the Flax Community Monitoring: Night Spotlighting section, where weevils and other animals were searched for on each marked flax plant, then counted and recorded by volunteers and the author during a scheduled FOMI work trips.

An additional search was scheduled a week after the release (March 8 - 9, 2020), to try to understand any immediate post-release behaviour of flax weevils, as I considered it possible that weevils may undertake significant movement after being translocated across the island.

Follow-up monitoring of marked weevils was primarily focused on the trial site of 80 flax plants; however, a couple of informal searches were also performed in late 2020 and 2021 outside the experiment area to attempt to find any weevils that may have left the experimental flax site. These informal searches involved spotlighting flax plants around the periphery of the trial site, spotlighting specifically for marked weevils only.

All marked weevil resighting events were recorded, and weevil movements analysed to understand their longevity and movement patterns.

Results and Discussion

The first flax weevil monitoring event took place one week after the release in March 2020, during a special monitoring trip done by one volunteer and the author, to see if any significant movement had occurred soon after the release of weevils at the trial site. On this first monitoring trip, almost all marked weevils recaptured were found on their release plants or on an adjacent plant, with minimal initial movement. On one plant six marked weevils (out of a possible total of 10) were found to be out feeding on the flax plant that they were released on. As we transitioned to the regular monitoring every two months in 2020, marked weevils began to be found beyond the plants that they were released on. Typically, they were found on plants nearby, although others travelled to adjacent transects further from their release plant. Summary information is available in Fig. 42.

In October 2020 the first marked weevils were located outside of the experimental site, on unmarked flax plants. In February 2021 a marked weevil was found well outside of the *Beauveria* trial site, on a flax plant 88 meters from its original release plant. This movement is unsurprising given what is known about ecology of other New Zealand weevils, including the critically endangered Canterbury knobbled weevil (*Hadramphus tuberculatus*). Fountain *et al*. (2013) found a *H. tuberculatus* weevil that moved 190 m in Burkes Pass Scenic Reserve. Another long-term weevil marking study done in New Zealand was that by Schops (1998) looking at the Chatham Island coxella weevil (*Hadramphus spinipennis*). Schops also found that the coxella weevil could travel up to 600 m, with the collapse of their host plants triggering long distance migration of weevils (Schops, 1998).

Overall, 168 marked weevil recapture events were recorded from March 2020 (the release date) to October 2021, the date of last flax weevil monitoring before thesis write-up. Of these 168 recaptures, 139 were flax weevils with unique markers, seen at least once after their release. Most marked weevils found were only resighted a single time after release, with 25 only found more than once (Table 12). Most of recaptured weevils that moved beyond their lines moved from the end of the C and D transects into the then healthier plants in the A and B transects. This followed the general trend of newly arriving weevils at the end of the site documented in Chapter 3.

Table 12 Summary of marked flax weevil recapture results on Mana Island.

Total number of weevils marked	**Total recapture events**	**Unique weevils resighted**	**Flax weevils found once**	**Flax weevils found twice**	**Flax weevils found three times**
430	168	139	114	21	4

Not unexpectedly, recapture rates of marked weevils were comparatively high shortly after the release of the weevils but slowed down relative to the total unmarked weevil sightings over time, as shown in Fig. 43. We marked 430 of the 1600 weevils we introduced to the site, which was a ratio of 26.9%. Our first two recovery sessions only recovered 7.5% and 7.1% of marked weevils out of all observed. A basic Lincoln-Petersen index (N = (M x S)/R, where N is a population estimate, M is 430, the number of marked weevils we released, S is the total number of weevils found in a monitoring session, and R is the number of marked weevils we recovered in the same monitoring session) suggests a much larger population than we released, with values of 5,748 to 6,029 weevils using the samples from first and second week after release (Lettink & Armstrong, 2003). The assumptions of Lincoln-Petersen are not met here as the site is open to immigration and migration, and there is an unknown value for tag loss, but the estimate is far higher than the 1600 weevils we expected at the site so soon after the release.

The oldest weevil sighted was ‘Yellow 54’, found in October 2021, after an eight-month gap in monitoring. This weevil was marked in March 2020 as an adult of unknown age, which suggests that flax weevils can live as adults for 20 months or longer. This longevity puts them on par with reported longevity in other large-bodied weevils in New Zealand (Table 13).

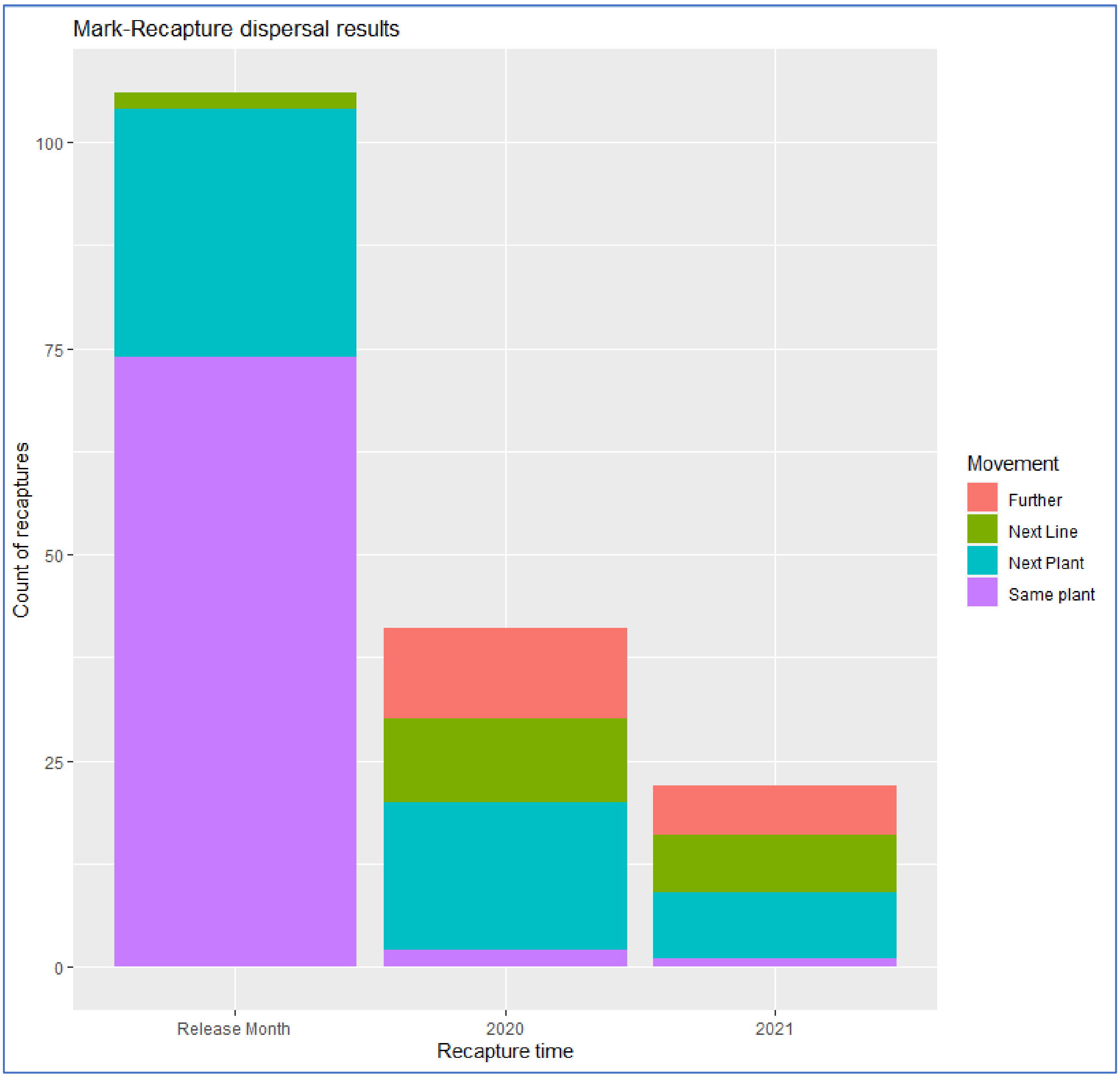

Figure 42. Results for the recapture of marked weevils. Categories are as follows; “Next plant” shows that the weevil was recovered on the same transect close to its release plant. “Next Line” means that the weevil was recovered from a transect adjacent to the one it was released on (e.g., a weevil release on plant B5 was count in the A or C lines). “Further” meant that the weevil had travelled across two transects (e.g., from A to D) or was found outside of the trial site.

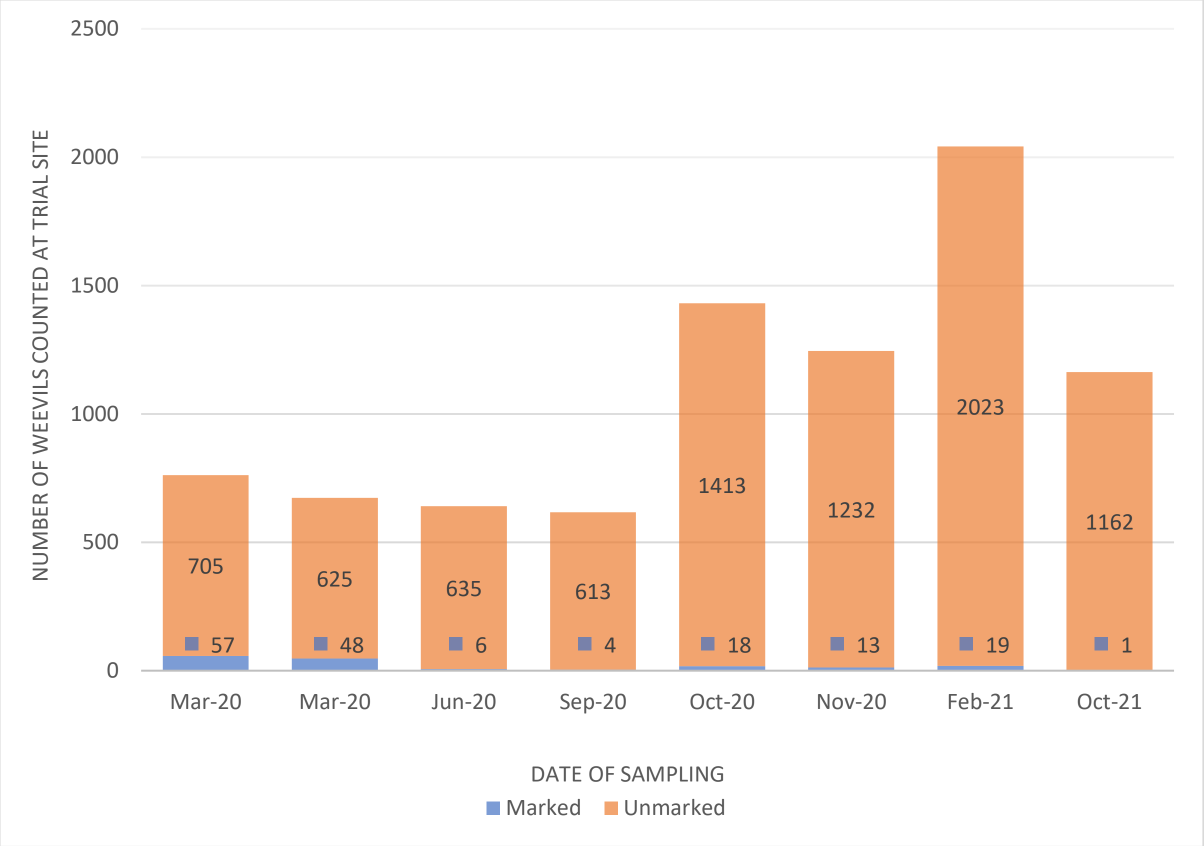

Figure 43. Recaptures of marked flax weevils (*Anagotus fairburni*) on Mana Island. Each count relates to the total count on the trial site in a standard night monitoring trip.

Table 13. Lifespan information for large-bodied weevils (Curculionidae) found in New Zealand.

Tribe	**Genus**	**Species**	**Maximum observed lifespan (months)**	**Survey method**	**Reference**
Molytini	*Karocolens*	*pittospori*	36	Captive rearing	Bennett (1987)
	Hadramphus	*spinnipenis*	45 (males) 28 (females)	Mark recapture	Schops (1998)
		tuberculatus	13	Captive rearing	Fountain (2016)
	Lyperobius	*huttoni*	30	Mark recapture	Bull (1966)
Aterpini	*Anagotus*	*stephenensis*	28	Mark recapture	(M. Anderson, unpublished)
		fairburni	20	Mark recapture	W. D Brockelsby (2022)

The extended longevity of New Zealand large-bodied weevils is interesting, as weevils are typically thought of as quick reproducing short-lived animals, often tied to seasonality or weather conditions, but here it is clear that several species within different tribes can live as long as some small mammals. It is possible that this longevity is just a function of their size, as Holm *et. al*. (2016) found a clear correlation between body size and longevity in geometrid moth species, noting a 1.3-fold increase in lifespan when body size increased two-fold (Holm, et al., 2016). It could also be that there is a good ecological or adaptive rationale for longevity in these weevils as they share very similar ecological life histories, being flightless specialist herbivores on one or two genera of plants with an ectophytic larval stage. This longevity may also relate to the cooler environmental conditions in temperate New Zealand. Insects have been shown to die faster in warmer environments as their metabolism speeds up and exhausts the insect's reserves faster (Wagner, Gagne, Sharpe, & Coulson, 1984; Brown, Hannebaum, Eaton-Clark, Booth, & O'Brien, 2022). Understanding the seasonality and longevity of this guild of weevils is of wider interest than the management of Mana Island, as many of the weevils listed in Table 13 have relict distributions and are considered nationally endangered (Leschen R. A., 2012).

Two marked weevils were subsequently found mating after release. 'Yellow 22' was a female who mated shortly after re-release at the trial site, in March 2020 (Fig. 45). Another marked female weevil, 'Blue 51' was found mating almost a year later in February 2021. The observation of Blue 51 mating in 2021 may imply that female weevils could be able to mate more than once in their lives, given that the female weevil was already at least 11 months old when this mating was observed. It is not impossible that Blue 51 had never mated yet and the mating observed in 2021 was its first, but I believe the more parsimonious explanation is that female flax weevils may mate multiple times over their lifespan.

Figure 44. A marked female flax weevil (*Anagotus fairburni*) ‘Yellow 22’, mating soon after release on Mana Island, March 2020. Photo: W. Brockelsby, CC-BY.

Further population estimates beyond the initial first two trips were not investigated using this flax weevil mark recapture data, as this wasn’t the primary focus of the work. The flax weevil population at the site was very open to immigration and death, being surrounded by non-marked flax plants which we were not surveying. For practical reasons we were unable to run subsequent nights of sampling, and our primary goal was to better understand how flax weevil behaviour at the site may impact our trial, and to attempt to learn more about their longevity. It is clear the flax weevil population at the site grew well beyond the 1600 weevils released, given our imperfect survey technique counted over 2042 weevils on one weekend.

This increase of the weevil population at the site and recapture rates after the translocation suggest that flax weevils may not have a strong density dependence trigger for migration, as seen in some insects (Fonseca & Hart, 1996; Rhainds, Shipp, Woodrow, & Anderson, 2005; Nowicki & Vrabec, 2011). This lack of a density dependency trigger for migration matches what Schops found on Mangere Island with coxella weevils. Schops (1998) hypothesised that the risk of finding food and a mate on an island where host plants are unevenly distributed outweighed the rewards from escaping intraspecific competition at the site. She further theorised that this resistance to early dispersal by the coxella weevils resulted in the over-exploitation of food plants and the eventual forced dispersal of all individuals to a distant host plant population. In good conditions on Mana Island, I have seen small numbers of flax weevils on flax plants that are quite far from other larger populations of weevils. These individuals have obviously dispersed earlier than their conspecifics, and it would be interesting to understand what cues there may be for this dispersal. It is possible that flax weevils show behavioural plasticity, with certain individuals showing a greater propensity to wander than others. Currently on Mana Island a flax weevil prone to dispersing early may gain a significant competitive advantage over weevils who stay put, given the ease with which such a weevil could potentially find new and unexploited host plants on the island. It is also possible that there is a mechanistic trigger behind dispersal, for example in maritime ringlet butterflies *Coenonympha nipisiquit* McDunnough, 1939, females were found to initially spend time ovipositing near their natal location and as they got older tended to become more likely to disperse to other habitats (Sei, 2008). Given the long life of the flax weevil, it would be interesting to understand whether older weevils are more likely to disperse than recently emerged adult flax weevils. Our low recapture rates for marked weevils at the flax trial site could possibly be a sign of age-triggered emigration rather than deaths.

In this study it was difficult to ascertain any quantitative assessment of tag loss, no weevils that had lost their marks were knowingly retrieved during this work. Related follow-up studies on Mana Island investigated this further, by double-marking weevils using the same methodology: 20 weevils were marked using the same bee tags and glue, with one tag on the upper elytra and one on the lower elytra. Seventeen of these weevils were eventually resighted and two marks had been lost from a total of 34 (Unpublished data, W D Brockelsby & C. Miskelly). This represents a tag loss rate of almost 6%, which could be used as a conservative basis for further quantitative mark recapture population work using this methodology. Our methodology worked well for the purposes of identifying individuals, especially easily from a distance, though it may not be practical for larger mark-recapture programs that aim to answer population questions. I’d recommend a lighter weight marking approach for efficient marking like the methods used by (Anderson, Hartley, & Wittmer, 2022) when monitoring Cook Strait click beetles *Amychus granulatus* (Broun, 1883) on Te Pākeka/Maud Island.

Chapter 5 General Discussion

Beauveria trial

Our understanding of the impact of applying *Beauveria* in the field is still ongoing, much of what we think is still subject to change as it involves long term processes. The true story of flax weevils on Mana Island will continue to be told past this work here. Restoration ecology is not a simple process, and this trial involved multiple phyla and their interactions. Best efforts are made here to describe what we have observed from the experimental manipulations, and to be understand what insights may be able to be gleaned to date.

Flax plant monitoring

In general, there wasn't a strong trend in our quantitative data that explained why some plants appeared healthier than others, except for the fact that A line plants have been heavily browsed by weevils for the least amount of time at the time of analysis. Most plants at the site in the B, C, and D lines were in a state of heavy collapse, with just a couple of plants as exceptions. Monitoring will continue into 2022 and beyond to further try see if conclusions shift over time, but at this stage it seems that this was an unsuccessful attempt to use *Beauveria pseudobassiana* to protect flax plants from collapsing in the face of thousands of flax weevils.

Soil moisture was a key variable in our original hypothesis, as it had been hypothesised that plants in damper conditions were more likely to survive, but no evidence of this was detectable in these results. It is possible that better measurements of soil moisture, using automated soil readers would show more of a trend than I could observe with the manual measurements taken around boat trip times.

One hypothesis that I was interested in was the periodic drowning hypothesis mentioned in Wehi (2007), where early flax plantation managers would periodically flood the flax to drown any animals living in the soil below the flax. At the trial site one low lying flax plant on the B line, B11, was a very large flax, often observed at least a foot underwater. It was often so wet at this plant that it would be impossible to arm the pitfall traps underneath it. In October 2021, I had expected this plant to be healthier, but found it much like the rest of the line, heavily collapsed with a score of 4.5. This suggests that winter inundation did not seem to affect the flax weevil larvae's survival in this plant. When I collected larvae for the larval head measurements described in Chapter 3, I did notice that inundating the larvae in ethanol was not a particularly humane or effective way to euthanise the larvae. They seemed to be able to survive total inundation in ethanol for at least 10 to 15 minutes. This is not that surprising, pupae of the tobacco hornworm *Manduca sexta* (Linnaeus, 1763) have been shown to be able to survive inundation for up to four days, with fifth instar larvae less resilient, surviving at least four hours of immersion (Woods & Lane, 2016).

Better understanding of the mechanics of the death of the flax plants and the flax weevil larvae's herbivory would be a valuable area to study, as the cause of death of the plants has not been thoroughly investigated in this work. Our working hypothesis on Mana Island is that it is a combination of drought stress over the dry months and weevil herbivory in the roots that interacts to reduce the plant's access to water, and ultimately causes their collapse. Cockayne (1919) describes the flax root as having a thin outer epidermis "skin" which allows water intake, if large weevil larvae living in the soil were regularly damaging this epidermis then it could be that plants are indeed dying of lack of water or nutrients due to root damage (Cockayne, 1919; Wehi, 2007). While direct herbivory and root damage seem to be the most likely mechanism behind plant mortality, many plant herbivores carry pathogens that cause the death of the host plant over time as well. Early 20th century commercial cultivation of *Phormium* was heavily affected by yellow leaf disease a specialised phytoplasma bacterium which was spread by an invertebrate host vector, the plant hopper (*Oliarus atkinsoni*) (Boyce & Newhook, 1953). It could be possible that some pathogen is an important mechanism in the death of the flax, rather than destruction of the roots and drought stress as we have assumed here. No obvious disease was noted during the work I did at the site.

When reviewing the plant collapse and soil core results it seemed possible that the trial results reflect a dosage response and that the level of *Beauveria pseudobassiana* spores required to successfully protect the flax bushes from the 1600 adult flax weevils and their offspring might be higher than what we used. Each flax plant was dosed with a 11.4×10^9 strength fungal mixture, but the ability for *Beauveria* to persist in the soil can depend on many abiotic and biotic factors such as; soil moisture for growth, direct UV light killing propagules, heavy rain washing out spores, good ground cover vegetation and even secondary hosts like collembola to act as vectors for infection (Blond, 2012; Vukicevich, Lowery, Bennett, & Hart, 2019). Dosage effects for *Beauveria* and other fungal entomopathogens are reported across literature, with LD_{50} (lethal dose required to kill 50% of insects) concentrations varying for some species and strains, depending on the dosage and method of fungus application (Watson, Geden, Long, & Rutz, 1995; Zaki, 2009; Sönmez, Demir, Bull, Butt, & Demirbağ, 2017). Overall, less flax plants collapsed under the *Beauveria* treatment than without it. One data point suggests itself to a dosage response from our trial. As discussed in Chapter 4, flax plant D5 had the highest *Beauveria* count from the soil core samples at the 10^{-4}/gm soil and was also the healthiest plant on the line by a good margin with a plant collapse score of just 2. This compares to an average score of 4.05 for the rest of D line's flax plants. Flax D5 was in a shady vegetated area a bit below the average lie of land when compared to other plants, so it is entirely possible that the conditions for the fungus to survive there were optimal. It will be interesting to follow the health of this plant in 2022 and beyond.

Timing and method of biocontrol releases can also be crucial to a successful biological control intervention, as it is important that the pathogen comes into direct contact with the host species (Fernandez, Groden, Vandenberg, & Furlong, 2001; Wraight & Ramos, 2015). I attempted to time the release to be in February or March, in synchrony with the observed egg laying period reported by Gourlay (1931). However, before this work we lacked any other data on flax weevil phenology, and so this timing was just a best guess based on limited data. Information gathered during this work suggest there may be an extended laying period across the spring and summer months (see Flax Weevil Ecology in this Chapter), and it may be more important to release *Beauveria* in optimal conditions for its persistence in the soil, rather than during the dry hot summer months in Feb and March when the soil is low in moisture content across the island. Improved methods to ensure the larvae come into contact with the *Beauveria* should be considered.

Soil core results showed positive *Beauveria* results for several plants that were in the control group, often on plants that were dead or close to dying. When read alongside the movement data generated for the adult weevils, this finding suggests that fungus able to be moved by adult weevils at the site, although not quickly enough to protect the plants. The idea that the more fungus resistant adult flax weevils spread *Beauveria* is not new. It is assumed that they brought *Beauveria* spores over with them from Te Pākeka/Maud Island when the weevils were originally translocated (Glare, 2017).

Another factor that may have contributed to the flax trial result was the number of weevils we chose to stress the plants with. When working with coxella weevils (*Hadramphus spinipennis*) Schops (2008) found a critical threshold of host plant to weevil density ratio of 18, meaning if there were on average more than 18 coxella weevils per plant it would lead to the local extinction of the *Aciphylla dieffenbachii* plants at the site. Looking at flax weevil count data when compared with flax plant collapse scores there is a suggestion that using our monitoring methods, sustained counts of 15 or more flax weevils per plant using our method is likely to lead to the eventual collapse of flax plants without intervention. Whether this is a true threshold is still speculative as flax plants are highly variable in size (and are much larger than *Aciphylla dieffenbachii*). However, the trial showed us that when 20 weevils were added to each of the site's plants, half of them had completely collapsed within 20 months. Weevil counts dropped to below to 10 weevils per plant when the plants were dead or near death, as the weevils migrated away from the dying bushes.

One of the key learnings from this work is that given the complexities in dealing with *in-situ* field trials like this, and the significant investment of money and time in setting up of a trial site it would be recommended to err on the side of slightly overdoing the dosage of *Beauveria* or any other fungal pathogen, within sensible boundaries. It would be interesting to try further applications of this strain of *B. pseudobasssiana* on the island at higher dosages to see if small patches of the plants could be protected. While this may not be scalable as a blanket prescription for the entire island, if it worked it could potentially give island managers a tool to use to protect important patches of flax e.g., if important areas of flax were identified as strongholds for the goldstripe gecko (*Woodworthia chrysosiretica*), or flax that had important cultural or historical values.

Much recent research is occurring in agricultural science on the possibility of plants' endophytic uptake of *Beauveria*, with some results showing strong benefits to plant growth, many plants uptaking fungus into their root systems and leaves (Jensen, Cabral, Enkegaard, & Steenberg, 2020; Macuphe, Oguntibeju, & Nchu, 2021; Mantzoukas, et al., 2021). It would be interesting to trial this with Mana Island flax and this native fungus strain to see whether flax might be able to also incorporate this strain of *Beauveria pseudobassiana*, and lead to being a feasible alternative to the manual application of a higher dose of a fungus in the field. Though the scalability of growing flax in this way may inhibit the desirability of this technique.

Flax community monitoring

The large numbers of flax weevils showing up at the site and the condition of the flax plants has undoubtedly had an impact on the animal community at the flax site, though this has been hard to measure quantitatively in the short period of 20 months after the weevil release. Even with the extended time available to the author during this write up, ecological change studies like this are best performed over a longer period than a thesis allows. Some trends are beginning to form but the data presented here is still provisional and follow up work will continue at the site in 2022 and onwards to better understand the long-term effects of weevil arrival and subsequent loss of large flax plants from the site.

All pitfall data analyses seem to suggest that no obvious change to the ground dwelling flax plant communities occurred directly after the *Beauveria* release. Limited searching for *Beauveria* infected animals was done by the author and volunteers while arming pitfall traps, and this turned up no direct evidence of animals of any species infected with *Beauveria pseudobassiana* at the site. This was quite promising as the species detected in pitfalls covered a range of ecological niches, but all share a habit of being primarily ground and soil-dwelling, and thus either directly susceptible to *Beauveria* (e.g., Arthropoda) or indirectly via trophic impacts at the site (e.g., *Oligosoma* sp.). Our introduction of *Beauveria pseudobassiana* didn't seem to affect the ground-dwelling soil community of the trial site directly. This may just be due to our low dosage rates, and it can't be completely ruled out that with higher application dosages or more time to persist other animals may show up with *Beauveria*. There was at least one other species of *Beauveria* detected at the site, occasionally turning up in our pitfalls on adult *Mimopeus* spp. beetles, but this was not the species or strain we released (T. Glare. pers. comm. 2021).

A possible source of bias in night monitoring data is the influence of increasing flax weevil counts. At the end of study, such was the weevil abundance on the flax plants, that it could make it difficult to count any other animals in the minute allowed for monitoring. The weevils were so conspicuous on the plants that it would have been easy for volunteer observers to miss other species, especially geckos that will actively hide when they hear noise or observe light, or cryptic immobile leaf-veined slugs. Our recorded declines in the Atharocophoridae slugs and *Woodworthia* geckos may be influenced by this observer bias, although no decline in other invertebrate taxa was observed. Irrespective of our monitoring methods, flax bushes are excellent habitat and refugia for the nocturnal animals, so while I believe Atharocophoridae slugs and *Woodworthia* geckos will persist at the site after the reduction of the flax plants at the site, I believe the implication that these species will see a decline in population to be a valid one.

Gecko night counts at the flax site showed a population structure (10 to 1) in favour of Raukawa geckos (*Woodworthia maculata*) over goldstripe geckos (*Woodworthia chrysosiretica*) at the site, though no major up effort was made to follow up the accuracy of these counts. Unpublished work done on other parts of the islands have found large flax bushes where this number is reversed, and Raukawa geckos are far less common than their goldstripe counterparts (Unpublished data, W D Brockelsby, C Miskelly, 2021). *Woodworthia* geckos can get to very high densities and these species' diets do overlap, with both goldstripe and Raukawa geckos known to feed on invertebrates, but goldstripe geckos were not reported to consume nectar by (Hare, 2016). Whether there is some competitive exclusion happening in the distributions of these two species on Mana Island would be interesting to investigate further.

Skink community composition at the trial site consisted of three species, the copper skink *Oligosoma aenum*, the northern grass skink *Oligosoma polychroma,* and the brown skink *Oligosoma zelandicum* (Gray, 1843). The copper skink was the most abundant, three times more common than the grass skink, with the brown skink comparatively scarce at the site[4]. As the flax disappears and more open rank grass takes over the site it is expected that the skink community composition will shift in favour of the heliothermic northern grass skink, over its cover-loving cousin the copper skink (van Winkel, Baling, & Hitchmough, 2018). Our catch data showed that these skinks were most detectable in late November or early December when compared to all other months we trapped, though pitfall traps were never run between the holiday period of late December to mid-February, and so no direct comparisons are possible for those times.

While our pitfall trapping was primarily designed to understand community changes at the flax plant trial site after the release of *Beauveria*, there is long-term value in continuing the monitoring beyond the scope of this trial. The monitoring programme provides an opportunity to shift the focus from what impact the fungus may have on the community, to understanding what the loss of the flax plants themselves is doing to animal communities at a typical Mana Island site. Less intensive, but continued monitoring of the pitfalls at the site would enable managers to understand the true impact of the loss of flax at the site.

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[4] *Oligosoma zelandicum* can look superficially like the other *Oligosoma* at the site, and it is likely the true number was underreported in our data.

Flax Weevil Ecology

Three years of monitoring flax and flax weevils on Mana Island enabled a lot of opportunity for observations on flax weevils, which I will attempt to summarise in this section. The experimental marking of the weevils showed their capacity for dispersal and longevity, and the regular night counting of large numbers of flax weevils from the trial site mid-outbreak and post-outbreak also meant that large numbers of individuals could be observed, of what is a normally comparatively cryptic animal found in relative low abundance in other sites. Much of this material is based on field observations.

Breeding and lifecycle ecology

Mating

The observed mating period of adult flax weevils on Mana Island was from June to March, and eggs were photographed from October to April suggesting a possible prolonged egg-laying season. Observations of marked weevils mating in February 2021 (Chapter 4) suggest that it is possible that mating occurs more than once in a weevil's life and that females remain fecund across their lifespan. Mating took place with the male on the back of the female (Fig. 45) and in some cases lasted for hours, some pairs kept overnight would not be separated in the morning. The female will often move freely with the (slightly) smaller male on her back. It is unclear whether this represents some form of mate guarding behaviour by the male, as is seen in many other beetle species (Dickinson, 1995; Harari, 2003; Chaudhary, 2015). No fighting or other intraspecific competitive behaviour was observed between flax weevils, although such behaviour (e.g., jostling for position) does occur in other weevils such as seen in giraffe weevils *Lasiorhynchus barbicornis* (Fabricus, 1775) (Painting, 2014).

Figure 45. Copulating flax weevils (*Anagotus fairburni*) on Mana Island, September 2019. The male grasps the female across the upper elytra with his forelegs while using the other legs to steady himself for copulation. Photo: W. Brockelsby, CC-BY.

Eggs and Oviposition

Eggs were identified based on Gourlay's descriptions (Gourlay, 1931) and based on their relative abundance, size, and association with flax. However, the identity of the eggs was not confirmed formally by rearing. The large eggs loosely spread around bases of infested flax plants are very likely to be flax weevil eggs. There are sympatric large-bodied Carabidae beetles on Mana Island (*Megadromus capito* and *Mecodema oblonglum*) which may have eggs of similar size, but *M. capito* is known to show parental care behaviour with their eggs and first instar larvae, and they and other Carabidae often lay eggs in underground chambers (Hutchison, 2001). The observed eggs also match photos of closely related weevils in Chile, *Aegorhinus superciliosus* (Parra, Mutis, Aguilera, Rebolledo, & Quiroz, 2009). No flax weevil parental care over eggs was observed on Mana Island, nor was oviposition directly observed.

Eggs were not seen laid in neat lines as described by Gourlay (1931) but were found loosely deposited in a variety of locations in the base of flax (Fig. 46). It is possible that Gourlay's (1931) neat rows of eggs was an artifact of artificial circumstances of his study (e.g., a lack of oviposition sites) rather than typical field behaviour.

Figure 46. Presumed flax weevil (*Anagotus fairburni*) eggs found in the base of a dying flax leaf in April 2019, on Mana Island. It is unclear whether these wet eggs were viable and would hatch. Photo: W. Brockelsby, CC-BY.

Figure 47. A single (presumed) flax weevil (*Anagotus fairburni)* egg laid near the base of a flax fan on Mana Island, October 2020. Photo: C. Stephens, CC-BY.

Larvae and Pupae

Larvae of all observable instars (Chapter 4) were found at the bases of flax plants in overlapping size cohorts, most likely reflecting a combination of a long oviposition season and long development. Excavation of flax plants showed that the early instar flax weevil larvae were typically found burrowed into the moist base of the flax fans. They would dig into soft green tissue and excavate an area of the flax leaf and cause distinctive yellowing damage to the tissue. They were mainly found on the outer leaves which were a bit older, as the central newer leaves were much firmer and covered in a thick protective liquid. As the weevil larvae grow, they migrate further down the plant before eventually burrowing into the flax plant root, where they excavate a chamber (Fig. 48). Different instars utilising slightly different sections of the flax plant may be an example of intraspecific resource partitioning of the primary host within the different life stages (Chen, 2021) . Partitioning occurs between adult flax weevils, which exploit the foliage, and the larvae, which exploit the root and base of the same plant without direct competition. Pupae weren't directly searched for, but a few pupal chambers like those described in Gourlay (1931) were found and one pupa was directly observed by FOMI volunteer Andrea Wilson in October 2020 (Fig. 49). No clearly teneral adults were observed during the brief visits to Mana Island.

Figure 48. A late instar flax weevil (*Anagotus fairburni*) larva in a chamber found in a flax plant root on Mana Island, May 2019. The larvae lie headfirst in the chamber and use their strong jaws to feed on the root. Photo: W. Brockelsby, CC-BY.

Figure 49. Pupa of a flax weevil (*Anagotus fairburni*) found on Mana Island in October 2020 by volunteer Andrea Wilson. Found under a dead flax plant, and shows a pupal chamber loosely constructed around it. Photo: W. Brockelsby, CC-BY.

Adult Weevils

Sexing live adult flax weevils is difficult. With other large New Zealand weevils, the curvature of the final abdominal segment is diagnostic (Craw, 1999; Fountain E. D., 2013), but in flax weevils no clear morphological differences were noticed.

Many other species of Coleoptera are sexually dimorphic, with females larger, e.g., the Stephen's Island Weevil *Anagotus stephenensis* (Anderson M. , 2018). Previous unpublished work in the Marlborough Sounds and Picton area had led us to believe that it might be possible to differentiate flax weevil sexes by weight (M. Anderson, pers. comm. 2019). To test this hypothesis 430 weevils were weighed using field micro scales with accuracy of 0.01 grams. These weights were recorded just prior to the weevils being marked with bee tags during the wider experimental *Beauveria* release. Weight data showed some evidence of a bimodal peak, possibly showing separate peaks for slightly lower weighted males and slightly heavier females (Fig. 50). Without a reliable way of determining between the sexes I didn't pursue this much further; however, two weevils that we determined to be females from their mating positions, weighed 2.26g and 2.34g respectively when they were originally marked (i.e., they were among the heaviest weevils weighed).

During fieldwork on Mana Island in 2019, weevil enthusiast Mark Anderson noticed that many weevils were showing a ‘black dot’ mark – due to a loss of scales in the middle of their elytra (Fig. 43). This ‘black dot’ mark was obvious at a distance, and we hypothesised that weevils with this black dot were mated females. During mating, the males would rest their rostra exactly where this mark occurred on the female elytra. Given mating events often last longer than 12 hours, it was possible that males leave a mark on the scales of the female’s elytra (Fig. 51). This was not verified by dissecting these weevils, but Schops (1998) reported this behaviour occurring in coxella weevil mating (*Hadramphus spinipennis*), and so this may be a useful if slightly unreliable way to sex weevils quickly in the field.

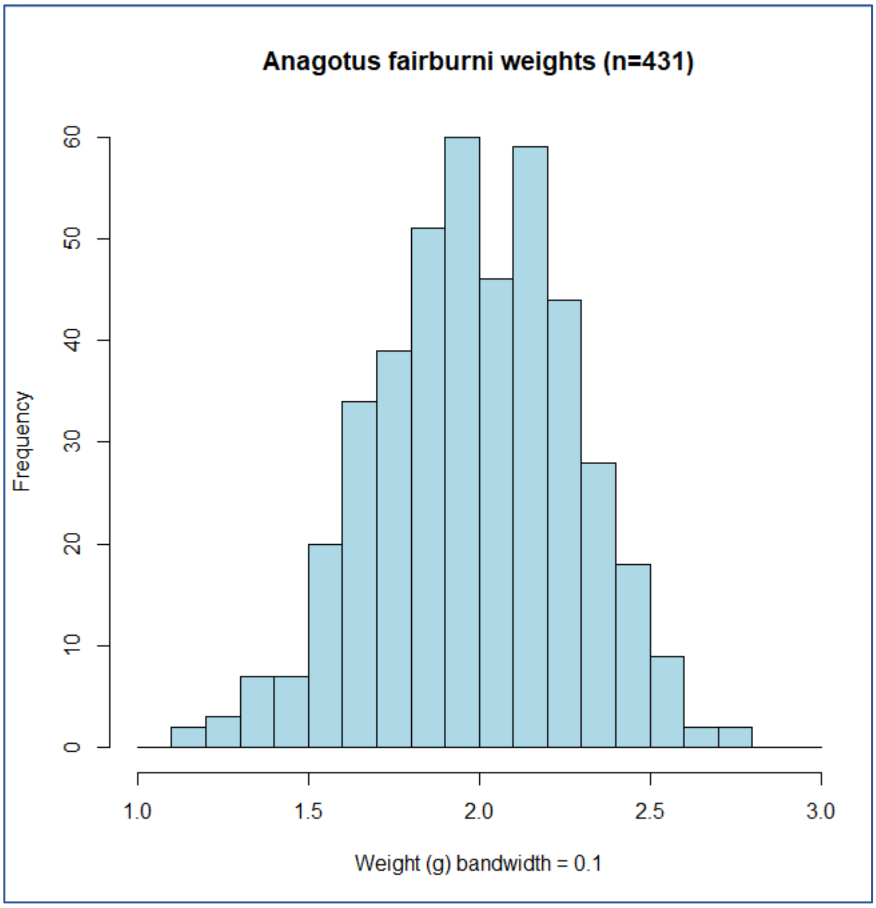

Figure 50. A histogram of the 431 *Anagotus fairburni* weights, showing slight bimodality, possibly due to lighter males peaking at less than two grams and females peaking at slightly over two grams.

Figure 51. A 'black dot' flax weevil (*Anagotus fairburni*), the dot is hypothesised to be a mating mark on a female weevil created by the mating position of the male weevil’s rostrum. Photo: W. Brockelsby, Mana Island, November 2019, CC-BY.

Dispersal behaviour and feeding

Individual marking of flax weevils (Chapter 4) revealed that they can move a minimum of 88 metres away from their release sites. Marked weevils regularly moved between flax plants and were active within a patch of plants. This habit of nocturnal wandering between plants may be a significant factor to the scarcity of flax weevils in areas with mammalian predators. Both rats and mice are nocturnally active, and a nocturnal flightless weevil encountered on the ground would be an easy and attractive item for them to prey on (Badan, 1986; McQueen, Diet of ship rats following a mast event in beech (Nothofagus spp.) forest, 2008).

Flowering flax is strongly attractive to flax weevils, both open and unopen flower stalks support huge densities of weevils feeding on them (Fig. 54). It was these huge numbers of weevils on flax stalks that first alerted Colin Miskelly (Te Papa) that there were larger than expected numbers of weevils on the island, which initiated this investigation. Any flax weevil monitoring done on other islands should be timed to coincide with flowering of the flax, to maximise the likelihood of finding weevils, particularly in areas with very sparse weevil populations. Schops (1998) found similar results with the coxella weevil (*Hadramphus spinipennis*), with individuals found readily on coxella flowers.

Figure 52. Flax weevils on a flax flower stalk at night on Mana Island, Nov 2013. Photo: C. Miskelly, Te Papa Blog.

I observed many flax weevils at night away from flax plants, on a range of other plants such as taupata (*Coprosma repens*) and *Muehlenbeckia australis*. Initially, I assumed that these weevils had attempted to disperse to flax plants but had failed to find flax and were unlikely to survive. However, after the wholesale collapse of the flax plants in the trial site in October 2021, I had the opportunity to observe large numbers of flax weevils off their main host plants, as they spread out from the site. It was during this monitoring trip that I found adult flax weevils feeding on an alternate host plant pohuehue (*Muehlenbeckia complexa*) (Fig. 55, 56).

Despite there being a large variety of plants around the flax site, and large numbers of weevils, about half a dozen adult flax weevils were seen to feed on pohuehue. It is perhaps not surprising that pohuehue is a suitable host, entomologist Brian Patrick (Wildlands Consultants) has found over 94 species of Lepidoptera feeding on this important plant, it and other *Muehlenbeckia* species are important for the ecology of native invertebrate communities (B Patrick, pers. comm., 2021). Other large-bodied weevils have been found to feed on alternative hosts, e.g., the coxella weevil

(*Hadramaphus spinipennis*) which is generally considered *Aciphylla dieffenbachia* specialist, was found feeding on Hoho, the Chatham Island lancewood (*Pseudopanax chathamicus*) (Schops, 1998).

This apparent ability of the flax weevils to feed on pohuehue is likely to be an example of a 'sink host' ecology, where the pohuehue plant acts as stepping-stone for the adult weevils to disperse widely and persist in the environment away from their preferred host, flax. This sink host mechanism has been investigated recently using laboratory experiments and field surveys by scientists looking at the dynamics of the herbivorous wheat curl mite (*Aceria tosichella*). It was found that while this mite was considered a specialist pest of wheat, it was able to persist in the environment on less preferred hosts such as wild grasses, and that these alternate hosts could be crucial to the persistence of a specialist herbivore during fluctuating host plant availability (Laska, 2021). It would be worth experimentally testing the suitability of pohuehue as a host plant for larvae, oviposition or adult longevity, and any future monitoring of flax weevils should pay special attention to the possibility that individuals may be found on other host plants.

The ability of flax weevils to feed on pohuehue has significant implications for the management of flax weevils on Mana Island. Previously it was thought that patches of flax that were distant from the weevil outbreak area might avoid being reached by the flightless weevils. Pohuehue is very abundant in the shrubland and coastal ecosystems on Mana Island. If adult flax weevils can survive for a while on this alternative host plant, the ability for the weevils to reach new patches of flax will be greatly expanded, and most flax patches on Mana Island are very likely to be colonised by flax weevils.

Figure 53. An adult flax weevil (*Anagotus fairburni*) feeding on an alternative host plant, *Muelenbeckia complexa*. Mana Island, October 27 2021. Photo: W. Brockelsby, CC-BY.

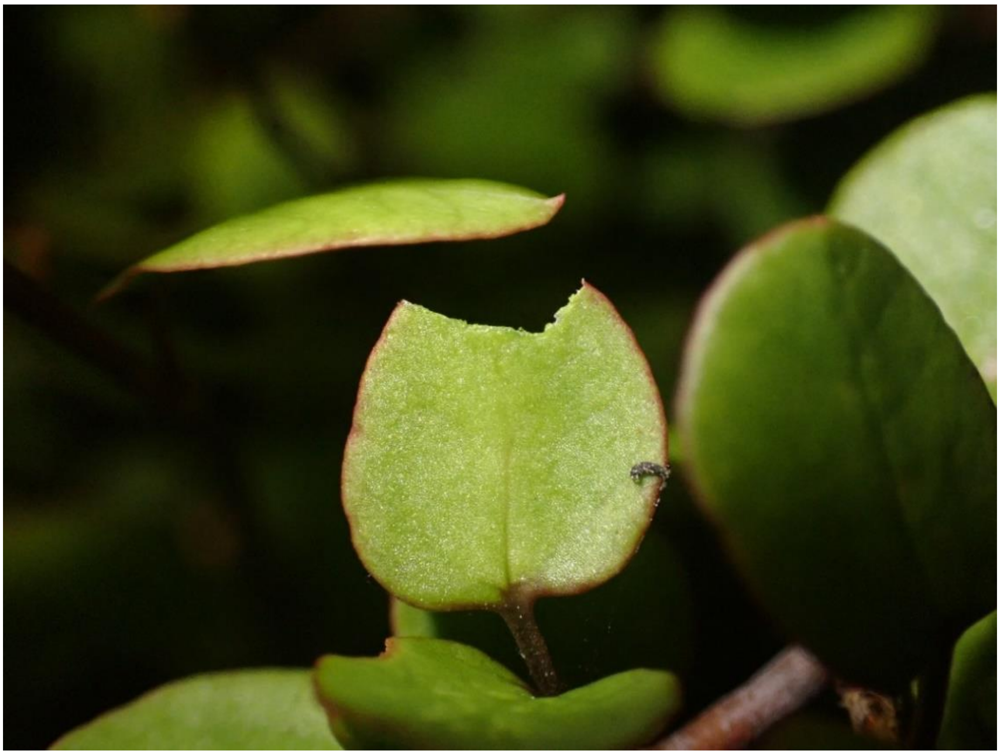

Figure 54. Feeding damage by a flax weevil (*Anagotus fairburni*) to a leaf of *Muehlenbeckia complexa*. This is the same leaf as in Fig. 55, with the flax weevil removed. Mana Island, October 27 2021. Photo: W. Brockelsby, CC-BY.

Chapter 6 Conclusions

The future of flax weevils on Mana Island

Introduction and re-introduction of a species is a tricky business; there are many variables that may cause unforeseen consequences. These consequences can be difficult to predict even when this species is well known to science, oftentimes the only solution is to give it a go and be prepared to adapt the management of the area as needed (Morrison S. A., 2014).

The goal of this project was to utilise management options (irrigation and a native entomopathogenic fungus as a bio-control agent) to attempt to limit the impact of flax weevil of Mana Island on its host plants, while also investigating ecological impacts on the flax-associated community of lizards and invertebrates. This form of "conservation biological control" using entomopathogenic fungi is more commonly employed in agricultural settings, where the purpose is to completely remove or drastically reduce the impact of insect pests on various crops. Here, this biological control technique is being used for the purpose of managing the ecological restoration of a conservation island. I could not fully address all aspects of flax weevil ecology within the timeframe of this work, although hopefully the observations recorded here can provide a base for future studies into this weevil and other conspecifics. Basic ecological information is important when attempting to understand broader population dynamics and without this information it can be difficult to make accurate predictions on possible trajectories. Even without full ecological data, it is still possible to hypothesise what may happen with the weevils on Mana Island over the long term.

The main drivers for the flax weevil population on Mana Island appear to be food and habitat availability, their reproductive potential, and their dispersal ability. As discussed in previous chapters, flax is an abundant plant on the island, the flax weevil's reproductive potential is high, and the weevils appear to have a sink host in *Muehlenbeckia complexa* to help them disperse between local patches of flax. It seems unlikely that flax weevils will be stopped from spreading across Mana Island. Flax weevils are already present over more than half the island and are expected to have reached all remaining areas within a decade.

The longer-term persistence of *B. pseudobassiana* in the soil, its pathogenicity and ability to infect its host will be important to help understand whether this fungus will be able to regulate the flax weevil population, and ultimately what the long-term flax weevil population will look like on Mana Island (Ostfield, Glass, & Keesing, 2005). Already there is visual evidence of the recovery of flax plants at the original site where *Beauveria* counts in the soil were high in 2017 (Glare, 2017). Epizootics of entomopathogenic fungi are common in agricultural and forestry systems, usually occurring once a crucial mix of host insect populations, timing and environment are right for such events to occur (Inglis, Goettel, Butt, & Strasser, 2001). Despite not observing any such outbreaks at the trial site on Mana Island, *Beauveria* appears to be readily spread by the adult weevils as they move across the island, albeit taking some time to build up in the soil. It does appear that the flax weevil strain of *Beauveria pseudobassiana* doesn't affect other insects on the island, at least at the application rates we trialled. In complex environments, population dynamics in insects with fungal entomopathogens are still not particularly well understood, when compared to agricultural or forestry systems, but it is possible to hypothesise some possible models of host population dynamics (Hesketh, 2010). One possible population trajectory for the flax weevil on Mana Island is the exponential population increase as weevils spread across the island, followed by a crash, and then finally plateauing as the fungus spreads and flax collapses. If the strain of *B. pseudobassiana* can persist in the soil for long periods of time in favourable conditions and is virulent enough to cause regular epizootics, this could be a likely outcome. Under this equilibrium model flax would recover over time once the fungus has spread throughout the island (Fig. 56). As previously mentioned, a study on the cockchafer beetle *Melolontha melolontha* showed that a deliberately released strain of *Beauveria brongniartii* persisted in the environment 14 years after its release, and the beetle population density was no longer reaching the damage threshold at any of its release sites (Enkerli, Widmer, & Keller, 2004).

Some argue that specialist pathogens encourage cyclical population peaks and crashes, something particularly reported in forest ecosystems with species of lepidoptera (Anderson & May, 1980; Anderson & May, 1981), although evidence for this is variable (Liu, Bonsall, & Godfray, 2007). It is possible, however, that rather than having flax weevil populations stabilise, we may see regular boom/bust cycles of flax weevil populations on the island.

It is also possible that the reality of Mana Island may be more complex than either of the two scenarios described above, and the flax weevil metapopulation across the island will develop into patchy local populations of weevils that over-exploit patches of flax locally but allow other areas of flax on the island time to regenerate. This was seen by Schops (1998) on Mangere Island for coxella weevils, though Schops did not report any natural enemies or pathogens. Coxella weevils seemed to be primarily limited by food availability (presence of their host plants) (Schops, 1998), and so perhaps this model is less likely to occur on Mana Island which is larger than Mangere Island (113 ha).

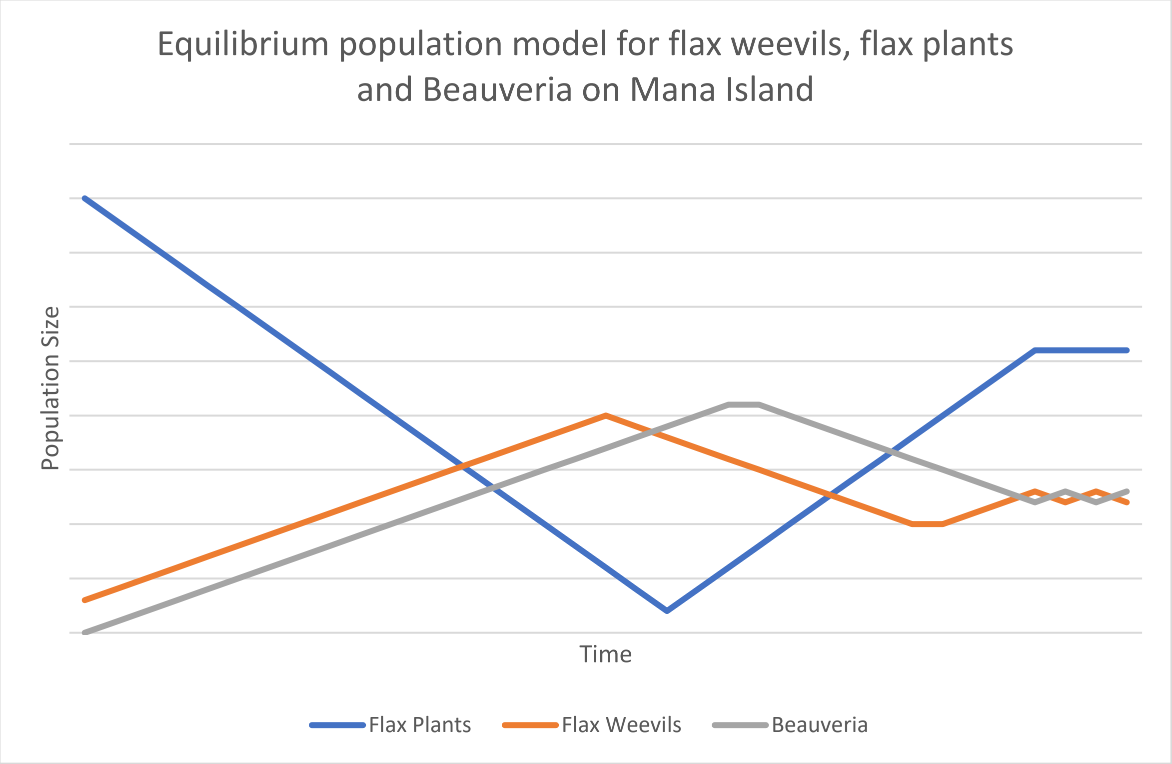

Figure 55. Theoretical population model for flax weevils on Mana Island, built on the assumption that *Beauveria* can build up in the soil sufficiently to control the population size of the weevils, allowing the limited recovery of flax plants on the island and regulating a stable weevil population.

On islands where flax has few natural herbivores, possibly due to deliberate translocation by people (Scheele & Smissen, 2010), flax plants can grow to massive sizes, towering above people. This is the current state of much of the flax in upper northern area of Mana Island, where great stands of flax can be seen at a considerable distance. Flax weevils have yet to reach this stand of flax. Back at the original weevil release site, where flax is now starting to recover, most regenerating plants show sign of weevil browse and flax doesn't appear to be reaching the huge size that existed there previously. It seems that with the introduction of a specialist herbivore, even with the *Beauveria* fungus in the soil, we may not see the return of the impressive large flax plants that can play host to large numbers of lizards and invertebrates.

As mentioned in the Chapter 2, the current state of flax weevil populations in New Zealand is characterised by its relict distribution in alpine areas and offshore islands. The ecological environment of weevils that existed in Pleistocene times is difficult to imagine, but they probably existed alongside many now extinct nocturnal predators like the laughing owl, flightless rails, and other nocturnal avifauna, as well as larger bodied reptiles like tuatara *Sphenodon punctatus* (Gray, 1842) and giant *Hoplodactylus* geckos. When the author discussed the situation on Mana Island with one conservation scientist, they [only somewhat jokingly] suggested that an introduction of tuatara might resolve the imbalance. Tuatara and flax weevils coexist on Takapourewa/Stephen's Island, where tuatara are highly abundant and flax weevils are comparatively rare (M Anderson, pers. comm., 2022). Maybe a nocturnal predator with a large enough mouth to directly predate on adult weevils, such as tuatara, might work synergistically alongside a soil fungal pathogen to reduce flax weevil numbers on Mana Island.

Regardless of which trajectory the flax weevil population takes, Mana Island is a perfect natural experiment for understanding more about the population ecology of a flightless insect and its fungal pathogen in a semi-natural system. After three years the site has changed remarkedly and it will be very interesting to see what will develop on the island over the next 20 years. Friends of Mana Island's regular working trips to Mana Island enabled long term monitoring of the flax site and were integral to this study. Without their logistics and volunteers' efforts and skills this interesting period of the island's history may have gone unrecorded.

Implications for future invertebrate translocations

The discussion I hear often about Mana Island and its flax weevils is that it is a cautionary tale, not one to be repeated lightly for fear of similar unexpected outcomes. While Mana is the exception rather than the rule when it comes to weevil translocations with its "hyper success", there are other local examples of restoration interventions gone wrong, well explored by Towns et al. (1997). One example followed the removal of kiore (*Rattus exulans*) from the Mokohinau Islands. Large swathes of pasture grasses triggered population outbreaks of the endemic chafer beetles from the genus *Odontria*. These generalist herbivores had cascading impacts on the threatened native flora of the island, as the food availability of pasture grasses and removal of a key predator drove their numbers to explode (Towns, Simberloff, & Atkinson, 1997). Despite this, very few argue that the removal of kiore was an overall bad outcome for the island.

The true lesson from Mana Island is to understand how invertebrate/insect translocations differ from bird and lizard introductions, which are better understood translocation targets. Many invertebrate species have high or very high reproductive potential and can quickly respond to favourable conditions and release from natural enemies. Even the flightless insect species are quite mobile. When considering the introduction of any invertebrate to a new area for conservation purposes, we need to attempt to understand the wider ecosystem of the area they may be shifted to and consider the following questions:

- What food availability is there in the ecosystem?
- What is the reproductive potential of the invertebrate?
- What enemies might limit the invertebrate's population if it grew exponentially?
- Should the invertebrate be screened for natural pathogens?

These questions are easier to answer in theory rather than in practice, as there is a real lack of ecological information for most rare invertebrates in complex natural or semi-natural environments. Regardless of whether definitive answers exist for any questions posed above, thinking through the potential answers will help a decision maker be aware of the uncertainty that may be involved in any translocation proposal.

It is also interesting to note the possibility that if there was a longer quarantine period enforced on the flax weevils during the original transfer to Mana Island, it may have picked up the *Beauveria pseudobassiana* on the weevils and the natural biocontrol may not have come across with them to Mana Island from Maud Island. Whether this would have been desirable or not, is interesting to consider as part of any future translocation in other circumstances, depending on the goals of the translocation.

The last thing the author wants to do is to encourage managers to be overly conservative about approving translocations of other specialist invertebrates, especially when it is a taxon at great risk of extinction. While the translocation of invertebrates is still a niche activity and is far less preferrable to the protection of existing invertebrate habitats and their communities, the need for translocation of threatened invertebrates may rise over the coming years as a warming climate is expected to intrude on many species' alpine refugia (Keegan, White, & Macinnis-Ng, 2022). In a warmer world, it is foreseeable that some large-bodied alpine invertebrates and other alpine fauna may need active management and secure populations established in new locations.

Finally, if you do translocate a species of invertebrate and they do very well in their new home – don't panic, it's a good thing. Cook Strait giant wētā (*Deinacrida rugosa*) were translocated to the predator-proof fenced ecosanctuary Zealandia, located in central Wellington, in 2007. Zealandia contained a host of natural invertebrate predators like tīeke *Philesturnus rufusater* (Lesson, 1828), ruru *Ninox novaeseelandiae* (Gmelin, 1788) and tuatara, but now the giant wētā are found throughout the valley and are attempting to disperse over the fence into the surrounding urban reserves (D. Shanahan, pers. comm., 2021; W D Brockelsby, unpublished data, 2022). Adapting to and successfully exploiting a new environment in the face of natural predators is just what these often-underestimated invertebrates do.

Post-script

After an eight-month gap in monitoring on the island, in June 2022 the FOMI work trips restarted and I was able to revisit the flax weevil site. What I found was a site quite changed from 2018 and even October 2021, on which most of the data of this thesis is based. Most of the flax plants were dead and the vegetation was beginning to transition from being flax-dominated, with poroporo (*Solanum laciniatum*) growing where flax used to be, scrambling vines and lianas climbing over dead flax, and divaricating *Coprosma* and other shrubs moving into the now free space. Without the aid of the waratah flax markers, it would've been difficult to see where some of the flax used to be. I felt disoriented, the site I knew very well now felt like a place I was less familiar with. Flax plants had continued to collapse (Table 14).

Night monitoring revealed weevils on every plant that still had leaves alive at the site, the total count was less than half the previous monitoring effort in October 2021, but still in the hundreds. Further up the island weevils had begun to move northwards, a feeding sign was seen in previously weevil free areas. The near total loss of flax at the site seems to be a likely state for quite a while. We plan to leave the flax marker waratahs in place, to see if flax plant recovery might take place over time once the weevils move on.

Table 14. Plant collapse scores in June 2022, after an 8 month gap in mointoring. Very few flax plants remain alive at the site.

Plant collapse score criteria	**Number of plants with each score, June 2022**	
	Control plants (no *Beauveria*)	**Plants treated with *Beauveria***
2 (few fans collapsed, but less than half the plant)		2
3 (heavily collapse of fans, clearly more than half the plant)		2
4 (plant mostly dead, but few fans still half-standing)	4	1
4.5 (some green left but close to dead leaves only)		1
5 (nothing green left, just a pile of dead leaves)	17	16
Totals	**21**	**22**

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Appendices

Appendix 1 Irrigation dates

Irrigated Plants Line A	Irrigated Plants B	Dates Tank Emptied
A4	B2	08/01/2020
A7	B4	16/01/2020
A8	B5	25/01/2020
A11	B7	6/02/2020
A14	B8	15/02/2020
A19	B9	24/02/2020
A20	B11	1/03/2020
	B10	8/03/2020
	B13	21/03/2020
	B15	8/02/2021
	B18	15/02/2021
	B20	27/02/2021

Appendix 2 *Beauveria* solution

The *Beauveria pseudobassiana* was transported to the island with dried propagules on rice (the growth substrate). The inoculated rice was prepared by Jenny Brookes at the Lincoln Bio-Protection Centre and was produced using the following methodology.

Preparation of Rice

Weigh out 500g of rice and place in a semi-transparent autoclave bag (295 x 600 mm). Add 110 mL of tap water. Place a metal tubing ring at the top of the bag, gather the end of the bag firmly around the ring and seal with autoclave tape ensuring there are no gaps. Expel all the air from the bag and place a cotton wool bung into the metal ring (not too thick as gas exchange is required during the growth process). Cap with a large square of foil. Place the bags flat on the autoclave trolley and make sure the rice is spread in a thin flat layer. Autoclave for 15 minutes at 121^{o}C. Once bags have cooled enough to handle, break up the rice so that it is friable in the bags.

Inoculating bags

Prepare 50 mL of SDB broth (40 g Dextrose, 10 g Neopeptone in 1 L of water) in 75 mL conical flasks, sterilise. Inoculate sterile broths with a loop of *Beauveria* spores and aseptically add the broth to the bags (50 mL per bag) by removing the cotton wool bung and carefully tipping the flask contents in. Pull the sides of the bag to ensure there is plenty of air inside, then replace the cotton wool bung and foil cap. Place bags flat on shelves at 23-25^{o}C, and ensure that the rice is in an even, thin layer across the entire bottom surface of the bag.

Growth

After 2-4 days the bags will require mixing, which is done by manually picking up each bag and carefully scrunching the bag to break up the first mycelial layer that has formed. Place the bags back flat on the shelves and ensure the rice is once again spread in an even, flat layer across the bottom surface of the bag. Bags were grown 2-4 weeks before needed to ensure enough growth.

Once this *Beauveria*-rice was on the island, a mixture was produced by Jenny Brookes on the day of the release 29/02/2020. *Beauveria* spores were harvested at base camp from 3 rice bags growing *B. pseudobassiana* which was originally isolated from Mana Island. One litre of 0.01% Triton X was added to each bag, swished around and lightly shaken for 2-3 minutes. The liquid was harvested through sterile muslin and combined giving a total volume of 2.8 litres collected. A 10 ml sample of concentrate was collected and kept in fridge until back at University for a CFU count (8.4 x

10^{-7}cfu's/ml). The concentrate was carried up to study site then approx. 136 ml of concentrated *Beauveria* solution was measured into a watering can then topped up to 4 litres with tap water.

Appendix 3 FOMI work trip weekends for flax weevil work

Date	(17-19) Aug-2018	(5-7) Oct-2018	(30-2) Nov-Dec-2018	(1-3) Mar-2019	(12-14) Apr-2019	(14-16) Jun-2019	(17-19) Aug-2019	(4-6) Oct-2019	(29-1) Nov-Dec-2019
	(28-2) Feb-Mar-2020	(20-22) Mar-2020	(12-14) Jun-2020	(4-6) Sep-2020	(16-18) Oct-2020	(27-29) Nov-2020	(4-7) Feb-2021	(25-26) Oct-2021	

Appendix 4 Plant health scores, October 2021

Plant	Plant Collapse Score	EPF
A4	3	Control
A7	2	Treatment
A8	4	Control
A11	2	Treatment
A14	3	Treatment
A19	3	Treatment
A20	4	Control
B2	4	Treatment
B4	4	Control
B5	4	Treatment
B7	4	Control
B8	4	Treatment
B9	4	Control
B10	4	Control
B11	4.5	Control
B13	4.5	Treatment
B15	4	Control
B18	3	Treatment
B20	5	Treatment
C1	5	Control
C3	4	Control
C5	4.5	Treatment

C6	5	Control
C8	4.5	Control
C10	5	Treatment
C12	4	Treatment
C13	2	Treatment
C15	4.5	Treatment
C17	5	Treatment
C19	5	Control
C20	4	Control
D1	3	Control
D4	3	Treatment
D5	2	Treatment
D6	5	Control
D7	3	Treatment
D9	5	Treatment
D11	4	Treatment
D12	4	Control
D13	4	Control
D15	5	Treatment
D18	4	Control
D20	5	Control

Appendix 5 Larvae capsule width measurements

Each individual flax plant I collected from was given a consecutive number. Measurements were taken three times for large larvae as there was variability with the electronic callipers, but small larvae only measured once in the lab.

Plant #	Measurements #1(mm)	Measurements #2(mm)	Measurements #3(mm)
12	2.53	2.54	2.6
12	2.22	2.32	2.27
11	3.78	3.8	3.96
11	3.7	3.66	3.85
11	2.25	2.32	2.1
11	1.38	1.53	1.5
10	4.28	4.1	4.06
10	2.41	2.34	2.28
10	2.27	2.14	2.13
10	1.4	1.37	1.33
10	1.43	1.37	1.29
10	1.3	1.25	1.18
10	1.27	1.26	1.08
10	0.99	0.92	0.91
9	3.61	3.58	3.55
9	3.34	3.29	3.26
9	3.32	3.23	3.2
9	2.63	2.55	2.51
9	2.4	2.36	2.35
9	2.25	2.21	2.06
9	2.1	2.04	2.01
9	2.09	2.09	1.94
9	1.86	1.82	1.71
9	1.87	1.77	1.66
9	1.78	1.77	1.72
9	1.59	1.58	1.53
9	1.53	1.5	1.44
9	1.48	1.42	1.39
9	1.41	1.39	1.38
9	1.37	1.35	1.26
9	1.39	1.37	1.19
9	1.29	1.27	1.19
9	1.22	1.19	1.13

9	1.23	1.15	1.13
9	1.17	1.15	1.03
9	1.14	1.07	1.05
9	1.11	1.06	1.01
9			0.196921
9			0.17918
9			0.1752
9			0.166921
9			0.161874
9			0.158675
9			0.137669
9			0.13049
9			0.124537
8	4.15	4.09	3.96
8	2.71	2.66	2.63
8	2.47	2.37	2.27
8	2.54	2.34	2.18
8	2.27	2.27	2.14
8	1.73	1.68	1.68
8	1.71	1.66	1.65
8	1.48	1.47	1.42
8	1.4	1.32	1.31
8	1.27	1.26	1.23
8	1.28	1.25	1.17
8	1.22	1.15	1.13
8			0.232956
8			0.155346
7	4.24	4.2	3.95
7	2.6	2.51	2.46
7	1.13	1.05	1.01
6	3.97	3.96	3.84
6	3.8	3.79	3.7
6	1.88	1.74	1.69
6	1.04	0.94	0.93
6	1	0.95	0.93
6			0.228351
6			0.210658
6			0.148862
5	3.98	3.92	3.89
5	3.67	3.66	3.51
5	3.6	3.5	3.4

5	1.44	1.4	1.36
5	1.22	1.15	1.11
4			0.178257
3	4	3.93	3.9
3	3.9	3.75	3.64
3	1.69	1.65	1.46
2	2.64	2.55	2.51
2	1.66	1.62	1.58
2	0.86	0.83	0.81
2			0.128153
1	3.93	3.89	3.86
1	3.51	3.47	3.43

Appendix 6 Weevil counts per line at flax plant trial site

Date	A Line	B Line	C Line	D Line	Sum	Marked Weevils
Aug-18	0	0	0	2	2	
Oct-18	1	7	5	4	17	
Nov-18	3	7	4	21	35	
Feb-19	1	10	5	18	34	
Apr-19	9	16	24	33	82	
Jun-19	2	9	28	40	79	
Aug-19	7	7	19	32	65	
Oct-19	9	36	31	23	99	
Nov-19	15	57	66	165	303	
Feb-20	48	167	96	197	508	
Mar-20	165	252	155	190	762	57
Mar-20	181	287	81	124	673	48
Jun-20	83	122	189	247	641	6
Sep-20	111	147	203	156	617	4
Oct-20	229	450	389	363	1431	18
Nov-20	210	434	319	282	1245	13
Feb-21	558	683	437	364	2042	19
Oct-21	645	232	131	155	1163	1