Neolema ogloblini: exploring a new option for the control of tradescantia (Tradescantia fluminensis)

"The endemic productions of New Zealand, are perfect one compared with another; but they are now rapidly yielding before the advancing legions of plants and animals introduced from Europe"

Charles Darwin; The Origin of Species, 1859/2003 (p: 195)

"No other plant of similar size has the ability to alter the form or shorten the life of a forest"

A. E. Esler on tradescantia; Forest Remnants of the Manawatu Lowlands: The Banks Lecture, 1962 (p: 257)

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Abstract

Invasive weeds pose one of the biggest threats to New Zealand's remaining native forest, and the effects are predicted to increase as the amount of invasive species continue to increase.

This thesis looks at the first biological control agent (*Neolema ogloblini*) released for the control of tradescantia (*Tradescantia fluminensis*), provides baseline data to aid later assessment of the efficacy of the control agents, and compares the two current methods of tradescantia control.

In glasshouse trials, I compared the effects of the biological control agent *N. ogloblini* and the traditional herbicide. This was assessed by measuring the survival and growth of two species of native seedlings planted underneath treated (or un-treated) tradescantia. Light reaching soil-level beneath the tradescantia canopy was also measured, as was dry biomass of the tradescantia. One seedling species (Kawakawa, *Macropiper excelsum*) growth rate did respond favourably to the significantly increased light and reduced tradescantia biomass following feeding by *N. ogloblini*, but the other species (Mahoe, *Melicytus ramiflorus*) did not. Survival rate was higher for all seedlings under tradescantia treated with *N. ogloblini* compared to those that were untreated or treated with herbicide.

I also set up and surveyed permanent plots in an area that has a long-standing swath of tradescantia. The data produced from this should aid in the assessment of the biological control agent if field trials are performed in the future at this site.

Finally, I compared the regrowth of tradescantia and other species into areas that were treated with mechanical or chemical control. The regrowth of tradescantia was not significantly different between the two methods, nor was the invasion and growth of other species.

Acknowledgements

I didn't plan to do a Masters, and I certainly didn't envisage that it would be on tiny beetles and an invasive weed, but with help from my supervisors, friends and family, I've done what I never expected to do.

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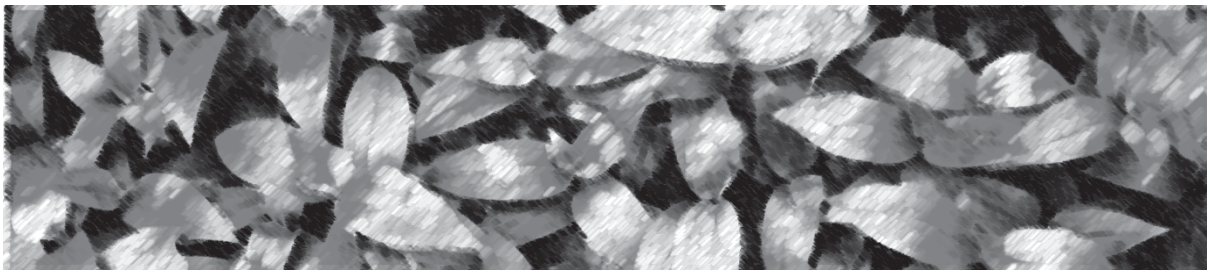


Weeds in New Zealand Forests

New Zealand's weed problem

Two thousand years ago, native forest covered over three quarters (possibly as high as 95%) of New Zealand's land mass (Fleet, 1986; McGlone, 1989). Now, after clearance by both Maori and European settlers, native forest cover is present on just under one quarter of New Zealand's land mass ((23%) Fleet, 1986; (24%) Ewers *et al.* 2006). This equates to about 14,000,000 ha of deforested land evenly split between the North and South Islands, with the residual forest mostly fragmented (Ewers *et al*. 2006). The remaining forests are under threat primarily from anthropogenic deforestation (Ewers *et al*. 2006, Fleet, 1984) and the invasion of alien plant species (Timmins and Williams, 1991; Wiser and Allen, 2006). Deforestation by humans is subject to a range of legal restraints (or the lack of them) and is therefore easier to reduce than the other main threat; weed species. The problems posed by weed species in New Zealand are among the most severe posed by invasive plants anywhere in the world (Julien *et al*. 2007).

Definition of a weed

The broadest definition of a weed is a plant that grows where it is not wanted. According to Esler (1988), weeds can be defined as having one (or more) of the following features: obstructive (restricting passage of humans, light, nutrients), suppressive (adversely affecting other plants), health and comfort endangering (toxic, irritant, allergen), quality impairing (pasture invasion, crop competition), damaging to

native vegetation, and likely to be a fire hazard. In the case of New Zealand's forests, weeds are both suppressive and damaging (Esler, 1988; Syrett, 2002; Van Driesche *et al*. 2010). These weeds are termed invasive, with the following definition taken from Owen (1998):

"Invasive weeds are plants that can significantly and adversely affect the long-term survival of native species, the integrity or sustainability of natural communities, or the genetic variation within indigenous species. "(p. 1)

This definition is very similar to the one provided by Williams and West (2000) for environmental weeds, a subset of invasive weeds that includes only non-agricultural weeds. For the purpose of this thesis, these two terms (invasive and environmental) will be considered the same.

Numbers and impacts of weeds on New Zealand forests

Owen (1998) noted that almost half of all vascular plants in New Zealand are introduced, about 19 000 species; and 2068 of those species are naturalised. Naturalised species are not automatically invasive weeds, but they do indicate their weedy potential (Randall, 2002). The 'Tens Rule' (Van Driesche, Hoddle and Center, 2008) predicts that ten percent of naturalised weed species in a country will become damaging species; meaning that approximately 200 species of the 1998 naturalised species list could (if the theory holds) become damaging. The National Pest Plant Accord currently lists at least 142 exotic plant species (some hybrids, subspecies and 'same-genera' species are not individually listed) as being unwanted weeds in New Zealand (Ministry for Primary Industries, 2012). The list by Howell (2008) had 328 species listed as environmental (invasive) weeds. Froude (2002) listed 174 weed species invading protected natural areas of New Zealand, and identified her top 24 weed species. These top 24 were rated by Syrett (2002) in terms of their environmental impact and the difficulty in managing them. The top five of the list are as follows; wandering Jew (*Tradescantia fluminensis*), Grey willow (*Salix cinerea*), Japanese honeysuckle (*Lonicera japonica*), old man's beard (*Clematis vitalba*) and smilax (*Asparagus asparagoides*). Tradescantia and old man's beard share the top spot as the 'worst' weeds in Syrett's ranking.

The greatest consequence of invasive weed incursions is the loss of native biodiversity (Van Driesche *et al.* 2010). According to Syrett (2002), over 60 native New Zealand plants are seriously threatened by invasive weeds, and another 16 are significantly impacted by them. Invasive weeds can also affect the survival of native animals; by displacing necessary native vegetation, allowing introduced competitors and predators increased entry to an area, or by reducing or destroying their habitat (Owen, 1998). In short, entire ecosystems can be affected by invasive weeds; the effects may cascade along the food and interaction webs of an ecosystem, affecting all individuals within (Esler, 1988; Van Driesche *et al.* 2010). The most threatening types of invasive weeds are thought to be woody species (grey willow), smothering vines (old man's beard, honeysuckle and smilax), and mat-forming herbaceous plants (tradescantia) (Wiser and Allen, 2006). These weeds have more permanent effects than other types, such as; forest ecosystem composition alteration through regeneration prevention (tradescantia), waterway alteration through wetland vegetation replacement (grey willow), and the creation of open spaces in forests by smothering the existing vegetation (old man's beard, honeysuckle, smilax) (Popay, Champion and James, 2010; NPPA, 2012).

Despite the effects already felt, New Zealand has few invasive weed species that have reached their full range as the invasion of alien plants is still at an early stage (Julien *et al.* 2007). Weed numbers, introduced and naturalised, are expected to increase, and so will the impacts that they bring.

Why is New Zealand so prone to invasive weeds?

In New Zealand, weeds tend to invade forests that have been disturbed by humans, introduced browsing animals or naturally; areas like landslide scars, small remnants, and canopy gaps (Wiser and Allen, 2006). Forest fragmentation (Fig. 1) as a result of human clearance is a common occurrence in New Zealand (Esler, 1962; Fleet, 1984; Ewers *et al.* 2006), with fragments suffering serious impacts on their internal structure (Hobbs, 2001). Landslides can be caused by natural events acting on naturally unstable soils (Crozier, *et al.* 1992) or as a result of human interference (Fleet, 1984 & 1986). Canopy gaps can also be natural (occasional tree falling) or not (selective logging or possum browsing; Fleet, 1984). In both cases, the natural events are rarer than those brought on by other causes (Fleet, 1984, Crozier *et al.* 1992). Browsing animals like deer, cattle, sheep, goats, along with pigs and possibly even rats can prevent regeneration and affect forest composition (Fleet, 1984; Atkinson and Cameron, 1993; Clayton, Wilson, Dickinson and West 2008; Smale, Dodd, Burns and Power 2008); with palatable plants being eaten and their niches kept open for unpalatable or weedy plants.

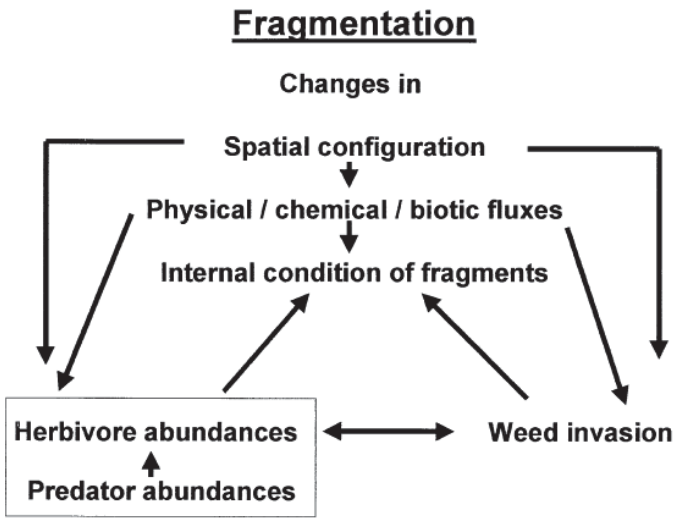


Figure 1: Fragmentation.
The direct effects from a change in spatial configuration and their relationships with one another. Taken from Hobbs, 2001.

Historically, before the introduction of such weed species, those disturbed areas mentioned above would have been colonised by the few native species that specialise in early colonisation (ruderals) (Wilson and Lee, 2012). Manuka (*Leptospermum*

scoparium) and Kanuka (*Kunzea ericoides*) were once the dominant woody species in early stage succession (Sullivan, Williams, & Timmins, 2007). This paucity of native ruderals could be a significant factor in New Zealand's vulnerability to weed invasion. In many areas, gorse (*Ulex europaeus*), elder (*Sambucus nigra*) and broom (*Cytisus scoparius*) now displace these native ruderals in slip scars, untended pasture and riversides, acting as a nurse crop for natives, but ultimately altering the trajectory of later successions (Williams, 1983; Esler, 1988; Wardle, 2002; Sullivan *et al.* 2007; Popay, Champion and James, 2010). Like gorse, elder and broom, the other introduced weeds have a longer history of colonising areas after human disturbance, and can often out-compete natives on nutrient rich soil (Craine, Lee and Walker 2006). Introduced weeds may also be better at using humans as dispersal vectors (Prinzing*et al.*2002). The degree of human impact is thought to be the most important influence on weed extent in a reserve or remnant (Timmins and Williams, 1991); the mere presence of visitors can increase disturbance levels (Lonsdale, 1999). The effects of human disturbance were discussed in the paper by Lusk, Hurrell and Lamoureaux (2012), which indicates that native species diversity improved and weed plant abundance decreased in remnants that were less disturbed. However, there are some weed species present in New Zealand that can penetrate intact forest (e.g. *Hieracium lepidulum*; Wiser, *et al.* 1998).

Biological Control

Definition of biological control

Biological control is the use of one organism (virus, bacteria, fungi, invertebrate or vertebrate) to control a pest organism (Lazarovits, Goettel and Vincent, 2007). Eilenberg, Hajek and Lomer (2001) define biological control as:

"The use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be." (p. 390)

There are three categories of biological control (Lazarovits, *et al*, 2007; Froude, 2002); Classical, Conservation and Inundative (Augmentation). When considering control of widespread invasive weeds, the classical version of biological control is best suited (Fowler, Syrett and Hill 2000; Van Driesche *et al*. 2010). For the purpose of this thesis, the term biological control will refer to classical biological control. The definition provided for classical biological control by Eilenberg *et al.* (2001) is:

"The intentional introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest control" (p. 391)

In simpler terms, a new organism (the biological control agent) is introduced; one that feeds on, or has a harmful effect on, the target organism. This agent usually comes from the target organism's country of origin, and has a long relationship with it. This relationship is re-established in the new country.

The aim of weed biological control is not eradication of the target weed, but a reduction in vigour (Syrett, 2002; Van Dreische, Hoddle and Center, 2008). This allows desirable plants to compete more successfully, and competition from the other plants can further suppress the target weed (Figure 2).

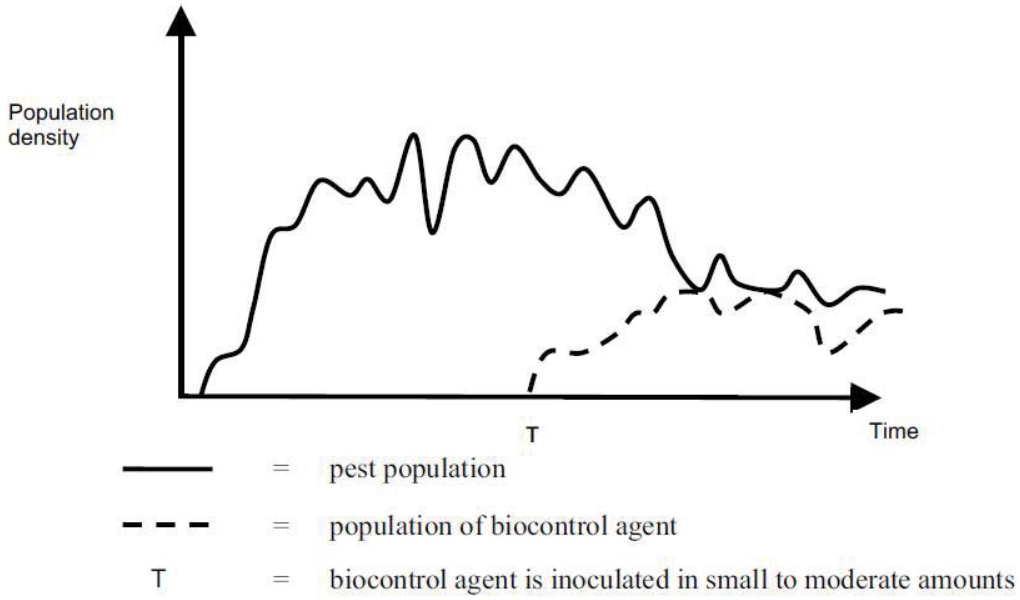


Figure 2: Classical biological control.
Population density of pest and agent over time. Note that pest density is reduced and then stabilised at a lower density.

History of Biological control; worldwide and in New Zealand

The first major use of classical biological control was in 1888, when the vedalia beetle (*Rodolia cardinalis*) was used to control the cottony-cushion scale (*Icerya purchasi*) in California (Doutt, 1964; Greathead, 1994). The literature on the first instance of biological control of weeds is not consistent. According to Doutt (1964), Holloway (1964), Greathead (1994) and Julien *et al.* (2007), the first instance of biological control for weeds was in 1902, when the search for a natural enemy of *Lantana camara* (a pest in Hawaii) started. But according to Syrett, Briese and Hoffman (2000), the first instance was in the 1860s, in Sri Lanka, against the prickly pear (*Opuntia vulgaris*) using the cochineal insect (*Dactylopius ceylonicus*). The first effective weed control program started in 1913 (or 1912, according to Julien *et al.* (2007)), in Australia against prickly pears (*Opuntia* spp.), resulting in 15 million hectares of infested pasture being cleared by multiple agents (Dodd, 1940; Holloway, 1964; Greathead, 1994). Early biological control efforts were not always well thought-out; the cane toad (*Bufo marinus*) was introduced to Australia in 1935 to control pests

among sugar cane crops (Gurr*et al.* 2000; Global Invasive Species Database, 2012f) with undesirable consequences.

One of the first instances of biological control in New Zealand was in the 1880s with the introduction of mustelids for the control of previously introduced rabbits (*Oryctolagus caniculus*) (Atkinson, 2006); an ill-fated program with negative consequences for New Zealand natives. According to Fowler and Withers (2006), the biological control of weeds and arthropods has been practised in New Zealand for at least 130 years. However, Holloway (1964) states that New Zealand first became interested in this form of weed control in 1927, with Dr R. J. Tillyard initiating the search for control agents of gorse and 'tansy ragwort' (Ragwort, *Jacobaea vulgaris*), both still nuisance species in New Zealand. Landcare Research (2012a) listed at least 53 species of arthropods and fungi as being present in New Zealand for the purpose of weed biological control, with a further nine species introducing themselves (or accidentally being introduced) and impacting on weed species, and two native species (*Anisoplaca pytoptera* & *Oemona hirta*) impacting on gorse .

Success, benefits and downsides

A biological control program, if successful, will work forever (Babendreier, 2007). The determination of whether a program is successful requires further attention. Froude (2002) gives three levels of success; agent establishment, damage to the pest plant, and damage to a level that reduces the vigour and extent of the pest plant. Establishment can be impeded by issues such as climate mismatching between original and host country (Robertson, Kriticos and Zachariades, 2008), insufficient release numbers (Fowler *et al.* 2006), and environmental stochasticity (Fowler, Syrett and Hill, 2000). Fowler, Syrett and Hill (2000) reported that the establishment rate for biological control agents in New Zealand was 76% (at the time), while Van Driesche, Hoddle and Center (2008) state that one third of introductions fail to establish. Attainment of the next level can also be impeded; climate mismatching and predation (Fowler, Syrett and Hill, 2000) could prevent significant progress. Paynter *et al* (2010, cited in Fowler et al. 2010) found that over 50% of those biological control agents established in New Zealand failed to contribute to the control of their host plant. It is important to note at this point that even in a (eventually) successful program, time is needed to allow the

agent to build up to the numbers needed to impact significantly on a host plant (Froude, 2002). Finally, even if a single agent has established and there is proven damage to the host plant, multiple agents may be required to reach the third level of success; substantial suppression (Denoth, Frid and Myers 2002; Van Driesche, Hoddle and Center 2008).

Provided the program has been successful, there are many benefits to using biological control instead of (or in conjunction with) more traditional methods. As Froude (2002) and Babendreier (2007) point out, when a biological control program is successful, there is a reduced need for the use of chemicals to control the pest species. Broad spectrum herbicides used against weed species can also be just as damaging against the native species (Harrington & Schmitz, 2007), while biological control agents can be selected for specificity to one species or a few closely related species (provided host testing is rigorous enough: Barrat *et al.* 2010; Simberloff 2012). Fowler, Syrett and Hill (2000) also mention the continuous action of the agent (provided it establishes), the long-term cost-effectiveness, the gradual impact, and the self-dispersal of the agents (assuming that the agents are mobile and the distance between weed species populations is not too large). An example of a successful biological control program in New Zealand is that of the St John's wort (*Hypericum perforatum*) by the lesser and greater St John's wort beetles (*Chrysolina hyperici* and *C. quadrigemina*, respectively). As an example of the program's effectiveness; 180 ha of the weed was cleared in 4 years by the lesser St John's wort beetle (Landcare Research, 2012b). Of all the biological control programs for invasive weeds in New Zealand, only the St John's wort project has so far been judged as a complete success, requiring no further input (Fowler *et al.* 2000). It is worth noting that these agents would not be introduced under the current rules surrounding biological control agent introductions (Groenteman, Fowler and Sullivan, 2011).

There are several drawbacks (also listed by Fowler *et al.* 2000) to biological control programs, like all control methods. It is initially quite expensive; one must travel to the pest plant's country of origin, spend time in that country researching, and bring potential agents back to be studied (with the potential to be rejected as unsuitable). Downstream effects in ecosystems are hard to predict; the agents may be preyed on by native predators, increasing the predator numbers and impacting on other (native)

prey species (Simberloff, 2012). The impact is not immediate, so the pest plant can still affect invaded areas and move to new areas while the agent population is increasing. Furthermore the agent may not perform as well as anticipated and be ineffective against the pest species, as have been the three agents released so far against old man's beard (Landcare Research, 2012b). The biomass reduction may also be too rapid; allowing new weeds to re-invade before natives get a chance (Reid, Morin, Downey *et al.* 2009). Also, biological control programs do not aim to eradicate the target pest species but reduce its vigour (Syrett, 2002; Van Driesche, Hoddle and Center, 2008), so if eradication of the pest species is the goal, a different or additional program will be required. Finally, in any weed control program (chemical, biological or otherwise), there is always the possibility of harmful knock-on effects (Fowler and Withers 2006). But an established biological control program is irreversible, and the possibility always exists that the agent will directly impact on different species (particularly natives or species of economic importance) and cause more damage. Fowler *et al.* (2000) provides three examples of where biological control agents released in New Zealand did damage to other vegetation, but all damage was minor or still being assessed at the time (damage later proving minor (Landcare Research, 2012b)).

Successful biological agents

There are many ways that an insect population can affect the performance of a plant (Crawley, 1989). They can affect flowering (destruction or reduction of flowers and flower buds directly or indirectly), fruit production (seed or fruit predation), post-dispersal seed mortality (seed predation after dispersal), seedling mortality, defoliation, growth & reproduction (plant modification through herbivory), competitive ability and mortality of established plants. There was no literature found by this author to suggest that a specific feeding guild is preferable over the others (foliage feeders, stem borers, seed predators, etc). Some plants are highly tolerant to particular forms of herbivory; a study by Gard *et al* in 2013 found that 90% defoliation of the common ragweed (*Ambrosia artemisiifolia*) did not obviously affect fitness. In these cases, it would be logical to find agents that affect plant fitness in other ways.

Beetles from the Chrysomelidae family (along with the Curculionidae family beetles) were found to be the most successful types of biological control agents in a review of 61 studies (Clewley et al. 2012). New Zealand's successful St John's wort project used two chrysomelid beetles, and all 20 of the beetles introduced to New Zealand as biological control agents are either from the Chrysomelidae (14) or Curculionidae (six) families.

Study Species; Tradescantia fluminensis.

A description of tradescantia

Class: Liliopsida

Order: Commelinales

Family: Commelinaceae

Species: *Tradescantia fluminensis* (*T. albiflora* Kunth often misapplied)

Common names: wandering Jew, tradescantia, white flowered wandering Jew, small leaf spiderwort, spiderwort, wandering Willie

Figure 3: Tradescantia.
Image taken from National Botanic Garden of Belgium (2013).

Tradescantia fluminensis Vell. (Family Commelinaceae), commonly known as tradescantia in New Zealand, is an agricultural weed native to South America (Brazil-Argentina: Burns, 2004; Uruguay: Thorp and Wilson, 2012). It is a succulent, frost

tender and shade tolerant perennial; able to grow at light levels at or above 5% of full-light (Standish, Robertson and Williams 2001; Popay, Champion and James, 2010). In its native range, it grows in rainforests and other damp shaded places (Barreto, 1997; cited in Global Invasive Species Database, 2012e). The stems are trailing, lightly rooting at the nodes and curving upwards at the tip; creating a thick carpet with stems laid over one another (Popay *et al.* 2010, Global Invasive Species Database, 2012e). It can reproduce both sexually and asexually; plants are able to grow from fragments as small as 1cm (Kelly & Skipworth 1984a). It has been introduced into New Zealand (Butcher and Kelly, 2011), Eastern Australia (Thorp and Wilson, 2012), Taiwan (Chihkai, ChienHui, and FuShan, 2008), Japan (Omori, 2008), South Africa (Foxcroft, Richardson, and Wilson, 2008), at least one of the Azores Archipelago islands (Silva and Smith, 2006), Italy and Russia (Samoilova *et al.* 2011), Spain (Froude, 2002), North America (USDA and NRCS, 2012), and Chile (Thorp and Wilson, 2012). Despite its low tolerance for cold weather, it has even become a casual urban weed in Belgium (Verloove, 2006; National Botanic Garden of Belgium, 2013).

Tradescantia in New Zealand; the effects

According to Butcher and Kelly (2011), tradescantia was introduced to New Zealand for ornamental purposes in 1910, while Kelly and Skipworth (1984a) say that it was first introduced by a Manawatu farmer in 1910 to help stabilise a steep bank. Froude (2002) considers tradescantia as being first recorded in New Zealand in 1916. Whenever and for whatever reason it was introduced, it is now found in frost free locations through-out the North Island and some locations in the South Island (Butcher and Kelly, 2011; Popay *et al.* 2010). It is considered an invasive weed; Esler (1988) listed tradescantia as being in the group of most threatening weeds of urban Auckland. It was listed by Froude (2002) as being in the top 24 weeds of New Zealand, and Syrett (2002) found that it was joint equal as the 'worst' weed. It cannot reproduce sexually in New Zealand for reasons as yet unknown, and so solely relies on vegetative reproduction (Froude, 2002; Popay *et al.* 2010, Global Invasive Species Database, 2012e). Dispersal throughout New Zealand is through garden waste, animal vectors, vehicles, water currents and human vectors (Global Invasive Species Database, 2012e). A recent study by Hurrell and Lusk (2012) found that fragments were able to survive up to two days immersion in sea water; so tradescantia can be also be spread to other

places by the sea currents. Under forest canopies, it can form areas of dense growth (over 60cms high) with 1400g found in one square metre (Esler, 1962; Kelly and Skipworth, 1984a). This maximum seems high; Standish *et al.* (*2001*) found a maximum of 819gm^2 dry biomass; it is possible that the amount provided by Kelly and Skipworth (1984a) referred to un-dried biomass.

Maule, Andrews, Morton, Jones and Daly (1995) proposed a set of attributes that contributes to the 'invasion strategy' for tradescantia in New Zealand, allowing it to invade forest remnants very successfully. It involves rapid growth, combined with vegetative reproduction, low irradiance level acclimation, longevity associated with slow growth in consolidated stands, and the ability to efficiently recycle nutrients. This seemingly contradicting statement (rapid growth and slow growth) is reflective of the different strategies tradescantia uses when faced with different light levels; quickly moving into areas of recent disturbance with high light levels, and then slowly turning over as the disturbance effects fade and the light dims. It cannot, however, move into intact forests as it requires a disturbance event to establish itself in an area, but once it has established a high biomass

Photo 1: Seedlings under tradescantia
This tradescantia has been parted to show the native seedlings below, which are not normally so apparent. The seedlings are still small and may die before they penetrate the tradescantia canopy.

sward in a disturbed area, it can persist apparently indefinitely (Global Invasive Species Group, 2012e)

The primary problem that tradescantia causes in New Zealand is the suppression of seedlings resulting in a decrease of native species richness and abundance (Standish *et al.* 2001). Standish *et al.* (2001) found the LD50 (tradescantia biomass at which 50% of seedlings are killed) for six native species varied from 12gm^2 dry weight for kawakawa (*Macropiper excelsum*) to 40gm^2 dry weight for kohekohe (*Dysoxylum spectabile*). These biomass levels are well below the maximum biomass: 695-819gm^2 dry weight. The reason for the native species intolerance to tradescantia is the decrease in light levels beneath the tradescantia carpet; as little as 1-2% of full light reaches the soil (Standish *et al.* 2001).

Another substantial impact is the correlated decline in invertebrate diversity and abundance (Standish, 2004; Toft, Harris, and Williams, 2001), possibly an effect of the plant's structure and microclimate. It also alters the rate of leaf litter decomposition and nutrient availability (Standish *et al.* 2004).

Treatment of tradescantia in New Zealand

Depending on the extent of an infestation, different methods of extermination are used. Small patches of tradescantia can be cleared by hand, but care must be taken to ensure every fragment is collected to prevent re-sprouting, and follow-up treatments may still be required to remove regrowth (Esler, 1988). The Department of Conservation and regional authorities control tradescantia mostly with triclopyr; using blanket or spot spraying first, and following up with spot spraying later on (Hurrell, James, Lusk, & Trolove, 2008; Lusk, Hurrell, & Lamoureaux, 2012). The Department of Conservation also suggests using herbicides containing glyphosate, picloram and amitrole (Department of Conservation, 2012). In a field trial done by Hurrell *et al.* (2008), triclopyr, fluroxypyr, glyphosate +fluroxypyr, metsulfuron-methyl +triclopyr and picloram +triclopyr all did significant damage to tradescantia. However, triclopyr and the other herbicides trialled successfully on tradescantia are also damaging to native species (Kelly and Skipworth, 1984b; Harrington and Schmitz, 2007; Hurrell *et al.* 2008), and herbicides also require follow-up treatment to ensure that tradescantia does not re-invade (Standish, 2002; Hurrell *et al*. 2009). In 2012, Hurrell *et al.*

calculated the amount of time, herbicide and money that was required to control tradescantia in Whakapohai Reserve, South Westland, New Zealand. Reducing and maintaining tradescantia cover to from 17.4% cover (7.6) to less than 0.01% of the reserve took eight years and required 32,986 L of made-up triclopyr herbicide, 965 working hours, and $180,000 (2010 NZD).

Not only is removal problematic and expensive, but instant (or near instant) removal of the plant may be harmful to other species. As mentioned before, herbicides can harm the native vegetation. Furthermore, a study found that the native New Zealand snail *Powelliphanta traversi* used tradescantia as habitat in the absence of native ground cover (Standish, Bennett, and Stringer, 2002b). The removal of this without concurrent replacement with native ground cover could greatly impact on the snails, and removal with the herbicide triclopyr could impact on later snail generations and possibly their prey (Standish, Bennett, and Stringer, 2002a) .

Low light levels are the main limiting factor that restricts tradescantia spread throughout a site (Kelly and Skipworth 1984a; Maule *et al.* 1995; Standish *et al.* 2001). A study by Standish (2002) found that shading out (reducing light below 5% full light) tradescantia was the most successful method in reducing tradescantia biomass, but this has little practical application in larger areas. Artificial shading requires large amounts of equipment and man-power to carry in and construct the shade, while natural shading using seedlings/saplings requires a long time period while the trees grow to produce the required shade. It does suggest that if native cover can be re-established, further control will be unnecessary in these areas. Areas where increased cover is not an option (waste areas, riparian zones) will need a different approach to control

Neolema ogloblini: *a new biological control agent*

A description of N. ogloblini

Class: Insecta

Order: Coleoptera

Family: Chrysomelidae

Species: *Neolema ogloblini*

Common name: Tradescantia leaf beetle

Photo 2: Three stages of tradescantia leaf beetle development. Larval form (top left), pupal cocoon (top right) and adult (bottom left).

In 2007, the tradescantia leaf beetle (*Neolema ogloblini*), a natural pest of tradescantia, was brought into New Zealand from Brazil by Landcare Research and finally released in 2011 (Landcare Research, 2012b; Fowler *et al*. 2013). It has established at several sites and appears to have failed at others, but it is still too early to judge the success as a biological control agent (Landcare Research, 2012b; Q. Paynter, personal communication, 20th December 2012).

This species was the most host-specific of several beetles surveyed in research trials, with only minor feeding on other plant species within the family Commelinaceae (Fowler *et al*., 2013). The larvae are the most damaging; with younger larvae forming feeding fronts and the older larvae feeding alone (Landcare Research, 2012b). Larvae can skeletonise entire leaves, while the adults are less damaging but can still consume entire leaves (Landcare Research, 2012b).

Little research or experimentation has been done on this beetle, either inside New Zealand or within its own native range. The only source of information on this species is the work done by Landcare Research before and after its recent release, and the recent paper by Fowler *et al*. (2013). Since 2011, at least 9755 individual tradescantia leaf beetles have been released at over 47 sites; Fowler *et al*. (2013) stated these amounts as being precise, we can assume that number has now increased from continued releases.

Photo 3: Comparative damage from larvae (left) and adults (right).
Note the 'windowing' on the leaf to the left, created when the larvae scrape epidermal tissue from the lower and upper surfaces. Meanwhile, adults consume sections from the edge of leaves, resulting in the ragged edge seen on the leaf to the right.

The goals

Standish (2001) states that a reduction of tradescantia biomass to 200gm^{-2} or below could be a realistic goal for a biological control program. The reduction of tradescantia biomass will be the measure by which this biological program is judged (Fowler *et al*. 2013). However, *N. ogloblini* is not expected to work alone; two other beetle species and a fungus provide complementary damage (to stems, foliage and growing tips). The two other beetle species (the stem beetle (*Lema basicostata*) and the tip beetle (*Neolema abbreviata*)) have been released, and the permission has recently been obtained for the yellow leaf spot fungus (*Kordyana* sp.) to be released following containment (Landcare Research, 2013; Fowler *et al*. 2013). However, any reduction in vigour that *N. ogloblini* can provide would be of benefit to any forest or forest remnant suffering from an invasion (Froude 2002). The gradual reduction in biomass that the agent(s) will likely provide should also reduce the risk of re-invasion by other weeds (Global Invasive Species Database, 2012e).

The study site; Monro's Bush

Photo 4: Monro's Bush, taken facing South-Southwest

Monro's Bush is only a few metres away from where tradescantia was first introduced to New Zealand in 1910 (Kelly and Skipworth 1984a), potentially making the forest remnant the longest occupied by tradescantia in New Zealand; over 100 years. Monro's stretches across the base of Monro's Hill, next to Massey University, Palmerston North (40°23.3' S, 175°36.7' E). It is a 2ha lowland forest remnant, currently subject to minimal disturbance by humans and stock is excluded (Kelly and Skipworth, 1984a). However, being so close to the University, it has been researched and used as a study site on many occasions (Esler 1962; Kelly and Skipworth 1984a; Standish *et al.* 2001; Standish 2002).

Esler (1962) provided a list of plant species found in Monro's Bush, although it was not known by that name at that time (Appendix 1). He noted a dense canopy of pukatea (*Laurelia nova-zelandiae*) and some other broad-leaved trees, with a few emergent kaihikatea (*Dacrycarpus dacrydioides*) trees and tradescantia as a prominent understory plant. Skipworth and Kelly (1984a) gave the canopy trees as tawa (*Beilschmiedia tawa*), karaka (*Corynocarpus laevigatus*), mahoe (*Melicytus ramiflorus*), titoki (*Alectryon excelsus*) and pukatea, while the most common understory plants were kawakawa and tradescantia.

Monro's Bush is adjacent to paddocks belonging to an agriculture research facility, and ground belonging to Massey University. It has a small stream running through it (not shown on Photo 5), which first passes through Massey University.

Photo 5: Monro's Bush, aerial photograph

The study site, with rough outlines of property and forest boundaries in red. The blue dot indicates the position of the photographer when the previous photo (Photo 4) was taken.

Image taken from Google maps (Google, 2013).

Aims of this study

In order to judge a biological control agent as successful or not, the impacts it has (or does not have) on the pest plant must be observed. Impacts in terms of plant size and growth must be assessed, and the impacts of the pest plant itself should be compared before and after the agent has been established.

The recent release of the first biological control agents for tradescantia has provided the opportunity to observe their effectiveness.

The aims of this thesis are as follows;

1. **To compare the effectiveness of the new biological control agent against traditional herbicide methods:**

It is hypothesised that the biological control agent will provide a level of control that will equal or exceed the level of control provided by the use of herbicide, with respect to survival and growth of seedlings and seeds planted into treated tradescantia.

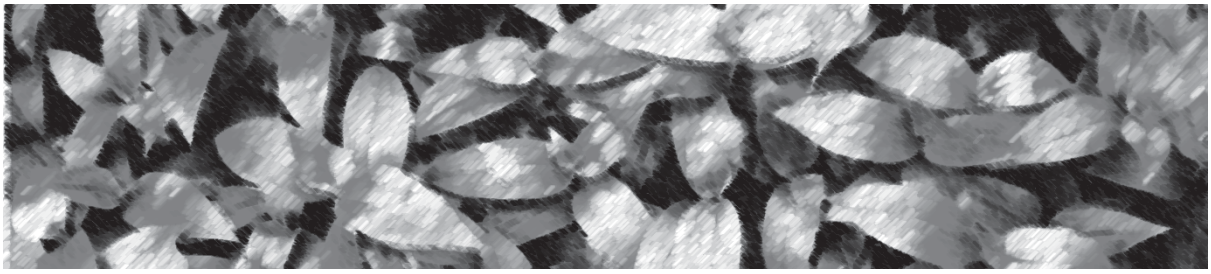
2. **To compare the regrowth of tradescantia when cleared back using herbicide versus by hand:**

It is hypothesised that the regrowth of tradescantia will be slower when re-invading an area treated with herbicides, but that regrowth of other vegetation (native or otherwise) will also be comparatively slower than in those areas cleared by hand.

3. **To provide a descriptive account of a remnant infested with tradescantia before the establishment of *N. ogloblini* and any other biological control agents:**

In order to observe and measure the effects that any biological control agent might have, a survey of an area should first be taken, to provide a base measurement with which to compare any changes.

Chapter Two: Measuring the impact of Neolema ogloblini



Introduction

Tradescantia (*Tradescantia fluminensis*) is now counted top-most among the worst invasive weeds of New Zealand (Esler, 1988; Froude, 2002; Syrett, 2002). The effects of tradescantia on native forests are numerous (Esler, 1988; Toft *et al*. 2001; Standish, 2004; Standish *et al*. 2001; Standish *et al*. 2004), but the primary concern is the prevention of forest regeneration. Many native seedlings are unable to tolerate the low light levels beneath the thick carpet, so germinate and die before reaching the light above the tradescantia canopy (Standish *et al*. 2001). Traditionally it has been controlled with herbicides (Department of Conservation, 2012), but this is labour and time expensive, not to mention harmful to other species (Kelly and Skipworth, 1984b; Harrington and Schmitz, 2007; Hurrell *et al.* 2008). These herbicides kill not only tradescantia but also native species present in the area. Further studies on different methods of control has found that light deprivation (shading out) is an effective means of control (Standish, 2002), but this method has not been put into use; possibly because of difficulties in implementing it. Three new biological control agents were recently released nation-wide (within tradescantia's range), the first of which is the study species; *Neolema ogloblini*, the tradescantia leaf beetle (Landcare Research 2013; Fowler *et al*. 2013). This species has established at several sites but failed to establish at others (Q. Paynter, personal communication, 20th December 2012) and there is currently no data on the beetles' effectiveness when compared to the traditional method of herbicide spraying.

The aim of this part of the study was to look at the differences between the level of control provided by herbicide and the tradescantia leaf beetle, and the results of each method when looking at the survival and growth of transplanted native seedlings and

the establishment rates of sown seeds. It is hypothesised that the biological control agent will reduce biomass to a level that allows increased survival and growth of the seedlings, due to increased light levels. Conversely, herbicide is hypothesised to harm many seedlings, but decrease biomass to a level far below that provided by the biological control agent.

Materials and Methods

Tradescantia fragments were planted into 160 plastic planter bags (18L) filled with long-term potting mix to a height of ten centimetres (Appendix Two). The 160 bags were split into two sets (Rep. 1 and Rep. 2); the two sets were started at different times and grown in separate places. Rep. 1 was grown and experimented upon in a temperature and light-controlled laboratory (16^{O}C and 10% of full light (averages)), while Rep. 2 was grown in shaded and heated glasshouse (13.5 ^{O}C and 19% of full light (averages)), and later experimented on in a different glasshouse (18.5 ^{O}C and 34% of full light (averages)). As the tradescantia established, three hollow plastic tubes (10cm high, 4cm diameter, 0.4cm thick) were inserted into each pot, equally spaced between one another and at least three centimetres from the pot edge (Photo 6, centre image) to provide a placeholder for seedlings to be inserted into later.

Typically, biomass of tradescantia in wild-situations can be calculated by inputting the average height and percentage cover of the tradescantia into an equation (Eq. 1).

$$\text{Biomass}(gm/s^2) = 0.014\ (\text{percentage cover} \times \text{height}).$$

Example: Average height is 146mm and percentage cover is 100%.
Biomass= 0.014x(100x146)
Biomass=200.2gm/s^2

Equation 1: Tradescantia biomass equation. Provided by Landcare Research.

However, this equation takes into account the built-up layers of vegetation that occur naturally; the potted plants were not natural and there may have not been sufficient time to accumulate such layers. The initial biomass at planting was not estimated, the starting point was instead based on the author's observations on the

growth of the potted plants, and time constraints. When experimentation on Rep. 1 and Rep. 2 was started, biomass and soil-level light of 20 random pots in each replicate were measured to provide starting point measurements (see below).

After the growing period was over, the 80 potted plants (pots) of each replicate were randomly allocated to one of four treatments; (1) traditional herbicide method (herbicide treatment), (2) new biological control agent (beetle treatment), (3) no control method (no treatment), and (4) bare soil control (control). Herbicide treatment pots were taken outside and sprayed (using a 500ml spray bottle) with Grazon® herbicide (active ingredient 600 g/L triclopyr; Dow AgroSciences (NZ) LTD, New Plymouth) at a rate of 0.72% ai (plus 0.1% Pulse surfactant (>800g/L active ingredient organomodified polydimethyl siloxane, NuFarm LTD, Auckland)) until the point of run-off (rate and type of application based on trials in Hurrell *et al*. 2008). Ten *N. ogloblini* beetles were added to the beetle treatment pots. The tradescantia in the control pots was removed (and the vegetation used to calculate the average biomass of the pots at the start). The tradescantia of the no treatment pots was left intact. All pots were enclosed in mesh bags to prevent beetle escape and cross-contamination; these mesh bags decreased the light available to the plants, but light inside remained above 5% of full light. The pots were left alone (except for a daily watering) for four weeks.

Photo 6: Replicate Two

Replicate two, before applying treatments (top image), applying treatments (middle left: applying herbicide; centre: a pot with vegetation removed; middle right: a beetle added to a plant), and the pots with their mesh bags tied up (bottom image; pink flags marked beetle treated plants)

During this time, 120 seedlings of mahoe and kawakawa (240 in total) were collected for each replicate. Seedlings for Rep. 1 were collected from the Manawatu Scenic Gorge Track, in the Manawatu district, close to Palmerston North. The seedlings for Rep. 2 were collected from a privately owned forest in Tolaga Bay, located on the East Cape. The seedlings were allowed to rest and grow in the same glasshouse/laboratory as their replicate pots. The species of the seedlings were two of those tested in the Standish (2001) paper in their response to tradescantia. Kawakawa was found to be relatively intolerant (LD50 12gm^2) while mahoe was moderately tolerant (LD50 28gm^2).

Photo 7: Kawakawa seedlings
Some seedlings collected for Rep. 1, in their tray awaiting transplantation.

After the four week-long rest and treatment period, the treated pots were randomly divided into two even groups with 10 pots of each treatment for each group, resulting in two 40-pot groups. The two groups were assigned to the two native seedlings collected beforehand, mahoe and kawakawa.

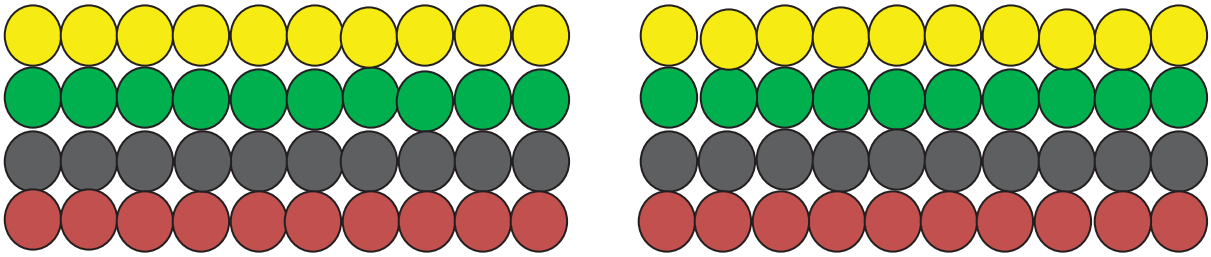


Figure 4: Pots divided between treatments and species
The four treatments are represented by the four rows of different colours, and the two main columns show the division of the treatments between the two different species of native seedlings.

The seedlings collected previously were now planted into the gaps that the hollow tubes (previously inserted) left behind when pulled out. This minimised disturbance to the tradescantia thatch. In Rep. 1, the seedlings of both species collected were of varying sizes and were arranged in groups according to their size; these groups were

evenly split between the treatments. Average measurements were taken of each group within each species; the largest leaf length, stem diameter and seedling height. In Rep. 2, the seedlings were more uniform in size, and individual measurements of the same variables were taken of each seedling (the position within individual bags recorded for each measured seedling). In addition, scarified seeds of mahoe and kawakawa (sourced from Proseed, Amberly, NZ) were also sown into the relevant pots, approx. 20 in each. The pots were now left for 12 weeks, except for a daily watering.

During this period, the beetle treatment pots were watched, with the aim of monitoring the progress of the beetles. Six weeks into the 12 week experiment on Rep. 1, it was noticed that beetle activity had dropped after the application of a fungicide (Yates (Watkins) copper oxychloride (500g active ingredient/kg product) at rate of 2.5g/L) to control a fungal infection of the tradescantia (suspected *Phoma* sp.). The beetle mortality rate was estimated to be 90%. Further beetles were not added. In Rep. 2, it was noticed after eight weeks that many plants were showing signs of definite larval and beetle damage. In fact, the extent of the damage was so great (Photo 8) that the decision was made to reduce the beetle populations in some pots.

Photo 8: Extensive damage
Contained within the mesh bags, in potentially ideal conditions, the beetles successfully multiplied. They impacted on the tradescantia to levels that were not considered natural or realistic.

Measurements were taken at soil level below the tradescantia canopy and compared to the light levels above the tradescantia canopy. If the measurements of a pot provided a percentage at or above 5%, the beetle population was decreased in that

pot by removing up to 30 beetles. This percentage was chosen because anywhere above 5% of full light in glasshouses should result in increased seedling growth (compared to lower light levels; Ebbet and Ogden, 1998), 5% was the maximum light recorded by Standish *et al.* (2001) under 200gm^2 of tradescantia, and I wished to keep the beetle damage within realistic levels. To remove beetles, the mesh bag was opened and left for a few minutes; the mesh bag was then folded down, and any obvious beetles or larvae were picked off. The mesh bag was then tied back up. This was effective in decreasing the population, but was not intensive enough to remove all individuals. This procedure was carried out once more, two weeks after the first instance. In the second instance, the bags that had previously been above the 5% mark in the first instance were measured for light levels but their beetle populations were not reduced further.

After the twelve week period, the Rep.2 pots and their respective plants were then destructively sampled. First, the mesh bags were removed from all pots. For each pot, light measurements were then taken from the canopy and sub-canopy positions as above. The tradescantia plant mass (dead or alive) was determined from each pot by drying the shoots at 60^{o}C for at 120 hours and then weighing. Any seedling still alive was re-measured, and those seedlings in Rep. 2 were matched back up to their original measurements through their position within the pot. Finally, the number of germinated seedlings from the seeds was recorded, as well as the number of leaves these seedlings possessed. In the pots where beetles were added, the vegetation was shaken gently (to dislodge adult beetles), and any obvious larvae were picked off. After 16 weeks, the same process above was used to sample Rep.1.

Competition trial (variable exclusion)

Because multiple seedlings were in each pot of the replicates, there was the potential for competition between the seedlings themselves. To measure this, a small competition trial with no tradescantia was set up to run alongside the main experiment. In this trial, 18 pots of soil were set up and split between the two native species trialled above (mahoe and kawakawa); 9 pots to each. The first three pots of each group contained only one seedling (no competition), the next three had three

seedlings each (what the main experiment had), and the final three had ten seedlings each (almost certain competition) (Photo 9). These seedlings were sourced from the

Manawatu Scenic Gorge Track, and were close to one another in size, and to the size of Rep.2 seedlings.

Photo 9: Competition Trial set up

Three competition pots, showing the positions of one, three and ten seedlings within the pots.

Statistics

All statistical tests were performed using Minitab version 16 (Mintab Inc., 2010). Hypotheses were tested at a 5% level of significance, and Tukey's grouping method was used where more than two populations were analysed, to control family error rate. This method produced 95% joint confidence intervals.

Results

Seedling germination

In both replicates, many seeds failed to germinate. Only kawakawa seeds germinated in Rep. 1, while no seeds germinated in Rep. 2. Therefore, comparison of germination percentage was not carried out.

Biomass

The differing dry biomasses of tradescantia from Rep. 1 in the three treatments were tested against one another and the initial measurements (taken when treatments were applied) using one-way ANOVAs with the Tukey method, resulting in 95% joint confidence intervals (Fig. 5). The treatments and the base measurement were all significantly different from one another (P<0.005); the un-manipulated pots (no treatment) had the largest dry biomass (grouping A, n: 20, StDev:15.38g, mean: 102.03g), followed by beetle treatment (grouping B, n:19, StDev13.89g, mean: 63.89g), initial measurements (grouping C, n:21, StDev: 15.02g, mean: 30.83g) and herbicide treatment (grouping D, n:20, StDev: 4.88g, mean: 10.95g).

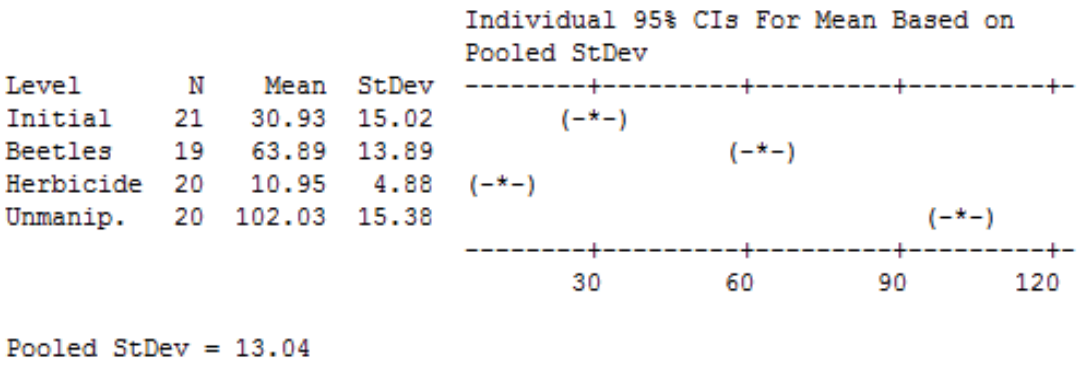


Figure 5: Confidence Intervals for biomass of different treatments
Here, the differing biomass of three treatments and the initial measurements are compared using an ANOVA.

The above measurements of biomass have been in grams, the amount found in each pot (23cm x 23cm = 529cm^2). The means and standard deviations from above were transformed into the grams per square metre by multiplying the biomass and standard deviations by 18.9 (10000/529) (Table 1).

Table 1: Table of biomass means and standard deviations

Here, the biomass per bag has been transformed into the biomass per square metre

	Mean (g/sqm)	Standard Deviation (g/sqm)
Initial	584.6880907	283.9319
Beetle	1207.750473	262.5709
Herbicide	206.9943289	92.24953
No Treatment	1928.733459	290.7372

Light Levels

A one-way ANOVA with Tukey's method was used to compare the soil-level light of the pre-treatment plants (initial) and the two different treatments in 95% joint confidence intervals (Fig. 6); un-manipulated and beetle treatment. There was a significant difference ($P<0.005$) in the soil-level light of the two treatments; the percentage of full light at soil level was higher (grouping A, n:20, StDev: 7.71%, mean: 8.54%) in those pots with beetle activity than in those with no beetle activity (grouping B, n:20, StDev: 0.261%, mean: 0.264%). Mean soil-level light was not significantly different ($P<0.005$) between the initial measurements (grouping B, n:20, StDev:1.009% mean: 0.8%) and the no treatment measurements.

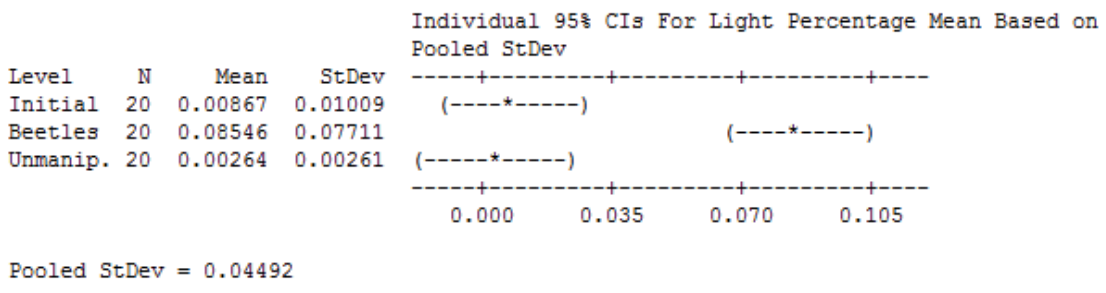


Figure 6: Confidence intervals for percentage of soil-level light.
This Anova chart compares the light ranges of two treatments (beetles and un-manipulated) and initial measurements

Seedling survival and comparative growth

Survival of planted seedlings in Rep 2 was best for the control pots (93.3%), followed by the beetle treatment pots (90%), followed by the un-manipulated (73%) and herbicide treatment (10%).

Despite high beetle mortality, replicate 1 has been included here, to show the difference in survival rates when the length of the experiment was extended to 16 weeks. In Replicate 1, the comparative seedling survival between treatments followed the same general pattern; Control (80%)>Beetles (20%)>Un-manipulated (10%)> Herbicide (0%).

Table 2: Survival rates of seedlings
Comparison of the survival of planted seedlings in the four treatments

Method	**Rep. 1(16 weeks)**	**Rep. 2(12 weeks)**
Control	0.8	0.93
Beetles	0.2	0.9
Un-manipulated	0.1	0.73
Herbicide	0	0.1

Only Rep. 2 is used in the remaining analyses. Base measurements of stem diameter, largest leaf length and height were compared against the new measurements of each seedling, and the difference (decrease/increase in size) calculated. In the results, this difference will be referred to as 'growth' unless a specific measurement is being explained.

One-way ANOVAs were performed to compare the growth of seedlings between the different treatments. ANOVAs were performed on the stem diameter, largest leaf length and height of all seedlings, irrespective of species (Fig. 7).

Analysis of stem diameter growth (Fig. 7, top CI chart) revealed that the 95% joint confidence intervals of beetles (n:53, StDev: 0.31mm, mean; 0.0047mm), herbicide (n:6, StDev:1.00mm, mean:0.3083mm) and un-manipulated (n:43, StDev:0.33mm, mean:0.0651mm) were not significantly different from one another (P>0.005, grouping B). Growth of stem size in the control 95% joint confidence interval was significantly

different (P<0.005, grouping A, n: 56, StDev: 0.9591mm, mean: 2.02mm); those seedlings in the control pots had significantly larger stem diameters.

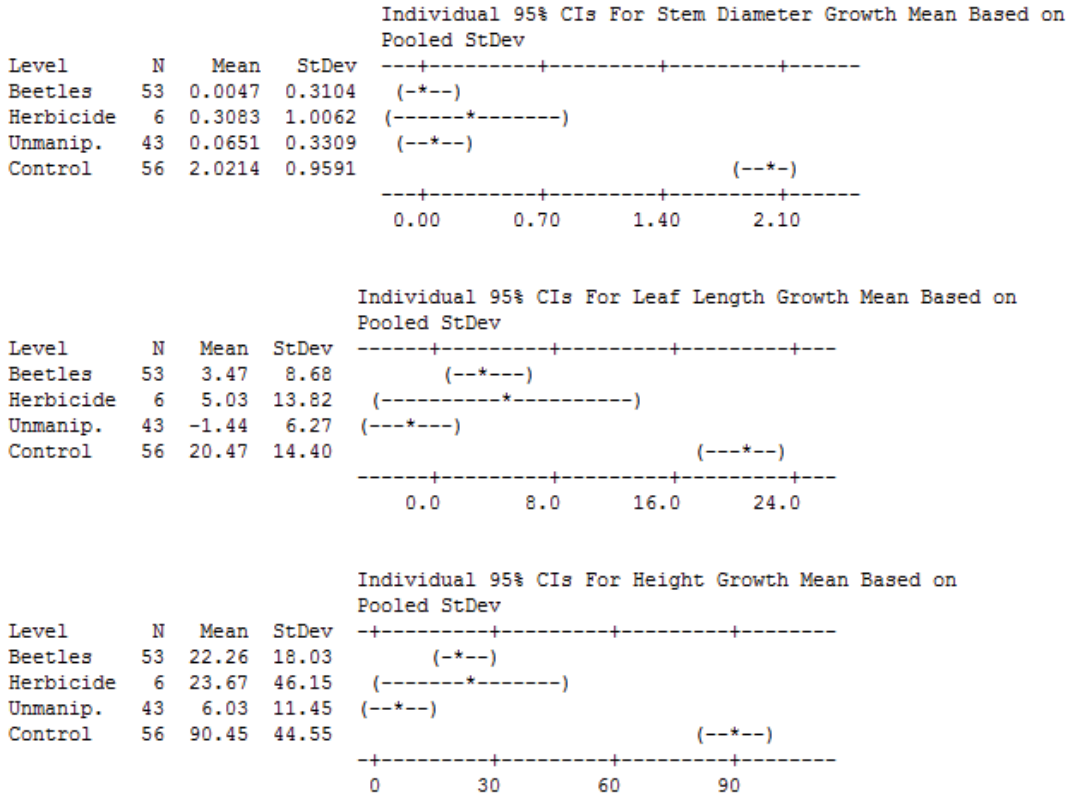


Figure 7: Confidence intervals of seedling variables
Confidence intervals for the growth of stem diameter (top), leaf length (middle) and height (bottom).

The results of the leaf length growth (Fig. 7, centre CI chart) were much the same. The 95% joint confidence intervals of beetles (n:53, StDev:8.63mm, mean: 3.47mm), herbicide (n:6, StDev:13.82mm, mean: 5.03mm), and un-manipulated(n:43, StDev: 6.27, mean: -1.44mm) showed no significant growth difference between one another (P>0.005, grouping B). Once again, the growth of leaf length in the control 95% joint confidence interval was significantly different from the growth of leaf length in the other treatments (P<0.005, grouping A, n:56, StDev:14.40mm, mean: 20.47mm); leaf length increased significantly.

The 95% joint confidence intervals for height growth (Fig. 7, bottom CI chart) were not significantly different (P>0.005, grouping B) when looking at the three treatment groups; beetles (n:53, StDev: 18.03mm, mean:22.26), herbicide (n:6, StDev: 46.15mm, mean: 23.64mm), and un-manipulated (n:43, StDev, 11.45mm, mean:11.45mm) had growth that overlapped. The control seedlings showed an increase in height that was

significantly different from the three treatments (P<0.005, grouping A, n: 56, StDev: 44.95mm, mean: 90.45mm).

The overall data was then divided between the species, and the same parameters put through ANOVAs.

Mahoe seedlings (Fig.8) returned similar results to the overall results. Only one mahoe seedling survived in the herbicide treatment pots, and so this treatment was discarded here.

95% joint confidence intervals showed that stem diameter growth (Fig. 8, top CI chart) was not significantly different (P>0.005, grouping B) between beetle (n:28, StDev:0.2952mm, mean:0.0018) and un-manipulated (n:16, StDev:0.3205mm, mean:0.0594mm), but stem diameter increased significantly for those seedlings in the control (P<0.005, grouping A, n:28, StDev:0.9258mm, mean:2.319mm).

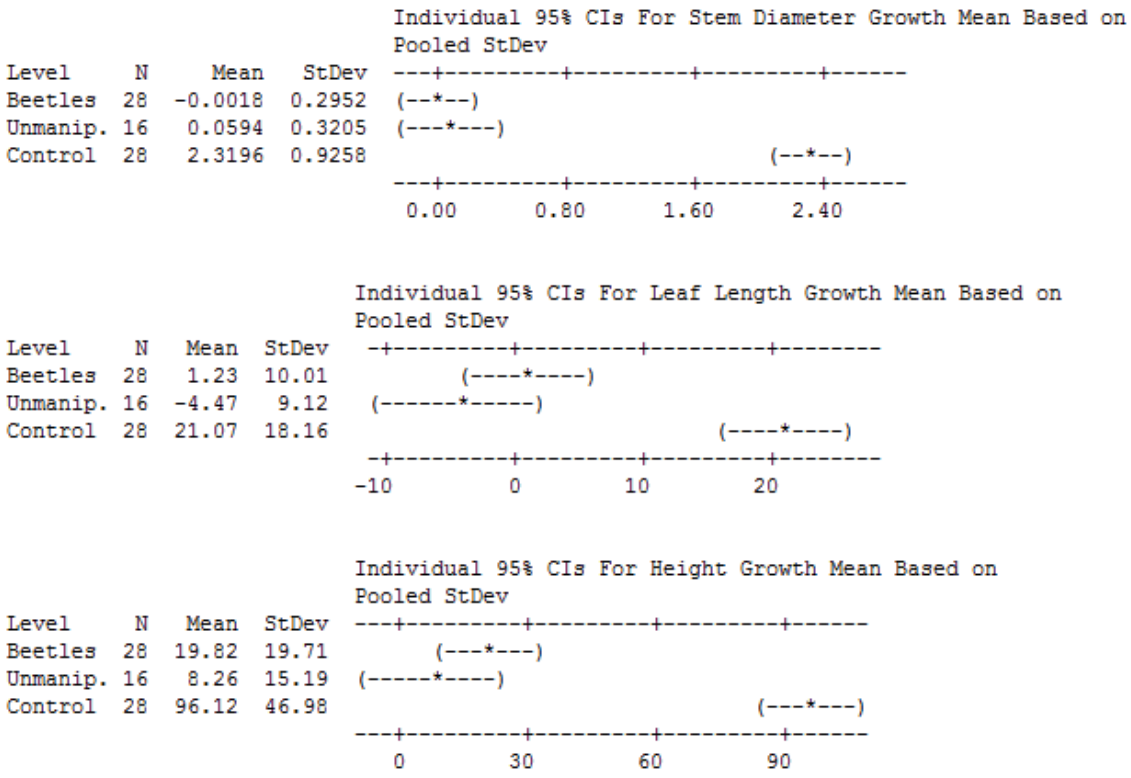


Figure 8: Confidence intervals of Mahoe variables.
Confidence intervals for the stem diameter (top), leaf length (middle) and height (bottom) growth of mahoe seedlings.

Leaf length 95% joint confidence intervals (Fig. 8, centre CI chart) indicated that growth was not significantly different (P>0.005, grouping B) between beetles(n:28,

StDev:10.01mm, mean:1.23mm) and un-manipulated (n:16, StDev:9.12, mean: -4.47mm), but was for the increase in length shown by the seedlings in control (P<0.005, grouping A, n:28, StDev:18.16mm, mean:21.07mm).

Height growth for mahoe (Fig. 8, bottom CI chart) was not significantly different (P>0.005, grouping B) for beetles (n:28, StDev:19.71mm, mean:19.82mm) and un-manipulated (n:16, StDev:15.19mm, mean:8.26mm) when looking at the 95% joint confidence intervals, but was for the increase in control seedling height (P<0.005, grouping A, n:28, StDev:46.98mm, mean:96.12mm).

Kawakawa seedlings (Fig. 9) showed the same trend for most of the variables, but did show significant difference (P<0.005) in some parameters other than those in the control.

Stem diameter growth (Fig. 9, Top CI chart) of those seedling in beetles (n:25, StDev:0.3327mm, mean:0.012mm), herbicide (n:5, StDev:0.498mm, mean:-0.06mm) and un-manipulated(n:27, StDev:0.3428mm, mean:0.0685mm) was not significantly different (P>0.005, grouping B) in the 95% joint confidence intervals, while the growth of those in control did increase significantly (P<0.005, grouping A, n:28, StDev:0.9122mm, mean:1.7232mm).

Leaf length growth (Fig. 9, centre CI chart) was different: control (grouping A, n:28, StDev:9.582mm, mean:19.873mm), beetles (grouping B, n:25, StDev:6.174mm, mean:5.98mm) and un-manipulated (grouping C, n:27, StDev:2.538mm, mean:0.357mm) were all significantly different (P<0.005) from one another in the 95% joint confidence intervals . Control had the largest leaf length increase, followed by beetles with the medium leaf length increase, and un-manipulated with the smallest leaf length increase (even a decrease in size). Herbicide treatment overlapped (grouping B C, n:5, StDev:8.939mm, mean:0.430mm) with un-manipulated and beetles, and so was not significantly different (P>0.005) from those two treatments.

The 95% joint confidence intervals for height growth (Fig.9, bottom CI chart) were much the same. Control (grouping A, n: 28, StDev: 42.07mm, mean: 84.78mm), beetles (grouping B, n:25, StDev: 15.90mm, mean:25mm) and un-manipulated (grouping C, n:27, StDev:8.58mm, mean:4.72mm) were all significantly (P:<0.005) different from one another. Control had the largest height increase, followed by the beetle's medium height increase, and the un-manipulated smallest height increase (even a potential decrease in height). Herbicide, like before, overlapped the confidence intervals of un-manipulated and beetles (grouping B C, n:5, StDev:8.14mm, mean:5.07mm) and so was not significantly different from these two treatments (P>0.005).

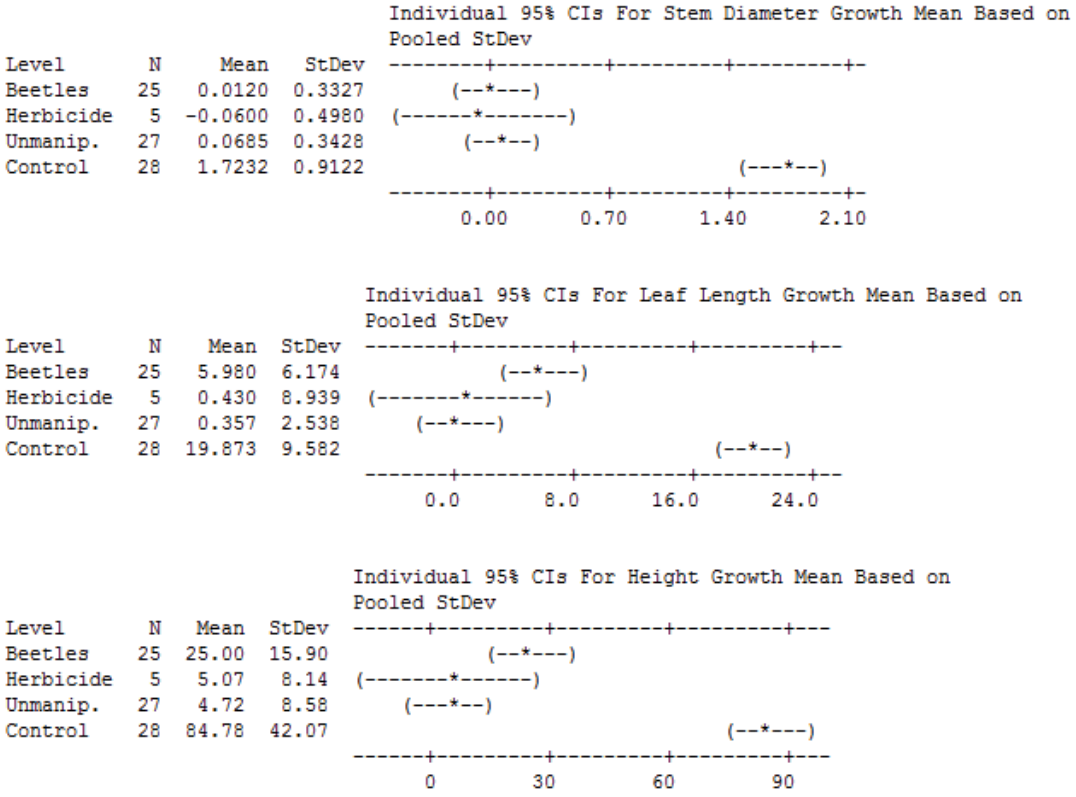


Figure 9: Confidence intervals of Kawakawa seedling variables
Confidence intervals for Kawakawa stem (top), leaf (middle) and height (bottom) growth.

In many instances, seedlings show a decrease in size from when they were first measured. This may have been as a result of measuring error by the author, but could also have been a response to less-than-ideal conditions, and so these measurements were included in the analysis.

Competition Trial

Due to transplantation shock and the time limitations, this trial was not completed. Soon after setup, many of the seedlings died, irrespective of competition degree. This was attributed to transplantation shock instead of competition, as even some seedlings in single-seedling pots died. Because of time constraints, the decision was made to not to restart this trial.

Discussion

First of all, attention should be drawn to the fact that this experiment was not performed in the field. The failure of the beetle to establish in many places has meant that the opportunity for field trials has been limited. Only in the light of this restriction, can the results be discussed.

The efficacy of the biological control agent has been assessed in four ways; the change in available light beneath the canopy, the change in tradescantia biomass, survival of native seedlings beneath the tradescantia canopy and the growth of those seedlings. The results of each assessment method will be discussed individually, then the overall results summarised.

Biomass

Beetle treatment pots showed a significant reduction in the growth rate of the tradescantia (in terms of biomass); while there was an increase from the pre-experiment size, it was not as high as the size of the un-manipulated plants. Conversely, herbicide did reduce the biomass to below that of the initial measurements, as expected.

Biomass in the initial measurements, un-manipulated pots and beetle treatment pots show levels far above the amount predicted by Standish *et al.* (2001) that would allow regeneration (200gm^2). Even the pots treated with herbicide had dead tradescantia that just exceeded that regeneration limit. The biomasses found in the beetle treated and un-manipulated pots are also far above what is normally found in the field (maximum 819gm^2), probably because of the constrained space preventing

the plant from sprawling. Most of the biomass in the beetle-treated pots came from the stems; these are likely to have less of an effect on the amount of light reaching the soil than leaves. Biomass and percentage cover are only the indicators of light availability below the tradescantia canopy; light was markedly different underneath these plants (discussed below).

The reduction of biomass due to beetle feeding here is unlikely to reflect what would happen in the field. The reason for this is the lack of dispersal in the individual pots (beyond what was done when light levels passed 5%), resulting in a population density that was most-likely unnatural. Fowler *et al.* (2013) discussed the possibility that the biological control agent in New Zealand would be released from specialised predation, and predation should not be an issue when populations are large, but it is the containment of the population that affects the applicability of this experiment to field situations. Adult movement (vertebrate or invertebrate) within an area will include movement to find resources lacking in the home range (Drake and Dingle, 2007; cited in Kim and Sappington, 2013). The possibility of low dispersal ability in *N. ogloblini* cannot be ruled out, but in this experiment the amount of tradescantia in many pots decreased to a level where adult movement was required in order to find new food and oviposition sites. In this case, adults have been limited by the amount of tradescantia available for oviposition sites, and the hatching larvae have been forced to feed intensively on this small area. The behaviour of the agent has not been studied, and they may respond to over-crowding with some form of population control. Some species have regulatory mechanisms that prevent overcrowding (Monro, 1967). However, it is important to note that the beetle populations may reach those high densities; we cannot predict their behaviour in the field and so we cannot say for certain what their densities in the wild will be.

While the population densities of the beetle pots were likely to be unnatural, it is encouraging that such a high tradescantia biomass can potentially be controlled by a large beetle population.

Light Levels

The light percentage at soil-level was significantly different between the beetle treatment (8.54%) pots and the un-manipulated (0.264%) pots. This increased light was likely to be the reason for the increased height and leaf size of the kawakawa seedlings.

While light levels were increased significantly from that of the initial measurements and the measurements under the un-manipulated plants, this may not occur in natural situations if beetle densities are lower.

Also, the 5% limit that was used to determine when beetle populations needed to be reduced was taken from a combination of the Ebbet and Ogden paper (1998), where podocarp species exhibited increased growth in glasshouses above 5% of full-light, and the 2001 paper by Standish *et al.*, where 5% was the highest value measured under (roughly) 200gm^2 of tradescantia in the field. However, that 5% light level may be lower or higher than the actual light limit that allows increased growth in angiosperms like kawakawa and mahoe. The mesh bags did reduce the amount of light that could enter the bags, but the amount that did (24%) was within the range of forest-floor light measured by some papers (2-30%: Ebbet and Ogden (1998); 1-30%: Standish *et al.* (2001)).

Seedling Growth

Growth of seedlings in the treatment pots was not significantly different from one another, while those seedlings in the control pots were significantly larger. When the data was split between the species, seedlings in the control pots were still significantly larger than those in the treatment pots. However, there was also a significant increase in kawakawa height and kawakawa leaf length for those seedlings in the beetle pots when compared to kawakawa seedlings in the un-manipulated pots. Mahoe seedling measurements did not differ significantly between the treatment pots. If this experiment had been run for a longer time period, it is possible that mahoe would have started respond to the increased light, and the results may have been clearer.

These results suggest that despite the increased light in the beetle treated pots, seedling growth did not always increase correspondingly. Kawakawa, despite being

less tolerant of tradescantia than mahoe (Standish *et al.* 2001), performed the best in the beetle treatment pots. It responded with an increase in leaf size, and an increase in height. While it is promising that the kawakawa seedlings did respond favourably to the treatment of tradescantia with the biological control agent, this may not translate to the field if the agent does not form dense populations.

Seedling Survival

The survival of seedlings was best in the control, as expected. The Replicate 2 control survival rate of 93% was regarded as the best possible rate in these circumstances (glasshouse conditions, transplantation shock), and the survival of seedlings in the treatments was compared against it. Comparative seedling survival was best under those plants treated with the new beetle biological control agent (90%), with the remaining treatments as follows: 73% (un-manipulated) and 10% (herbicide). In this assessment of success, the biological control agent performed as hypothesised; survival was better for seedlings in those pots treated by beetles compared with those treated with herbicide or not treated at all. The survival rate of seedlings in the beetle treatment pots was comparable to that of the seedlings without any tradescantia at all (control). This also suggests that a longer experiment could have translated into clearer results for seedling performance.

The reason for the low seedling survival in the herbicide pots can be attributed to the residual activity of herbicide in the soils. Triclopyr has a reported half-life (in moist & irradiated soils) of 308hr (12-13 days), but when soils are not moist, the degradation of triclopyr is slower (Graebing, Frank and Chib, 2003). Irradiation (in the form of sunlight) was present and probably dried the soils out. After 28 days (rest period before seedlings go in) the herbicide present in the soils of the herbicide pots could have been as low as one-quarter of the starting amount, but was likely to be higher. Even this reduced amount seems to have been detrimental to the native seedlings, thus the low survival rate. Survival of seedlings in the herbicide pots may have been higher if the rest period between herbicide application and seedling insertion was longer, or if the soils had been kept moist for longer. A limitation of this experiment is the lack of replication; the beetle treatment in the first replicate failed, and was also left for a longer time period, so was not directly available for comparison with Rep. 2.

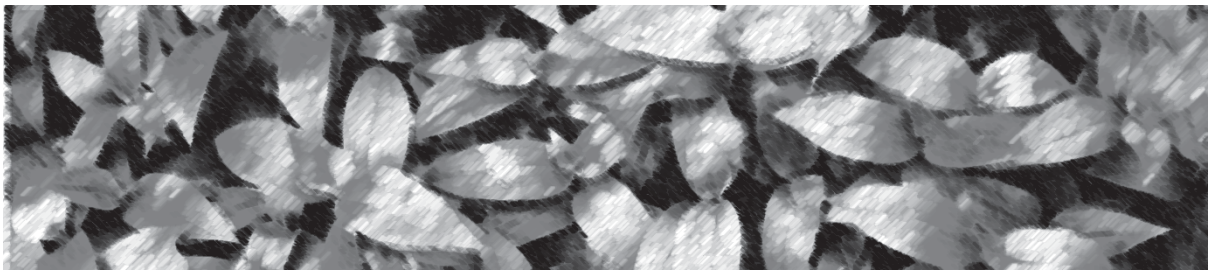
In reference to natural situations, some conclusions can be drawn. Where herbicide is applied to tradescantia in the wild, the herbicide could still be present in damaging amounts at least 26 days after application (provided the ideal moist and irradiated conditions are present (unlikely)). Survival of seedlings and other native plants that are present during application, or seed germination in the application area during this time, is unlikely.

Summary

In this study, the new biological control agent has provided some significant results in glasshouse situations, namely the increase of light levels and reduction of biomass.

It is recommended that the agent is trialled again in the field, with and without the other agents. In this way, their success in controlling tradescantia can be fully assessed.

Chapter Three: A description of an impacted site before control agents establish



Introduction

The biological control program for tradescantia was recently started (Landcare Research, 2012a; Fowler *et al*. 2013) and the effects of it have yet to be seen. In order to observe and quantify any changes in a site where a biological control agent has been introduced, baseline measurements should be taken. These can be compared against those measurements taken at a later date, to describe changes due to biological control. In the case of tradescantia, the most obvious (arguably the most important) change would be the increase in species richness and number of native seedlings.

Monro's Bush (40^{o}23.3' S, 175^{o}36.7' E) is located at the base of Monro's Hill, near Massey University in Palmerston North. It is just metres away from the site of the first reported introduction of tradescantia to New Zealand (Kelly and Skipworth, 1984a). It's location close to the Massey University campus means that it has been used multiple times as a study site (Esler 1962; Kelly and Skipworth 1984a; Standish *et al.* 2001; Standish 2002). In 1962, Esler provided a list of the plant species present at the site, and Kelly and Skipworth (1984a) gave a brief overview of the main plant species. However, the long-standing tradescantia occupation of Monro's may have changed the site, and merely including species names on a list does not indicate numbers, age of individuals, and location.

The aim of this section is to provide comparable measurements of species presence and numbers in a site before the tradescantia biological control program is established.

Materials and Methods

Existing vegetation

Four plots (5x5m) were set up in haphazardly chosen locations in Monro's Bush. Quadrats were set up with fibreglass poles at the plot corners and the locations are plotted on the map provided in Appendix Three. These plots were then surveyed, based on the method used by Allen and McLennan (1983). The numbers and species of all trees (trunk diameter over 3cm), saplings (trunk under 3cm, but higher than 135cm), lianes, vines, epiphytes and tree ferns within the plot were all recorded. Four small quadrats (.5x.5m) were randomly taken within the larger plot (Photo 10). A brightly coloured marker was tossed into the plot from each side, and the quadrat centred over that marker. This allowed the quadrat to include those plants that were within the sapling definition, but larger and higher than what the quadrat could slip over. Within these quadrats, the species and numbers of all plants over the height of 12 centimetres were recorded, apart from tradescantia. If tradescantia was found within the quadrat, a photo was taken directly above the quadrat and the measurements of six random stems within the quadrat were taken, to estimate the

Photo 10: Photo taken for assessment of tradescantia cover and biomass.

percentage of cover and biomass within the entire plot (See Chapter Four (4.4.1) for more details on this method).

Potential vegetation: Seed bank

The seed bank was assessed by taking haphazard soil samples. The first set was taken on the 29th of March, 2012, and the second on the 31st of October, 2012. These samples were refrigerated for 19 days at 4 ^{o}C (as used in Fountain and Outred, 1991; cited by Standish *et al.* 2001). The samples were then spread out over potting mix and dolomite, and watered daily for three months. Any seedlings that did grow were identified and recorded.

Statistics

Minitab version 16 (Minitab Inc., 2010) and Microsoft® Office Excel® version 12 (Microsoft Corporation, 2006) was used to perform statistical analyses. Digital Sampling Method version 1.00 (Landcare Research Ltd, 2003) was used to estimate tradescantia cover.

Results

Existing vegetation

A table (Table 3) containing all species found in the plots was created. It is not intended to be a complete list of all species present in Monro's but a list of what was present in the plots surveyed. Elder (*Sambucus nigra*) and bindweed (*Calystegia sylvatica*) were new additions, these species not being present in the list compiled by Esler in 1962 (and therefore, supposedly not in Monro's at that time). Both are exotic.

Pearson's correlations were performed between the tradescantia biomass and the vegetation groups defined by Allen and McLennan (1983), except the tree fern group which had no individuals measured. There was no relationship ($r<0.1$) between the lianes/vines group ($n=4$, $r=-0.0189$) and tradescantia biomass. There was a weak ($0.1<r<0.3$) positive relationship between tradescantia biomass and the seedling/small plant group ($n=4$, $r=0.1216$). Moderate positive relationships ($0.3<r<0.7$) were indicated between tradescantia biomass and the saplings group ($n=4$, $r=0.6809$), the epiphyte group ($n=4$, $r=0.5817$), and the tree group ($n=4$, $r=0.0498$).

Table 3: List of plant species found in Munro's Bush by the author.

Species with an asterisk are exotic species

Trees	Shrubs	Vines and Lianes	Ferns and Fern Allies	Herbaceous Plants
Alectyron excelsus	*Macropiper excelsum*	*Calystegia silvatica**	*Asplenium flaccidum*	*Collospermum hastatum*
Beilschemiedia tawa	*Streblus heterophyllus*	*Calystegia tuguriorum*	*Microsorum scandens*	*Tradescantia fluminensis**
Coprosma robusta		*Metrosideros perforata*		
Corynocarpus laevigatus		*Muehlenbeckia australis*		
Laurelia nova-zelandiae		*Ripogonum scandens*		
Melicytus ramiflorus		*Parsonsia heterophylla*		
Pittosporum eugeniodides				
*Sambucus nigra**				

A Pearson's correlation was also done to examine any potential relationship between kawakawa (*Macropiper excelsum*) and the tradescantia biomass. There were no relationships ($r<0.1$) between biomass and kawakawa overall (shrubs and saplings, $n=4$, $r=-0.0499$) and kawakawa shrubs ($n=4$, $r=0.0931$), and a moderate positive relationship with kawakawa saplings ($n=4$, $r=-0.3014$).

Pearson's correlations were also performed to examine the relationship of tradescantia biomass to total (native and exotic) species richness, native species richness and exotic species richness. Total species richness and exotic species richness had strong positive relationships($r>0.7$) with biomass; total species richness ($n=4$, $r=0.8174$) and exotic numbers ($n=4$, $r=0.7468$). Native species richness had a moderate relationship ($n=4$, $r=06809$).

In the permanent plots, the numbers of individuals in each vegetation group were recorded, as well as the percentage of cover and the biomass of tradescantia (Table 4). This list shows the different amounts of trees, saplings, lianes/vines, epiphytes, tree ferns and small plants/seedlings in each plot, as well as the biomass and percentage cover of tradescantia in each plot.

Table 4: Numbers of individuals in vegetation groups and tradescantia details

This table presents the raw data without identification of species. Seedlings and small plants are not common, and tree ferns are totally absent. This does not represent the forest as a whole.

Plot	Trees	Saplings	Lianes and Vines	Epiphytes	Tree Ferns	Seedlings & small plants	Average estimated Biomass m^2 of tradescantia	Average Proportion Cover of tradescantia
One	12	2	4	0	0	0	122.8	0.300226
Two	5	2	6	1	0	0	408.5	0.8525
Three	3	0	6	0	0	0	345.1	0.1275
Four	17	2	0	2	0	2	150.3	0.5025

Two months after the plots were set up, it was discovered that stock had entered Monro's and grazed on the tradescantia of the 'South' bank (Appendix Three) where Plot Three was located (Photo 11). This plot was deemed unusable for later work; the grazing had reduced the tradescantia to 3cm in height in some places and soil had been churned up in many places; this could affect the presence of seedlings and saplings later on. There also appeared to be grazing on some karaka saplings. The marker pegs for the plot were removed and the plot abandoned. Figures 12, 13 and 14 show the graphs of species and individuals in each vegetation groups, for the three

Photo 11: Photo Three, after grazing.
The tradescantia has been reduced to stalks averaging 3cm in height.

remaining plots.

Plot one (Fig. 10) was notable because it had the lowest amount of vegetation groups recorded. Only trees, saplings and lianes/vines were recorded as present, and no seedlings were recorded. An invasive weed was present; Elder.

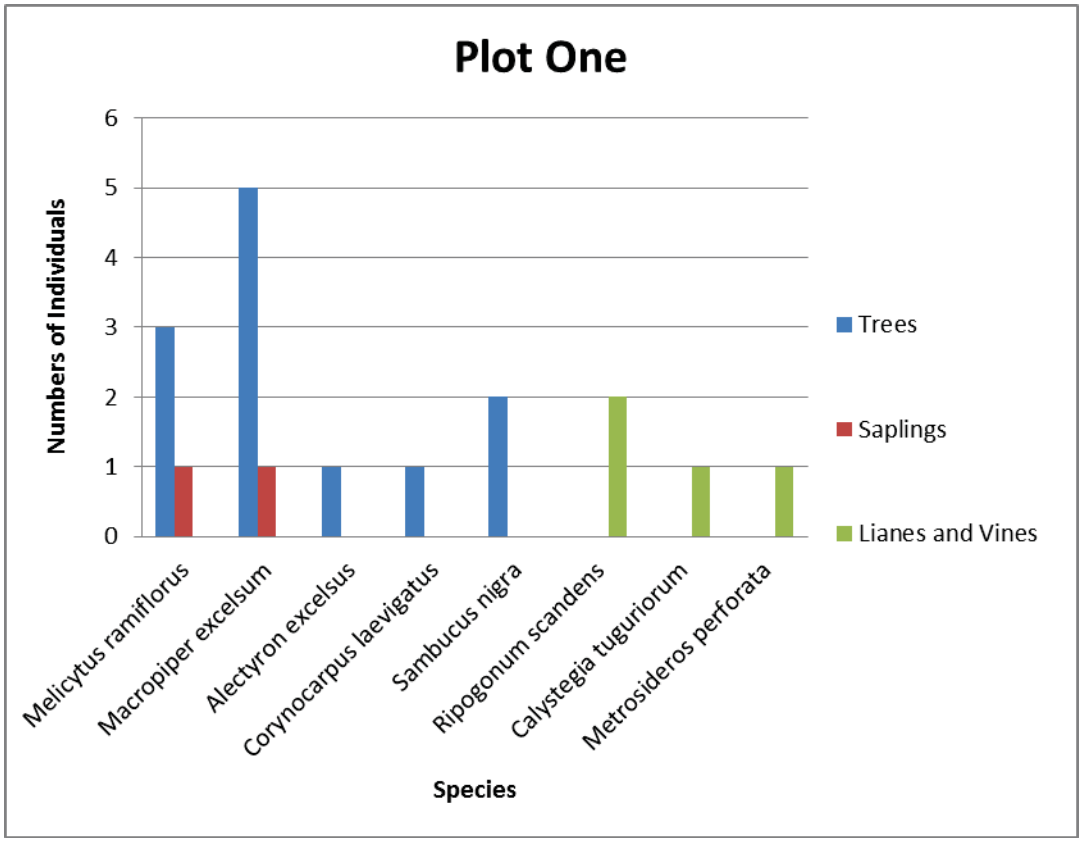


Figure 10: Species in vegetation groups of Plot One
Graph showing the species present in Plot One, and the numbers of individuals in each group

Plot two (Fig. 11) had the highest amount of species present despite having the highest tradescantia biomass of all the plots assessed (408.5 g/m^2). However, two of the nine species graphed were introduced species; elder was present, as well as the exotic bindweed. It also lacked kawakawa, the most prevalent species in the other two plots. The number of kahakaha (*Collospermum hastatum*) individuals was given as one, because the height at which the plant was located made it difficult to accurately assess numbers.

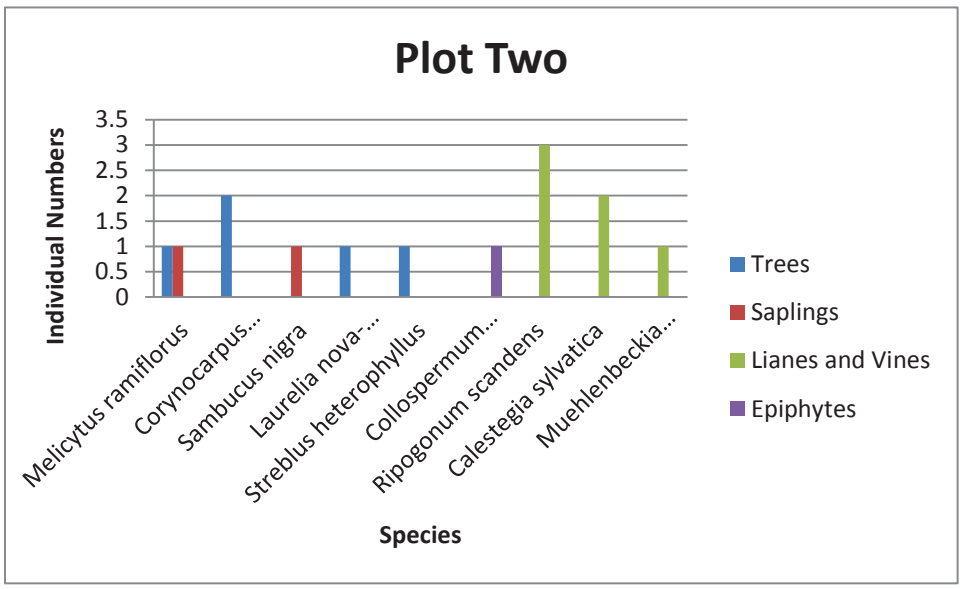


Figure 11: Species in vegetation groups of Plot Two
Graph showing the species present in Plot Two, and the numbers of individuals in each group

Plot Four (Fig. 12) contained the only ferns (living as epiphytes) recorded in this census, and the most trees. Over half of the trees were kawakawa, and no weed species were recorded in this plot.

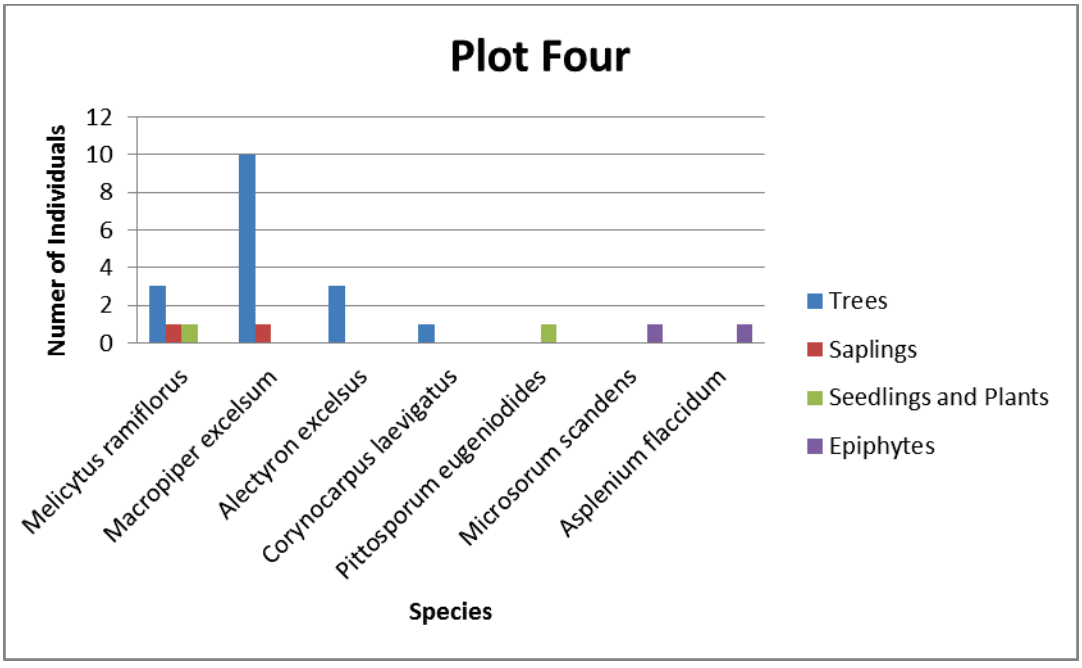


Figure 12: Species in vegetation groups of Plot Four.
Graph showing the species present in Plot Four, and the number of individuals in each vegetation group.

Potential vegetation: Seed bank

This experiment returned mixed results. Out of the eight total samples, only two (both March samples) grew seedlings. Four of the seven seedlings grown were not natives or normal weed species, but were horticultural species; three *Brassica* sp. Plants (Photo 12) and one chard (*Beta vulgaris* subsp. *cicla*) or spinach plant (*Spinacia oleracea*). The other three were identified as mahoe. These results are not indicative of anything beyond the paucity of quick-growing species in the seed bank.

Photo 12: Two large *Brassica* species in seed-bank sample

Discussion

Existing vegetation: Correlations

Because this section is more qualitative, rigorous statistical analyses were not performed. The sample sizes are not large and so trends seen are indicative only

There was no relationship between tradescantia biomass (referred to as biomass hereafter) and lianes/vines. However, the presence of other weed species in this group (bindweed) may be confounding this relationship; facilitated entrance of the weed species by the presence of tradescantia is possible. Also, he growth habits of many vines may mean that they are relatively free of the effects of tradescantia. Supplejack and the two bindweeds (*C. sylvatica* and *tuguriorum*) grow from rhizomes (Metcalf, 2009); so have the energy to reach above the tradescantia carpet. Kaihua (*Parsonsia heterophylla*), black vine (*Muehlenbeckia australis*) and rata (*Metrosideros perforata*)

do not possess rhizomes but the bases of the trees they use to ascend may provide more space and light, or these species may be better adapted for low light levels. Conversely, they may be all affected by tradescantia, with even the lowest amount measured here being detrimental to the plants.

This study found a weak relationship between biomass and tree presence. Tradescantia does affect regeneration (Standish *et al.* 2001) and it has been present in the site for close to a century (Kelly and Skipworth, 1984a), but the tree grouping is wide (truck 3cm diameter and above), distribution of tradescantia throughout the site is patchy, and disturbance of the site (research, possible selective logging, occasional stock ingress) could have also affected tree presence. It has been reported that an individual mechanically removed tradescantia from the 'north' side of the site (Appendix Three) on many occasions (A. Robertson, personal communication, 28th May 2013)

The weakly positive relationship between seedlings/small plants and biomass was unexpected and is attributed more to the haphazard nature of seedling/small plant sampling than the effect of tradescantia biomass. .. The lowest biomass recorded here is 122 gm^2, below the biomass of 200gm^2 suggested by Standish *et al.* (2001) as being impenetrable by most seedlings. However, many species in that same paper showed LD50s well below the 100gm^2 mark. The most tolerant species in that study was kohekohe (*Dysoxylum spectabile*) at 40 gm^2, which is not present in the site. The most tolerant species in the Monro's site that was tested in Standish's paper was 30gm^2, for Karaka (*Corynocarpus laevigatus*). So increasing biomass should have no apparent trend in this study if the lowest amount measured is already above the limit which prevents significant regeneration by many species in the site.

The moderate relationship between biomass and saplings was unexpected. Saplings are necessarily younger than trees and their presence may not overlap with many of the potential events listed above, leaving biomass as their main predictor. However, this study returned a positive correlation between the two (increasing biomass, increasing sapling numbers). This is not what is expected, given that regeneration is diminished by the increase in biomass (Standish *et al.* 2001). Small sample size and recent site disturbance are possible reasons for this.

The moderate positive relationship between epiphytes and biomass is not backed up by the growth habits of epiphytes. By definition, epiphytes grow or perch on other plants (Metcalfe, 2009); so tradescantia is less likely to affect the plants that grow this way. But the effect of biomass on trees suitable for epiphytic growth could be the indirect reason behind this; a high biomass means less adult trees and thus less room for epiphytes. However, the lack of adult trees in itself could be the reason for both; fewer trees means increased light levels which allows increased biomass (Standish *et al.* 2001), and less trees means less epiphyte habitat.

There was a weak relationship between biomass and kawakawa seedlings, but no relationship between biomass and kawakawa trees. A negative relationship would be expected, due to the low tolerance of kawakawa (Standish *et al.* 2001). But the relationships found here are based on small sample sizes and are only correlative. There may be a number of unknown factors contributing to trends measured here. Furthermore, the biomasses measured were far above the LD50 of kawakawa ($12gm^2$); so the impacts should be the same at all sites. The smallest biomass measured was $122.8gm^2$, far above that of LD50 of kawakawa; so it is expected that there would be no difference in the number of kawakawa seedlings that did penetrate the tradescantia canopy in any of the plots.

Total species richness and exotic species numbers were strongly and positively related to biomass. Weed presence in a site can facilitate the invasion of other weed species (disturbance levels increased), while eventually decreasing native abundance, so early on in an invasion, total species richness would increase (weed and native species combined). The moderately positive relationship with native species is not backed up by the 2001 paper by Standish *et al.* But it must be remembered that some individuals in this survey are long-lived trees (tawa (*Beilschmiedia tawa*), mahoe, titoki (*Alectryon excelsus*)), or species relatively free of the effects of biomass (like Kahakaha; *Collospermum hastatum*). In the case of trees, their presence currently increases total and native species richness, but they are prevented from successfully reproducing in the area and once the adult trees die, species richness will drop. Also, when those large adult trees die, habitat for epiphytes will be seriously reduced as they are forced to occupy smaller and smaller trees.

Existing vegetation; Description

A description of this site by the establishment of the permanent plots has provided areas available for re-surveying during and after the establishment of one, or all, of the biological control agents for tradescantia, to explore changes in the site.

Potential vegetation; Seed bank

The paucity of germinated seeds in the seed banks samples could be reflective of the small amount of time allowed for germination. Many species may take longer to germinate after stratification and it is possible that more time would have produced more species in the samples.

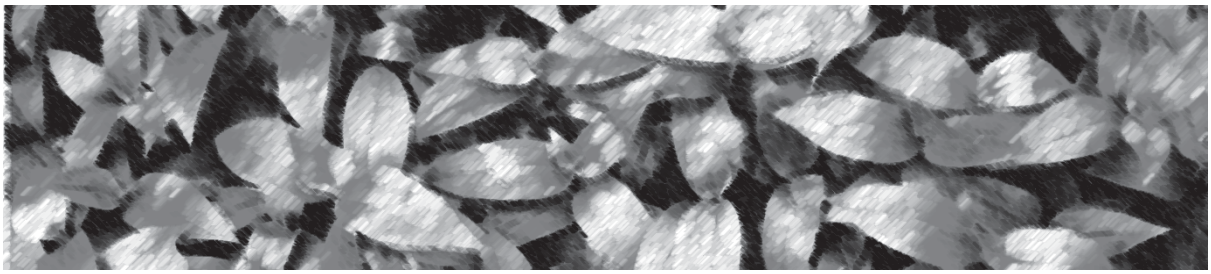
Summary and recommendations

This section was not intended to repeat earlier studies of the tradescantia-seedling relationship, but establish permanent plots for the purpose of re-surveying at a later date. The data gathered should provide a good baseline to compare against should a tradescantia biological control program be established here. Exploring the data that was gathered from the establishment of permanent plots was correlative only and the small sample size means there is no power to detect all but the strongest trends.

It is recommended that one, or all, of the biological control agents for tradescantia be re-released at Monro's, although not in the original release site, which has proven prone to stock ingress. The 'north' side of the site was not browsed during the recent incursion, and this side is also where the remaining permanent plots are located (See Appendix Three).

It is also recommended that the three remaining permanent plots be surveyed during and after the establishment of any biological control agent. This will allow the documentation of any change and in particular, whether the release of seedlings from tradescantia is achieved.

Chapter Four: Comparative regrowth of Tradescantia fluminensis



Introduction

When looking at the control of tradescantia, hand-weeding and herbicide application provide the most visual impact, but these methods are not necessarily the most effective (Standish, 2002). However, they are currently the only options readily available and the only options used by regional councils and the Department of Conservation ((Hurrell *et al.* 2008; Lusk, Hurrell, and Lamoureaux, 2012). The recent releases of the biological control agents for tradescantia have not yet had time to be effective (Landcare Research, 2012b; Q. Paynter, personal communication, 20th December 2012), and the recommendation of shading out by Standish (2002) is difficult to carry out in large areas.

The comparison of different methods for clearing tradescantia has been tested before (Standish, 2002), and this experiment is intended as a repetition of previous work done. In contrast to Standish's work (where hand-weeding was best), it is hypothesised that the tradescantia will be slowest to reinvade the areas where herbicides have been used. The reasoning behind this was the residual effects of the herbicide were expected to prevent tradescantia from re-invading. Those plots that were hand-weeded would have no such deterrent.

Materials and Methods

Eight plots (2x2m) were marked out in areas of tradescantia growth in the Monro's bush site. They were all located on the 'South' side of the stream that runs through the site (Appendix Three), because this area contained the largest areas of continuous dense tradescantia. This ensured that a minimum of native plants and trees would be harmed with the herbicide used in this experiment. Four of the plots were randomly selected to be cleared by hand, and the remaining plots were left to be cleared with herbicide. The hand-cleared plots were cleared by cutting the borders with a machete, and rolling the vegetation up like a carpet. Every effort was made to collect all fragments of tradescantia from the soil. The herbicide treated plots were knapsack-sprayed with Grazon® herbicide (active ingredient 600 gram/litre triclopyr; Dow AgroSciences (NZ) LTD, New Plymouth), at a rate of 0.72% plus 0.1% Pulse surfactant (>800g/L active ingredient organomodified polydimethyl siloxane, NuFarm LTD, Auckland), until the point of run-off (rate and type of application based on trials in

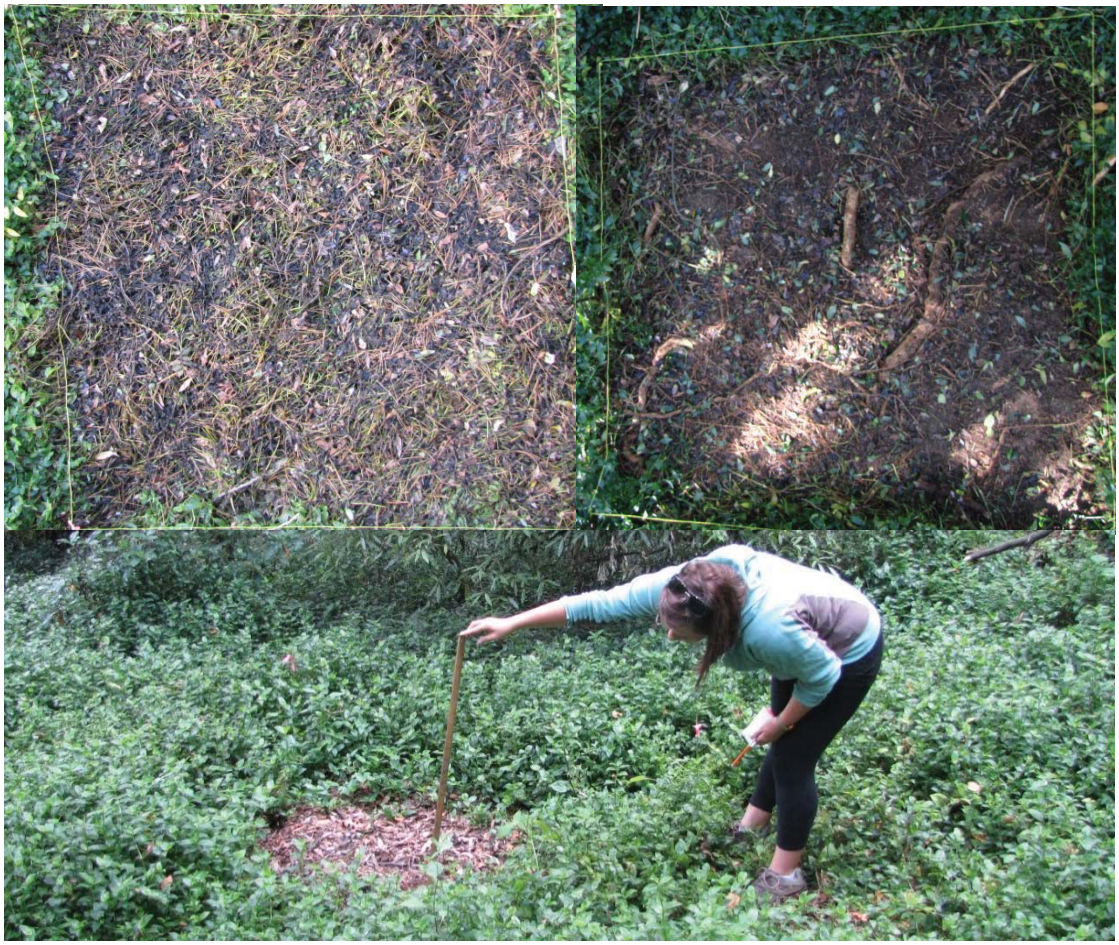


Photo 13: Plots, and measuring technique.
Two different methods of clearing; hand-clearing (top left) and herbicide application (top right). The image on the bottom shows the author measuring stems.

Hurrell *et al*. 2008). Herbicide application was performed on a fine day, with no wind. Photos were taken from directly above the plots every 10-15 days (Photo 13, top images). After 42 days the heights of 6 random stems remaining or growing back into the cleared plots were taken as well. Stem measurements were taken by standing on the edge of the plot, and leaning over to read the measurements (Photo 13, bottom image).

The interval plot photos were put through Digital Sampling Method version 1.00 (Landcare Research Ltd, 2003) to randomly select 200 points in a plot. The border of each plot was marked out in a photo, and the random points were generated within the marked area. Each individual point was then assigned one of four codes, depending on what the point had landed on. The letter T meant that tradescantia was at that point, D indicated that bare soil, leaf litter or dead tradescantia was there. Ov was the code for other vegetation, native or not, and Ob was used when the point was obscured (light flecks, glare). These 200 coded points were used to find the percentage of tradescantia cover (as well as habitable ground and other vegetation) over time in the 8 different plots.

Statistics

Digital Sampling Method version 1.00 (Landcare Research Ltd, 2003) was used to generate data from the series of time lapse photographs. Microsoft® Office Excel® version 12 (Microsoft Corporation, 2006) and Minitab version 16 (Mintab Inc., 2010). was used to perform all calculations and statistical operations.

Results

Percentage of cover

The tradescantia percentage cover of the four plots for each clearance method was averaged and the two averages plotted against one another on a time series graph (Fig.13).

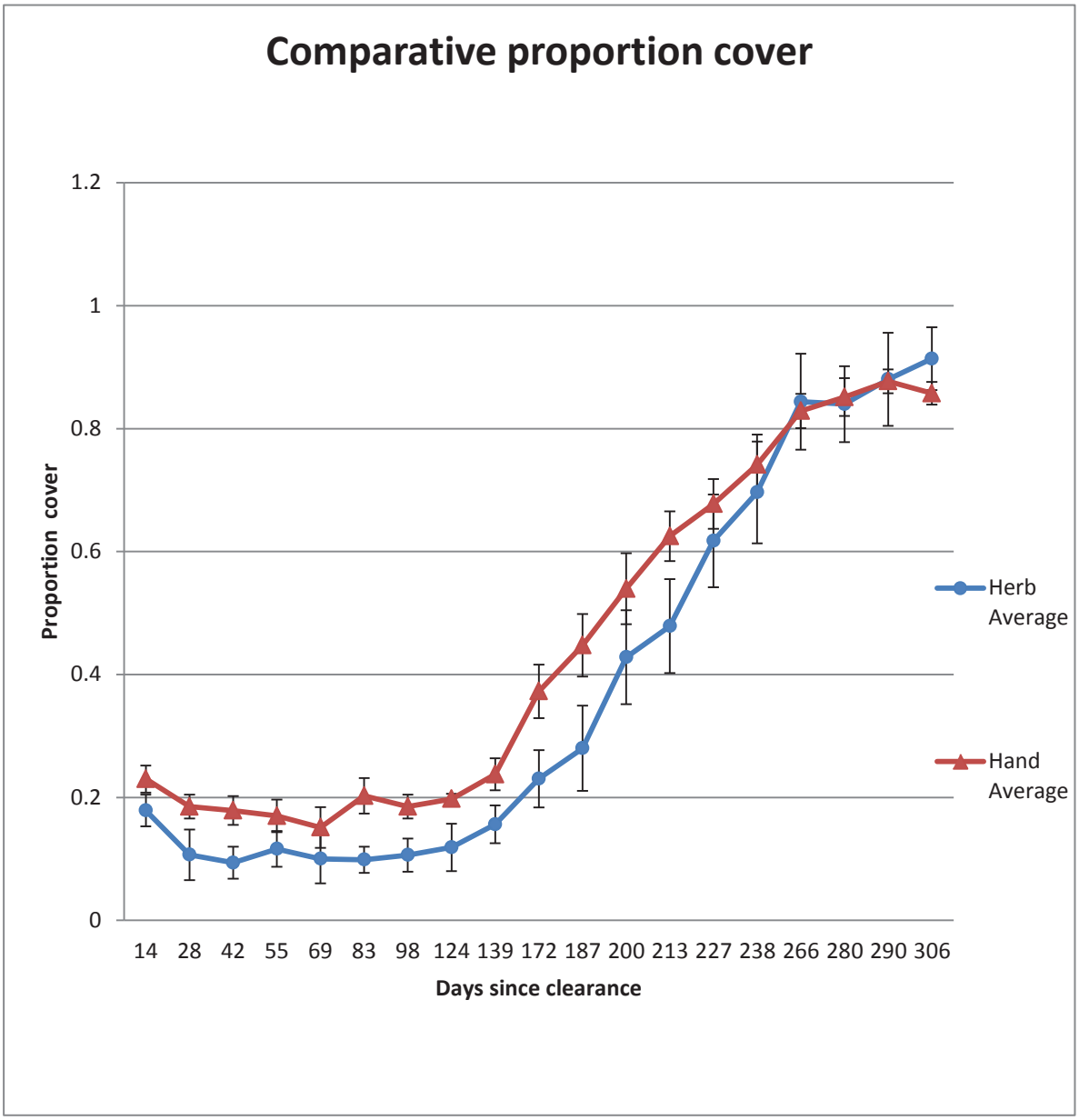


Figure 13: Time series comparing the percentage cover regrowth and standard error of two different treatments as proportions

The data suggests that those plots cleared by hand were re-invaded faster than sprayed plots. However, during the final stages of re-invasion (60% to 100%, Day 200 to Day 306), the two methods were indistinguishable.

Biomass

Biomass was calculated using a previously mentioned equation (Eq. 1). The percentages previously calculated for each plot were used to calculate biomass regrowth in terms of biomass for each individual plot.

$$\text{Biomass } (gm/s^2) = 0.014 \text{ (percentage cover x height).}$$

Equation 1: Equation for estimating biomass of tradescantia, from Landcare Research.

The averages for each method, and the standard errors were calculated, and plotted on a time series graph (Fig. 14). The standard error bars overlapped at all observation points, suggesting that biomass regrowth was not significantly different between the methods.

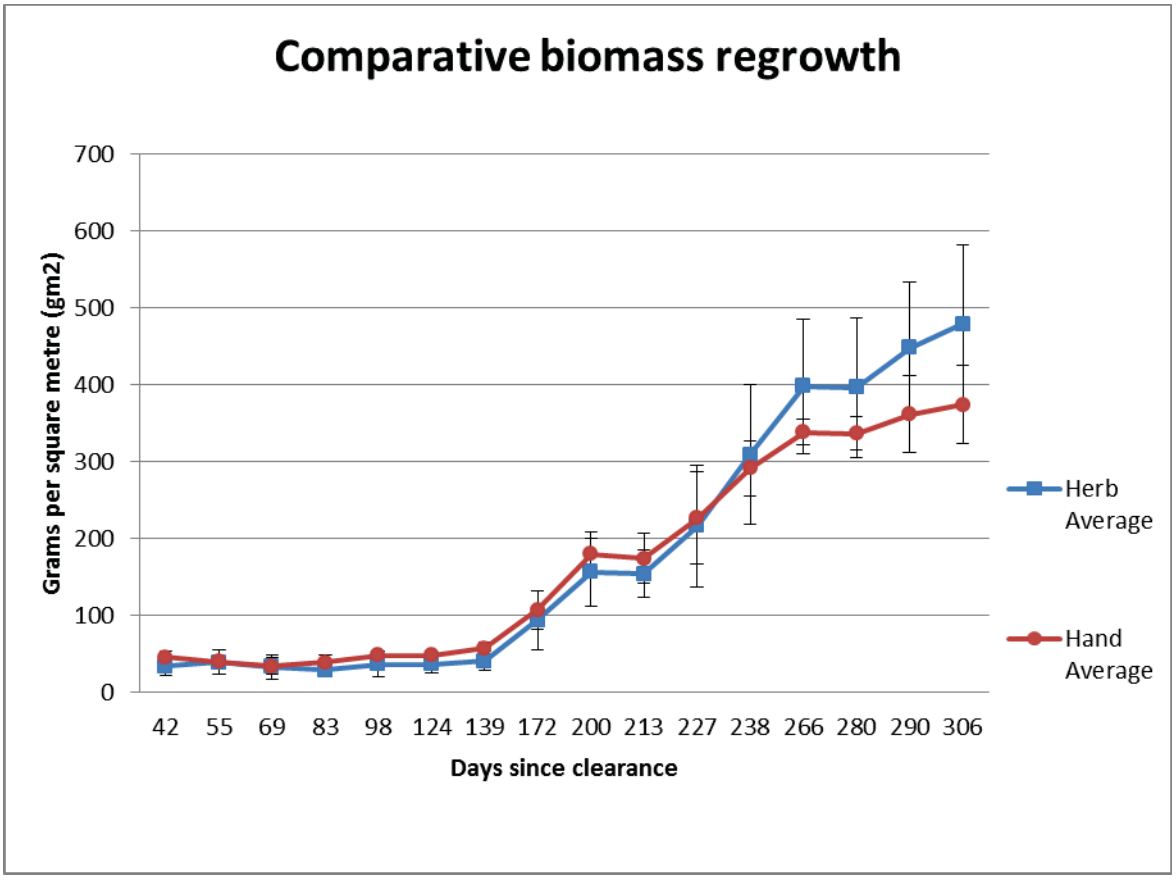


Figure 14: Timeline of biomass regrowth and standard error for herbicide and hand-clearance methods

Other Vegetation

The amount (percentage cover) of other plant species was also analysed. The method used to estimate tradescantia percentage cover also provided other plant species percentage cover, although the species were not identified.

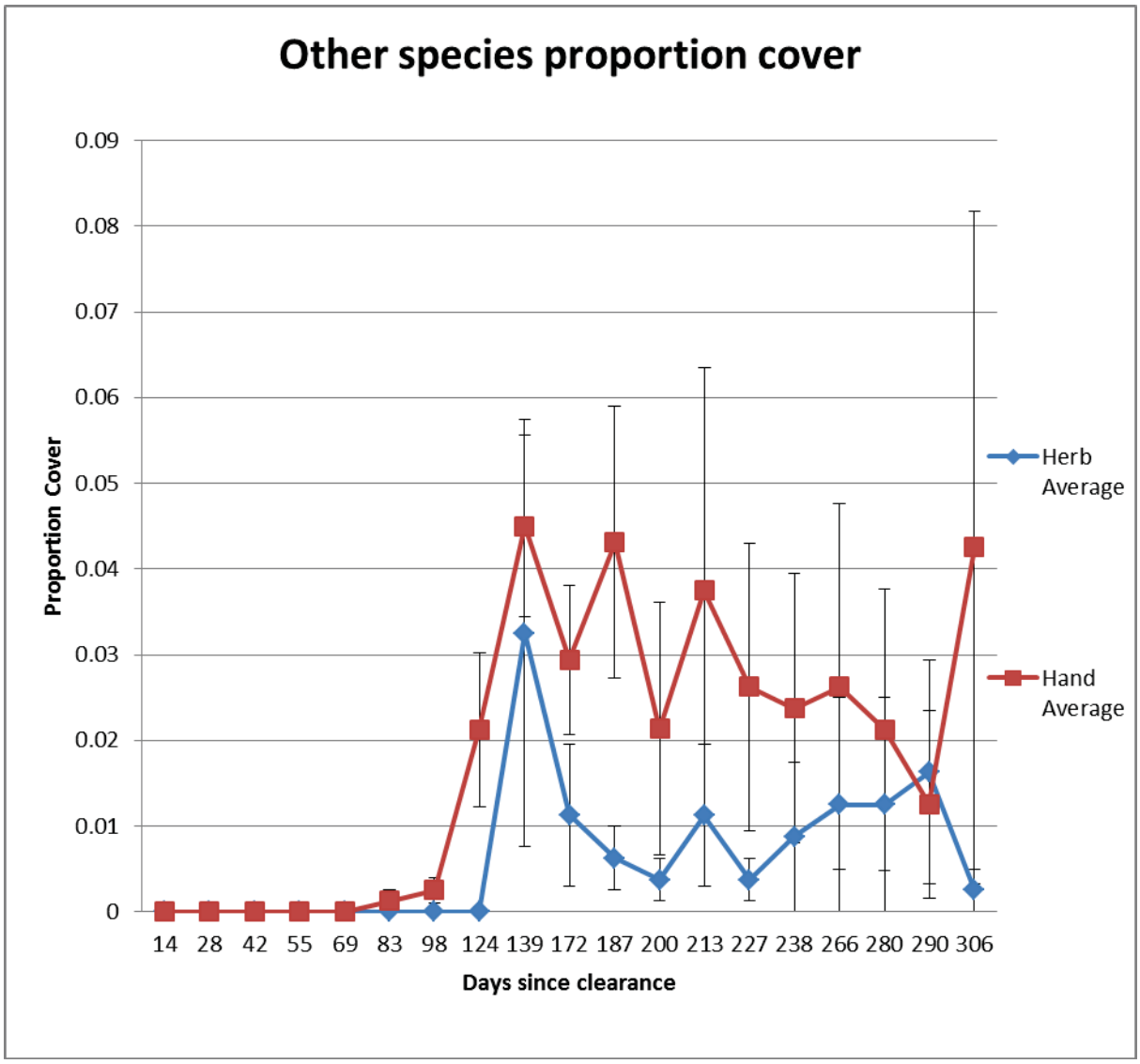


Figure 15: Percentage cover and standard error of other vegetation, compared as proportions

The initial 98 days show no significant difference in the percentage cover of other species. Percentage cover of other species began to differ after 124 days, with the hand-cleared plots averaging a higher percentage on many occasions, however variability between and within sampling times was high.

The eight individual plot measurements were smoothed using a three point moving average. The data was then transformed using Arcsine transformation, and one point between days 124 and 306 was randomly selected. The eight measurements for that

point were divided by their method of control, and a two-sample t-test performed. The result was not significant (P>0.05).

Discussion

The results did not support the hypothesis that tradescantia will take longer to re-invade areas treated with herbicide. Other plant species regrowth did not significantly differ either.

Invasion by other species (in terms of percentage cover) appeared to trend higher for the plots cleared by hand for days 124 to 306, and this would be expected due to the residual effects of the herbicide. However the data was highly variable and a t-test suggested that this difference was not significant. Many plant species that were initially observed in the cleared plots were fast growing weed species, or newly germinated native seedlings. The remaining plant species present once tradescantia cover passed 60% were weed species (author's personal observation). It is reasonable to assume that as tradescantia increased, the space for other plants decreased, and any established plants would eventually be smothered unless they could match or exceed the rate at which tradescantia grew.

The results produced by this experiment suggest a different conclusion than that reached by Standish (2002); here, the two methods are not significantly different in the regrowth (percentage cover) of tradescantia. The reason for this could be due to one (or more) of many differences in how the experiments were carried out.

Standish (2002) recorded percentage cover monthly or bi-monthly, while this experiment recorded in 10-20 day (average) increments. The older experiment also ran over a longer time period (over 600 days) and had repeat applications of the control methods, while this experiment ran for just over 300 days and had one application of the control methods. This experiment did not measure the effects of the different seasons on the regrowth, while the older experiment did take this into account and ran different experiments with control method applications timed in for either winter or summer. In this study, the herbicide application occurred just before a period of slower growth (autumn, winter) and those plots experienced a regrowth rate that was

slower than if the regrowth occurred during a period faster growth (i.e; spring, summer).

In summary, this study found that when a single clearance event occurs in autumn, comparative regrowth is not markedly different in the end. Other plant species do appear soon after clearance, but quickly decrease as tradescantia increases.

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Appendix One

1962 species list for 'Massey College' reserve, from Esler (1962). Older synonyms used by Esler have been replaced.

Trees	**Shrubs**	**Ferns and Allies**	**Herbaceous**
Alectryon excelsus	*Brachyglottis repanda*	*Adiantum formosum*	*Astelia solandri*
Aristotelia serrata	*Haloragis erecta*	*Asplenium bulbiferum*	*Carex solandri*
Beilschmiedia tawa	*Macropiper excelsum*	*A. polyodon*	*C. ternaria*
Coprosma australis	*Pittosporum cornifolium*	*A. flaccidum*	*Collospermum hastatum*
C. robusta	*Streblus heterophyllus*	*A. lucidum*	*Solanum nodiflorum*
Cordyline australis		*Blechnum filiforme*	
Corynocarpus laevigatus	**Vines**	*Cyathea dealbata*	
Dacrycarpus dacrydioides	*Calstegia tuguriorum*	*C. medullaris*	**Orchids**
Griselina lucida	*Freycinetia banksii*	*Histiopteris incisa*	*Winika cunninghamii*
Hoheria sexstylosa	*Fuchsia perscandens*	*Lasteopsis glabella*	
Laurelia nova-zelandiae	*Metrosideros colensoi*	*Microsorum pustulatum*	
Melicytus ramiflorus	*M. diffusa*	*M. scandens*	**Weeds**
Myoporum laetum	*M. perforata*	*Pellaea falcate*	*Tradescantia fluminensis*
Myrsine australis	*Muehlenbeckia australis*	*P. rotundifola*	
Pennantia corymbosa	*Parsonsia heterophylla*	*Pneumatopteris pennigera*	
Pittosporum eugeniodes	*Passiflora tetrandra*	*Pteridium esculentum*	
P. tenuifolium	*Rhipogonum scandens*		
Plagianthus regis	*Rubus australis*		
Psedopanax arboreus	*R. schmidelioides*		
Schefflera digitata			
Sophora microphylla			
Syzygium marie			

Appendix Two

Long-term potting mix materials

Added to 100L Daltons™ base mix:

- 200g Woodace® Long-Term 8-9 month slow release fertiliser
    - 18% Nitrogen (N), 5% Phosphate (P), 10% Potassium (K)
- 100g Woodace® Flowering Plant 3-4 month slow release fertiliser
    - 14% N, 14%P, 14%K
- 150g Dolomite

100L Daltons™ Base Mix contains:

- 50% Pinus Radiata bark, with calcium and ammonium nitrate (C.A.N) and moisture added, pH controlled, composted for 15-18 weeks
- 30% Pinus Radiata shredded bark fibre, with C.A.N. and moisture added, pH controlled, composted for 15-18 weeks
- 20% Pacific pumice
- 1Kg/m^3 Serpentine super

Appendix Three

Monro's Bush (image orientated North), with approximate boundaries (red), the Turitea Stream (dark blue), and the smaller stream (light blue) that divides Monro's. The 'south side' and 'north side' of Monro's refers to the areas south and north of the dividing stream. Latitude and longitude of each pemanent plot is as follows; Plot One: 40 23 07.67120 S 175 36 42.32156 E, Plot Two: 40 23 08.45214 S 175 36 40.78234 E, Plot Four: 40 23 06.62261 S 175 36 42.78994 E. Image taken from Google Earth (Google, 2013).

Appendix Four

Numbers of individual plants found in each survey plot. Note that *Collospermum hastatum* was counted as one individual due to it's inaccessibility.

	Plot One			
	Trees	Saplings	Lianes and Vines	Epiphytes
Melicytus ramiflorus	3	1		
Macropiper excelsum	5	1		
Alectyron excelsus	1			
Corynocarpus laevigatus	1			
Sambucus nigra	2			
Ripogonum scandens			2	
Calystegia tuguriorum			1	
Metrosideros perforata			1	
	Plot Two			
	Trees	Saplings	Lianes and Vines	Epiphytes
Melicytus ramiflorus	1	1		
Corynocarpus laevigatus	2			
Sambucus nigra		1		
Laurelia nova-zelandiae	1			
Streblus heterophyllus	1			
Collospermum hastatum				1
Ripogonum scandens			3	
Calestegia sylvatica			2	
Muehlenbeckia australis			1	
	Plot 3			
	Trees	Lianes and Vines		
Melicytus ramiflorus	1			
Coprosma robusta	1			
Beilschemiedia tawa	1			
Ripogonum scandens		5		
Parsonsia heterophylla		1		
	Plot 4			
	Trees	Saplings	Seedlings and Plants	Epiphytes
Melicytus ramiflorus	3	1	1	
Macropiper excelsum	10	1		
Alectyron excelsus	3			
Corynocarpus laevigatus	1			
Pittosporum eugeniodides			1	
Microsorum scandens				1
Asplenium flaccidum				1