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

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Abstract The leaf beetle *Neolema ogloblini* was released in 2011 as a biological control agent for *Tradescantia fluminensis*, a major warm temperate forest environmental weed in New Zealand. To assess whether *N. ogloblini* can suppress *T. fluminensis* and improve native seedling growth and survival, a glasshouse experiment was established. Kawakawa (*Macropiper excelsum*) and mahoe (*Melicytus ramiflorus*) seedlings were planted underneath uncontrolled *T. fluminensis* and compared with seedlings (1) under *T. fluminensis* damaged by *N. ogloblini*, (2) under *T. fluminensis* sprayed with herbicide (triclopyr) and (3) released from competition by manually removing *T. fluminensis*. Seedlings did not grow faster in response to reduced *T. fluminensis* biomass and increased light levels following feeding by *N. ogloblini* over the 12-week experiment. However, seedling survival rates were higher (kawakawa 87% and mahoe 93%) with *N. ogloblini* feeding than herbicide-treatment (kawakawa 17% and mahoe 3%) *T. fluminensis*. Survival in uncontrolled *T. fluminensis* (kawakawa 90% and mahoe 57%) varied for the two species. This experiment suggests that regeneration of native plants may benefit from damage to *T. fluminensis* caused by *N. ogloblini* feeding in the field.

Keywords tradescantia, *Tradescantia fluminensis*, tradescantia leaf beetle, *Neolema ogloblini*, biological control, environmental weeds.

INTRODUCTION

Tradescantia fluminensis (Commelinaceae) is one of New Zealand's worst invasive temperate forest weeds (Syrett 2002). The effects of *T. fluminensis* on native forests are numerous (Esler 1988; Toft et al. 2001; Standish et al. 2001, 2004; Standish 2004), but the primary concern is the prevention of forest regeneration. Many germinating native seedlings are unable to tolerate the low light levels found underneath thick *T. fluminensis* stands commonly found in New Zealand (Standish et al. 2001). Traditionally *T. fluminensis* has been controlled with herbicides (Department of

Conservation 2012), but this is labour intensive and harms or kills many non-target species (Kelly & Skipworth 1984; Harrington & Schmitz 2007; Hurrell et al. 2008). Further studies on different methods of control have found that light deprivation (shading out to 2–5% of full light) is an effective means of reducing *T. fluminensis* biomass (Standish 2002), but this is difficult to implement over large areas. Alternatively, increasing light availability at ground level to above 5% of full light should result in increased native seedling growth under *T. fluminensis*

(Ebbett & Ogden 1998). Five percent was also the maximum light (measured as incident radiation) recorded by Standish et al. (2001) under 200 g/m² of *T. fluminensis*, the highest density of *T. fluminensis* that they found would allow forest regeneration. Another proposed method to increase light availability to native seedlings growing under *T. fluminensis* is biological control (Syrett 2002). Three new coleopteran biocontrol agents have been recently sourced, tested, cleared for release by the Environmental Protection Agency, and released into New Zealand (Fowler et al. 2013; Landcare Research 2013). The first species to be released, *Neolema ogloblini* (Chrysomelidae), has now established at several sites and is causing high levels of damage (S. Fowler, Landcare Research, personal communication, 11 March 2015). However, there are currently no data on the beetles' effectiveness.

The aim of this study was to measure the reduction in *T. fluminensis* cover, the hypothesised subsequent increase in light reaching ground level, and the impact on native seedlings following biocontrol. The study also compared results from biocontrol against herbicide treatments in relation to impacts on seedling survival and growth of two native forest species. At the time of this experiment, the biocontrol agent had not established well in the field, and so a glasshouse experiment was decided upon.

MATERIALS AND METHODS

Tradescantia fluminensis fragments (3–5 per pot, 5–10 cm long) were planted into eighty 18-litre polythene planter bags two-thirds filled with long-term potting mix (Woodace® Short-term 3–4 month and Long-Term 8–9 month slow release fertiliser) made in the Plant Growth Unit of Massey University, Palmerston North, and kept in a shaded and heated glasshouse at an average temperature of 13.5°C and 19% of full light. After 12 weeks the plants were shifted to a different glasshouse at an average temperature of 18.5°C and 34% of full light. As the *T. fluminensis* established, three hollow plastic tubes (10 cm high, 4 cm diameter, 0.4 cm thick) were inserted into the topsoil of each pot to provide a placeholder for insertion of native seedlings at a later date.

Kawakawa (*Macropiper excelsum*, Piperaceae) and mahoe (*Melicytus ramiflorus*, Violaceae) were chosen for this study because both species have previously been identified by Standish et al. (2001) to be relatively intolerant and moderately tolerant of *T. fluminensis* cover respectively. Seedlings of both species (size range 11–96 mm) were collected from a native forest site in the Gisborne region (38°21'53.04"S 178°13'42.45"E). These were potted into the same standard long-term potting mix, and the seedlings were left to grow in a glasshouse.

Four weeks later, the 80 potted plants were haphazardly allocated to one of four treatments: (1) herbicide-treatment, (2) beetle-treatment, (3) no-treatment (*T. fluminensis* was left undisturbed), and (4) seedlings-only (*T. fluminensis* manually removed). The herbicide-treatment pots were treated following procedures described in Hurrell et al. (2008). Thus, Grazon® (active ingredient 600 g/litre triclopyr) was applied at a rate of 0.72% ai plus 0.1% Pulse surfactant (800 g/litre active ingredient organomodified polydimethyl siloxane) with a knapsack sprayer to run-off. Beetle-treatment pots had ten *N. ogloblini* beetles added to each, *T. fluminensis* in the no-treatment pots was left intact and *T. fluminensis* in the seedling-only pots was removed and used to calculate the average biomass of all the pots at the start of the experiment. All pots were enclosed in mesh bags to prevent beetle escape and cross-contamination. The mesh bags decreased the light available to the plants (already restricted by the glasshouse) to 25% full light, which is above the minimum level required by *T. fluminensis* to grow (5%; Standish et al. 2001).

A further 4 weeks later, all 80 pots were haphazardly divided into two even groups with ten pots from each treatment in each group. The two groups were assigned to either kawakawa or mahoe and haphazardly allocated seedlings (three per pot). The initial size of each seedling, longest leaf length, stem diameter and seedling height, was measured with callipers. Seedlings were planted into the gaps left behind by removal of the hollow plastic tubes inserted earlier, thus minimising the disturbance to the *T. fluminensis* thatch, and each individual seedling had their

position within each pot recorded. The planting itself was done as quickly as possible, to prevent beetle escape from the beetle-treatment pots.

During the next 12 weeks the plants were watered daily from above and the beetle-treatment pots were monitored for beetle feeding damage. After 8 weeks many beetle-treatment pots had extensive adult and second-generation larval feeding damage so the decision was made to reduce beetle numbers in pots with heavily damaged *T. fluminensis* foliage. On a clear day, light levels were measured (under the mesh bags) at soil level in the centre of the pots below the *T. fluminensis* canopy and compared with those above the *T. fluminensis* canopy. Throughout this process, light measurements were being taken outside the glasshouse, and these values were used to scale the measurements taken within the glasshouse to “full light” values. If the calculated full light measurement was at or above 5% of full light, as many adult beetles as could be found were removed from the pot to avoid imposing unrealistic feeding damage. Five percent of full light was chosen because it was the lowest light measurement that would allow increased seedling growth in glasshouse situations (Ebbett & Ogden 1998), and the maximum light level recorded by Standish et al. (2001) under 200 g/m² of *T. fluminensis*, the suggested threshold density for forest regeneration (decision-making process discussed further in Discussion).

At 12 weeks after the seedlings were planted, the mesh bags were removed from all pots, light measurements of the no-treatment and beetle-treatment pots were taken at soil level and from above the *T. fluminensis* canopy using the same method as before. *Tradescantia fluminensis* biomass (dead or alive) in the herbicide treatment (19 pots dead, one alive), beetle-treatment and no-treatment pots was determined by removing any beetles and/or larvae from beetle-treatment plants and drying the leaves, stems, stolons and roots from individual pots at 60°C for 120 h before weighing. Any native seedlings that were still alive were re-measured.

The biomass of *T. fluminensis* across all treatments was analysed using an ANOVA in SAS ver. 9.3. The proportion of light penetrating

through the *T. fluminensis* canopy to the soil surface in the various treatments was compared using an ANOVA applied to log_e-transformed proportion values, although for illustration the non-transformed proportions were expressed as an interval plot in Minitab ver. 16. Seedling survival rates were compared using a generalised linear model with binomial distributions through logit link function in Minitab ver. 16. The seedling size differences (differences from initial measurements) of differing treatments were tested using LMMs (linear mixed models) to take account of correlation among seedlings grown in the same pots, in SAS ver. 9.3.

RESULTS

The three treatments containing *T. fluminensis* (dead or alive) produced significantly different levels of biomass ($P < 0.0001$). The herbicide-treatment retarded *T. fluminensis* growth the most, while the beetle-treatment pots had a higher biomass than herbicide-treatment pots but less than the no-treatment pots, which had the highest biomass (Figure 1). The ANOVA of light proportions in beetle-treatment and no-treatment pots showed that significantly more light was penetrating through beetle-treatment pots (8.5%; 95% CI 4.9–12.1%) compared with no-treatment pots (0.26%; 95% CI 0.14–0.38%) ($P < 0.001$).

Figure 1 *Tradescantia fluminensis* biomass (g/m²) under three treatments. Error bars indicate standard errors.

Tradescantia fluminensis treatments significantly affected the welfare of the transplanted woody seedlings (Figure 2). The mean survival of seedlings in the herbicide-treatment was significantly lower than survival in the other three treatments for both species ($P < 0.001$). Kawakawa seedling survival in the beetle-treatment pots was not significantly different when compared to the remaining treatments ($P = 0.688$, $P = 0.398$ for no-treatment and seedlings-only respectively). Mahoe seedling survival in the beetle-treatment pots was significantly higher than in the no-treatment pots ($P = 0.004$), and not significantly different from the seedling-only treatment pots ($P = 1$).

Regarding seedling size differences, when comparing no-treatment pots to beetle-treatment pots, there was no significant difference in seedling stem diameter ($P = 0.763$, $P = 0.726$ for kawakawa and mahoe respectively), leaf length ($P = 0.100$, $P = 0.390$ for kawakawa and mahoe respectively) or seedling height ($P = 0.077$, $P = 0.374$ for kawakawa and mahoe respectively). There were not enough surviving mahoe seedlings from the herbicide treatment to include in the analysis.

DISCUSSION

This study has shown three significant responses in potted *T. fluminensis* and competing seedlings to the presence of the biological control

agent *N. ogloblini*: a significant reduction in *T. fluminensis* biomass due to beetle feeding, an increase in available light at soil level beneath the *T. fluminensis* canopy, and improved survival of native mahoe seedlings.

Biomass of *T. fluminensis* was significantly reduced by the beetle-feeding, although the mean biomass figures from beetle-treatment pots (1207 g/m^2) still reached levels well above the threshold for native seedling regeneration estimated by Standish et al. (2001) (200 g/m^2). However, the removal of supposed excess beetles may have prevented a stronger impact. Even the herbicide-treatment pots (207 g/m^2) just exceeded that regeneration limit, despite the fact that it was composed of (for the most-part) dead vegetation. The *T. fluminensis* biomasses found in the beetle-treated and no-treatment pots also exceed the level normally found in the field (maximum 819 g/m^2).

There is still some uncertainty about how much *T. fluminensis* needs to be suppressed to allow forest regeneration. In this experiment, it was decided that the beetle numbers should be reduced once 5% of full light was reached. This 5% light threshold was based on a combination of the work of Ebbett & Ogden (1998), who showed that New Zealand podocarp species had positive growth rates in glasshouses where light was above 5% of

Figure 2 Survival means and standard errors of mahoe and kawakawa seedlings under four treatments.

full-light, and Standish et al. (2001), where 5% light levels was the highest value measured under ca 200 g/m² of *T. fluminensis* in the field – the point at which native seedlings could be regularly seen penetrating the *T. fluminensis*. Five percent of full light is also within the range previously found at the soil level under forest canopies in New Zealand (Ebbett & Ogden 1998; Standish et al. 2001). As the main aim of the experiment was to discover if the hypothesised increased light would benefit native seedlings, 5% was deemed to be the minimum needed and beetles were removed before further feeding damage occurred in confinement. In retrospect, the authors would not have elected to remove these beetles, given the recent results from established populations (S. Fowler, Landcare Research, personal communication, 11 March 2015).

The reduced biomass (despite removal of supposed excess beetles) in the beetle-treatment pots did result in significantly increased ground-light levels. Light levels in these pots averaged 8.5%, while those pots with no treatment averaged 0.26%. This increased light is likely to be the reason for the better survival of mahoe seedlings in the beetle-treated pots compared to no-treatment pots and the failure of seedlings from both species to respond further, developmentally, may have been due to the relatively short duration (12 weeks) of the experiment. In Standish et al. (2001), it was suggested that mahoe was the more shade-tolerant of the two species, which may be the reason behind the increased survival in beetle-treatment pots when compared to kawakawa. Kawakawa (90% survival) survived the no-treatment better than mahoe (57%). Standish et al. (2001) investigated presence/absence of naturally occurring seedlings in varying levels of *T. fluminensis* biomass, and suggested that kawakawa was not as tolerant of *T. fluminensis* as mahoe. It is thought that a longer experiment would have created the effect seen in Standish et al. (2001), but it is possible that because seedlings were planted into the *T. fluminensis* sward, they had enough carbohydrate reserves to survive the experiment (Myers & Kitajima 2007).

The results show that under these experimental conditions, the biocontrol agent

N. ogloblini has the potential to remove enough *T. fluminensis* to allow native seedlings to grow through an established thatch, which does not currently occur without either herbicide application or mechanical weeding. However, whether or not the beetles will perform in field conditions remains unknown. For instance, what density will the beetles reach when not caged onto individual pots? Generally insect movement can be expected to find new resources (Kim & Sappington 2013) and *N. ogloblini* may disperse before reaching the densities used in the present experiment. If this occurs it may prevent beetles from removing enough *T. fluminensis* to benefit native forest seedlings. A second unknown is predation of beetles in the field. Fowler et al. (2013) discussed the hope that the biological control agents in New Zealand will be successfully released from specialised natural enemies, and speculated that generalist predation will not be an issue if populations are large. However, it is possible that high densities of beetles may attract generalist invertebrate and/or vertebrate predators such as birds that may suppress beetles below the densities required for sufficient control. Recent observations in the field suggest that some of these worries may be unfounded; established populations are damaging *T. fluminensis* significantly and appear to be living in large populations without over-dispersion occurring (S. Fowler, Landcare Research, personal communication, 11 March 2015).

Finally, a side-note on the effects of herbicides on *T. fluminensis* and native seedlings. In the experiment, the application of herbicide (4 weeks previously) significantly damaged the native seedlings, presumably due to residual activity in the soil. Triclopyr has a reported half-life of 12–13 days in moist and sun-exposed soils, but when soils dry out the degradation of triclopyr is slower (Graebing et al. 2003). Drying and re-wetting of the soil surface from regular aerial watering occurred prior to planting seedlings into treatment pots in the present experiment, but the dead *T. fluminensis* biomass likely acted as a shield, preventing irradiation of the soils. This non-target damage to native seedlings is the main drawback of using herbicides (like those containing triclopyr)

as a control strategy for *T. fluminensis* in forest remnants (Kelly & Skipworth 1984; Standish 2002). When herbicide is applied to *T. fluminensis* in the field, it not only kills native woody seedlings but could still be present at damaging levels for at least 28 days after application thus jeopardising regeneration of non-target native species from any germinating seed.

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