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**Ecosystem – service provision by the biofuel feedstock plant,
*Miscanthus x giganteus***

A dissertation
submitted in partial fulfilment
of the requirements for the Degree of
Bachelor of Agricultural Science with Honours

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Joseph Zonneveld

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Dairy expansion on the Canterbury Plains has led to a gross simplification of the agricultural landscape, particularly in the form of shelterbelt removal to allow for the implementation of pivot irrigators. This decline in landscape structure can be seen in multiple agroecosystems worldwide and has been linked to decreases in key ecosystem services such as the biological control of insects. This trial evaluated selected ecological effects surrounding the improvement of landscape complexity through the inclusion of the biofuel feedstock plant *Miscanthus x giganteus* as a shelterbelt on a Canterbury dairy farm.

This information was collected through four separate experiments:

1. The use of tracking tunnels to identify potential ecosystem disservices provided by *M. x giganteus* in the form of harbouring rodent populations. This was compared with standard Canterbury post-and-wire fence lines in addition to *Cortaderia selloana* shelterbelts
2. The use of 'bumblebee motels' to identify any differences in nesting rates by bumblebees between *M. x giganteus* shelter and a standard Canterbury dairy farm fence line
3. Invertebrate monitoring to identify any potential differences in natural enemy and pest populations between *M. x giganteus* shelter and a standard Canterbury dairy farm fence line
4. A glasshouse experiment measuring the growth effects of various strain mixtures of the beneficial fungus *Trichoderma atroviride* on the performance of *M. x giganteus* seedlings

Results from tracking tunnel analysis indicated no significant differences in rodent populations between *M. x giganteus*, *C. selloana* and unimproved fence lines. This was, however, thought to be due to relatively few experimental replicates. Trends indicating *C. selloana* harbouring higher rodent numbers than *M. x giganteus* were identified.

Bumblebee motel monitoring yielded no information concerning bumblebee nesting preference due to the timing in which the Honours project was undertaken. Motels were instead used as another measure of rodent populations, indicating a significant difference in these populations between *M. giganteus* shelter and unimproved fence lines.

Invertebrate population monitoring revealed a significant increase in natural enemy populations (2.8 times higher) between *M. x giganteus* shelter and unimproved fence lines. This has potential to increase potential biological control of pasture pest species, a key ecosystem service.

Three out of five *T. atroviride* strain mixtures significantly increased the performance of *M. x giganteus* plants compared to a control. Mixture PR6 was identified as forming the most beneficial symbiosis, increasing plant height by 28%, shoot numbers by 39% and chlorophyll content by 15%.

Keywords: *Miscanthus x giganteus*, ecosystem services, shelterbelt, biofuel feedstock, functional invertebrate biodiversity, rodent pests, habitat structure, bumblebee motel, tracking tunnel, *Trichoderma atroviride*

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Chapter 1

Introduction

Miscanthus x giganteus Keng. (Poales; Poaceae) is a sterile hybrid perennial grass bioenergy crop which has been identified for growing in New Zealand (NZ) based on two main traits:

1. A potential for large biomass yields (Heaton et al., 2014) when grown on prime as well as marginal land as a biofuel feedstock crop (Heaton et al., 2008; Fargione et al., 2010)
2. An ability to increase the structural complexity of landscapes (Semere et al., 2006) and the resulting ecosystem services (Gurr et al., 2001; Landis et al., 2008)

Miscanthus species are thought to originate in Japan, where they have been traditionally used for thousands of years as forage and thatching (Stewart et al., 2009). *M. x giganteus* was first identified in the 1930s as a naturally occurring hybrid of *Miscanthus sinensis* (Anderson.) and *Miscanthus sacchariflorus* (Maxim.) (Heaton et al., 2014). Following the 1970s oil crisis and the resulting search for more sustainable sources of energy, *M. x giganteus* was identified as an ideal lignocellulosic bioenergy crop (Heaton et al., 2014). Research into whole-field growth of *M. x giganteus* in Europe has displayed its potential for consistent high annual yield with low-input management (Lewandowski et al., 2000), partly due to its potential to re-grow annually from rhizomes post-harvest (Purdy et al., 2013). However, there is little information recorded on the growth of *M. x giganteus* on marginal land, e.g. in the form of shelterbelts. This area of information will be investigated in the following study through the growth of *M. x giganteus* as a shelterbelt on irrigated Canterbury dairy farms.

1.1 Biofuels: Progress, Prospects and Impediments to Development

‘Climate change is one of the greatest threats posed to the future of human-kind and the world’ (Hawking, 2012). According to a report by the Intergovernmental Panel on Climate Change (IPCC), climate change has resulted in a mean temperature increase of 0.2°C per decade between 1975 and 1996 (Carere et al., 2008), a rate which is expected to rise with the melting of ice caps (Lemke et al., 2007). Although climate change is a natural process, the current *rate* of global climate change is more rapid and very unusual in the context of past changes (IPCC, 2007). Indeed, there is no evidence that the current rate of temperature increase has been matched in the last 50 million years (IPCC, 2007).

The impact of this change on global climate is complex and extensive, with some major examples including:

- Higher maximum annual temperatures, with increased summer drying over most mid-latitude continental interiors and associated risk of drought and heat waves (McCarthy et al., 2001)
- Increase in tropical cyclone peak wind intensities as well as mean and peak precipitation intensities, which in turn will increase flooding and landslides (McCarthy et al., 2001)
- A global sea level rise of 1.8 mm/yr \pm 0.1 (Douglas, 1991)
- Increased climate variability with more frequent droughts, heat waves, and storms, leading to an increased uncertainty in food production over nearly all land areas (McCarthy et al., 2001; Fuhrer et al., 2014)

It is widely regarded that the largest factor affecting these rising temperatures is an increase in anthropogenic greenhouse gases (Carere et al., 2008). These are released from a range of human activities, although the largest contributor (accounting for 60% of greenhouse warming resulting from anthropogenic sources) is CO₂ released from the burning of fossil fuels (Brown et al., 1998). Fossil fuels consist of coal, oil, petroleum, and natural gas products, which accounted for 83.6% of the total energy consumed in the USA between 2009 and 2013 (World Bank, 2014). As world energy consumption has been predicted to increase by 54% between 2001 and 2025, actions must be taken to generate energy from other sources to avoid unnecessarily escalating the effects of climate change (Carere et al., 2008).

Biofuels is an 'umbrella' term referring to a range of fuels derived immediately from living matter (Oxford, 2014). Renewable diesel is a category which fits under the biofuels umbrella and refers to fuels which are chemically identical to petroleum-based diesel but are made of recently living biomass using a thermal depolymerisation process (Yoon, 2014). Biofuels such as renewable diesel are considered to be a viably sustainable alternative to fossil fuels (Tilman et al., 2009; Fargione et al., 2010; Zhuang et al., 2013), and have the potential to reduce CO₂ emissions by 60-90% relative to conventional petroleum fuels (Brown et al., 1998). As a result, many crops are now purpose-grown for biofuel with a considerable proportion of all maize (*Zea mays L.*) grain produced in the USA since the late 2000s being devoted to the production of first-generation biofuels (Zhuang et al., 2013). These are biofuels which are derived from sources such as starch or sugar, common compounds in food crops (Naik et al., 2010). Indeed, the USA government has set a target of producing 79 billion litres of biofuel (mainly in the form of ethanol from maize grain) annually by 2022 (Energy

Independence and Security Act, 2007), requiring an estimated 85% of all maize-producing areas in the USA (Monfreda et al., 2008). The world's population is estimated to increase to 9.6 billion by 2050 (UN, 2014), necessitating an estimated 70% increase in current agricultural production (FOA, 2014).

For biofuels to truly be produced in a sustainable manner, they must be produced with minimal impact on food production (Tilman et al., 2009). However, the USA's target of increasing biofuel production through maize (Energy Independence and Security Act, 2007) is not possible without seriously jeopardizing food security (Fargione et al., 2010; Davis et al., 2012). Another factor affecting the sustainability of biofuels is the net level of greenhouse gas emissions involved in their production (Crutzen et al., 2008). Crops with high-input requirements such as maize have high CO₂ emissions from agrichemical production/application in addition to nitrous oxide (N₂O) emissions from nitrogenous fertiliser application (Pickett et al., 2008) – a greenhouse gas that has a global warming potential 296 times higher than CO₂ (IPCC, 2007). Therefore, the ideal biofuel crop should be grown without competing with food crops (Tilman et al., 2009) (e.g. on marginal land), with low input requirements (Pickett et al., 2008) while still providing high annual yields (Lewandowski et al., 2000) – categories which maize is far from fulfilling.

A favourable alternative to traditional food crops (e.g. maize) in the production of biofuel is the use of perennial grass bioenergy crops such as *M. x giganteus* (Heaton et al., 2008; Fargione et al., 2010). *M. x giganteus* is a perennial, warm-season grass hybrid (Heaton et al., 2014), which, when compared against maize, has been recorded to yield more than double the amount of biomass (21.5 t DM/ha-1/yr-1) and 39% more ethanol given current conversion technologies (Zhuang et al., 2013). Moreover, it has been estimated that *M. x giganteus* has the potential to fulfil the USA's mandate of 79 billion litres of annual biofuel production (Energy Independence and Security Act, 2007), using only half of the cropland and two-thirds of the water required for maize production (Zhuang et al., 2013). The widespread growth of *M. x giganteus* will free up current bioenergy maize for food production, making it possible to achieve the USA's biofuel mandates without exceedingly exacerbating the competition between food and fuel (Heaton et al., 2014).

It is therefore theoretically possible to reduce anthropogenic greenhouse gases and climate change without adversely effecting food security. This can feasibly be achieved through the large-scale use of *M. x giganteus* in bioenergy production, or through other similarly promising biofuel feedstocks such as switchgrass (*Panicum virgatum* L.), which has been shown to produce 540% more renewable energy than non-renewable energy consumed in its production (Schmer et al., 2008). Although many promising alternatives to *M. x giganteus* (such as switchgrass) have been identified, their production will not be analysed in depth in this dissertation.

1.2 Provision of Ecosystem Services by *Miscanthus*

‘What good are all those species that man cannot eat or sell?’ (Odum, 1971). This realistic message expresses how farmers and society as a whole will not undertake measures to increase landscape complexity and biodiversity without recuperating quantifiable benefits to mankind. Once naturally occurring ecosystem functions can be quantified as directly or indirectly benefitting human populations, they can be re-classified as ‘Ecosystem Services’ (Constanza et al., 1997). Some of these ecosystem services, such as biological control, can be readily identified by farmers. However many services also occur which provide greater societal benefits (e.g. recreation, water cycling etc.) (Grigg et al., 2011) and can be harder to quantify in terms of Keynesian economics (Czech, 2009).

Growing crops in vast monocultures, as in maize production, results in a gross simplification of the agricultural landscape (i.e. monoculture at the extreme) (Price, 2008). This reduces the potential for ecosystem services, such as the biological control of insect pest species (Thies et al., 1999; Landis et al., 2008). As a result of the USA’s mandate of increasing biofuel production (Energy Independence and Security Act, 2007), maize acreage increased 19% in the USA over a one-year period, 2006-2007 (Landis et al., 2008). This reduction in landscape complexity has been estimated to have reduced biological control (a key ecosystem service) by 24%, at an estimated cost of \$58 million in reduced yield and increased pesticide use (Landis et al., 2008).

By increasing landscape complexity through agricultural diversification, ecosystem services such as biological control can be increased, reducing crop damage (Thies et al., 1999; Landis et al., 2008). The identification of *M. x giganteus* as a viable alternative bioenergy crop to maize (Heaton et al., 2008; Fargione et al., 2010) creates the potential to achieve this agricultural diversification – increasing the potential for multiple ecosystem services (Landis et al., 2008). Indeed, *M. x giganteus* has been shown to increase invertebrate diversity among many taxa (Semere et al., 2007), mainly due to its low-input agrochemical requirements (Semere et al., 2007; Heaton et al., 2014). This increased biodiversity provides key food resources for birds, notably during their breeding season (Semere et al., 2007; Bellamy et al., 2009). Bird populations have recently been shown to be heavily impacted by pesticide use, namely neonicotinoids (Hallman et al., 2014), therefore the provision of low-agrochemical habitats such as those provided by *M. x giganteus* are key for farmland bird biodiversity and potential ecosystem-service provision.

Similar to maize expansion in the USA, dairy expansion in NZ has also resulted in a large simplification of landscape complexity (Jay, 2007). In Canterbury this has occurred in the form of extensive shelterbelt vegetation removal to allow for the implementation of pivot irrigators (Goulter, 2010). This removal of woody vegetation such as Canterbury shelterbelts, in conjunction with increasing agrochemical use, has caused rapid declines in invertebrate biodiversity in NZ and worldwide (Tilman

et al., 2001; MEA, 2005; Semere et al., 2007)- a widely accepted indicator of agroecosystem health (Gregory et al., 2005). Attributes of *M. x giganteus* such as its tall, 4m high (Jones et al., 2001), yet flexible growth habit make it an ideal candidate to replace these felled shelterbelts without hindering pivot irrigation (Littlejohn C., personal communication).

Growing *M. x giganteus* in the context of a shelterbelt on Canterbury dairy farms therefore has the potential to increase landscape complexity, invertebrate/vertebrate biodiversity, ecosystem health, and the resulting ecosystem services.

1.3 Miscanthus on Canterbury Dairy Farms

This Honours study supplements and expands on a larger doctorate programme being undertaken by Chris Littlejohn, funded by Westland Milk and Dairy NZ. This involves *M. x giganteus* growing in 4m wide strips alongside paddock edges to act as a shelterbelt on a pivot-irrigated Canterbury dairy farm. Once *M. x giganteus* has become established (into its 2nd season) it has the potential to be harvested annually as a biofuel crop. This allows for a diversification of farm income in addition to an increase in landscape complexity (improving potential ecosystem services), with minimal reduction of the pastoral grazing area.



Figure 1: 4m wide *M. giganteus* shelterbelt, photo taken during winter senescence of the crop

This Honours study aimed to identify and quantify the ecosystem-service provision of *M. x giganteus* in the Canterbury dairy farm scenario. The study is of notable interest especially as this ecosystem approach has been critically scarce in dairy systems worldwide (Fukuda et al. 2011).

This information was collected and analysed using four separate experiments:

5. The use of tracking tunnels to identify potential ecosystem disservices provided by *M. x giganteus* in the form of harbouring rodent populations. This was compared with standard Canterbury post-and-wire fence lines in addition to *Cortaderia selloana* shelterbelts
6. The use of 'bumblebee motels' to identify any differences in nesting preference by bumblebees between *M. x giganteus* shelter and a standard Canterbury dairy farm fence line
7. Invertebrate monitoring to identify any potential differences in natural enemy and pest populations between *M. x giganteus* shelter and a standard Canterbury dairy farm fence line
8. A glasshouse experiment measuring the growth effects of various strain mixtures of the beneficial fungus *Trichoderma atroviride* (P. Karst, 1892) on the early performance of *M. x giganteus*

The combination of these studies will provide critical information surrounding the wider ecological benefits and disservices, if any, of *M. x giganteus* on Canterbury dairy farms – an area of little previous research.

Chapter 2

Methods

This Honours study was centred on four independent experiments, undertaken concurrently from 3 March until 13 November 2014. These experiments were carried out using a total of five *M. x giganteus* shelterbelts, four located on an Aylesbury Rd study farm (dairy) and one on a nearby Karetu farm (support block, grazing non-milking stock). Both farms were irrigated using pivot irrigators and experienced similar environmental conditions throughout the trial.

Experiments carried out in this study included:

1. Tracking tunnel rodent population monitoring
2. Bumblebee population monitoring
3. Measurement of invertebrate diversity
4. Glass-house monitoring of the growth effects of *T. atroviride* mixtures on *M. x giganteus*

2.1 Tracking tunnels rodent population monitoring

A key potential disservice of shelterbelts is their capability to shelter rodent populations (Maisonneuve et al., 2001). This potential ecosystem-disservice was compared between the four available *M. x giganteus* shelterbelts, four nearby *Cortaderia selloana* (pampas grass) shelterbelts and four representatives of standard Canterbury dairy farm fence lines (two electric wires). Comparisons were carried out using tracking tunnels, a technique which is considered well-suited to providing simultaneous comparisons of the relative abundance of rodents (Gillies & Williams, 2013). The technique was carried out as prescribed in a Department of Conservation (DOC) protocol by Gillies and Williams (2013) which provides standardised methodology for consistent tracking tunnel use.

Five tunnels were placed at 10m spacing inside each of the four *M. x giganteus* shelterbelts, as well as in each of the four *C. selloana* shelterbelts and along each of four representative Canterbury dairy farm fence lines (controls). That is, there were 60 tracking tunnels: 20 inside *M. x giganteus* shelterbelts, 20 inside *C. selloana* shelterbelts and 20 along fence lines.

Tunnels were placed on 6 August 2014, pegged to the ground in an un-baited state. They were then left for three weeks for resident animals to become conditioned to their presence (Gillies & Williams, 2013). The tunnels were then loaded with a tracking card and baited with peanut butter surrounded

by black dye, as in the DOC protocol. After 24h, tracking cards were collected. Data comprised a proportion of each tracking line (5 tunnels) with rodent footprints – creating an index of the relative abundance of rodents in each sample area. This index was then averaged over each shelter/fence line treatment. An additional set of repeated measurements using the same process were carried out again from 1-2 October 2014.

Statistically the experiment was laid out in a randomised block design, with one *M. x giganteus*, *C. selloana* and fence line block (3 blocks in total). Replicates were made up of tracking tunnel lines within each block (e.g. one tracking tunnel line of five tunnels = one replicate). This led to four replicates within each block (four tracking tunnel lines per block).

Statistical analysis of the data was carried out as follows:

- Calculation of the average proportion of tracking tunnels showing the presence of rodents within each block (*M. x giganteus*, *C. selloana* and fence line)
- Calculation of standard deviation and standard error for each block
- The use of an analysis of variance (ANOVA) to indicate any significant differences between the means of each block
- Results (mean abundance of rodents) were expressed graphically with standard error of each block expressed as error bars

2.2 Bumblebee motel monitoring

Bumblebees were monitored in this study using a variation of the Lincoln University bumblebee motel design (Lincoln University, 2014).

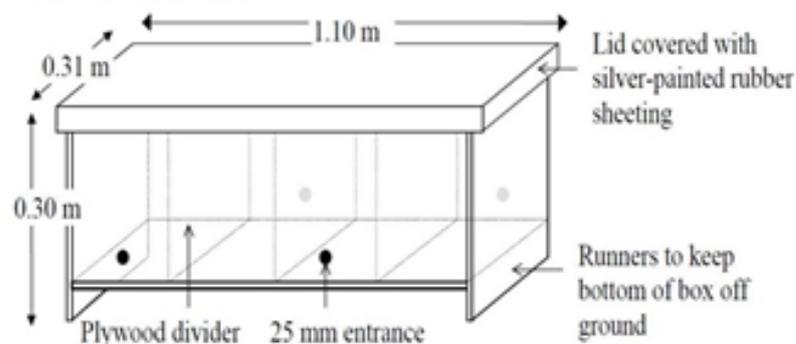


Figure 2: Lincoln University bumblebee motel design

This design was varied (creating the 'sub-plots' in this experiment) in an attempt to improve the motels ability to repel rodents; allowing for higher rates of colonisation by bumble bees and

providing information around potential future improvements to the design. Once treatments were applied to individual motels, they were spread over five available *M. x giganteus* shelterbelts and five areas of adjacent paddock fence lines.

Each of these five shelterbelts had four bumblebee motels evenly spaced inside the shelterbelt and four motels (control treatments) spread along fence lines in adjacent paddocks – leading to a total of forty motels. Each motel (plot) had four compartments (sub-plots) inside it, allowing four different treatments to be assigned to each motel, being replicated forty times.

These four treatments included:

1. Raising the entry hole to 10cm off the ground so rodent will not be able to reach the hole
2. Raising the hole 10cm and applying a 25cm² perspex surface around the entry hole that rodent/rats cannot grip onto
3. Raising the hole 10cm adding a small amount of projecting PVC piping around the entrance hole to stop rats/rodent entering
4. Control treatment – no changes from original design (entrance hole at base of motel)



Figure 3: Bumblebee motel along fence line with four entrance type treatments

This led to a total of 40 motels; 20 placed inside the *M. x giganteus* shelter and 20 along an unimproved fence line. Each motel contained 4 sub-plots, each containing one of each treatment type. This created a total of 40 replicates of each treatment - with a total of 160 plots. The assignment of treatments to the four sub-plots inside each motel was assigned using a randomised block design in genstat to remove any effect of compartment position on results. In addition, the

order of operations (e.g., setting up and assessment) was randomised between the two areas (*M. x giganteus* shelter and unimproved fence lines) within each paddock by way of a coin toss.

The measurements taken included the presence of rodents (which also occupy the motels) and the presence of bumblebees which were identified as either *Bombus hortorum* (Linnaeus, 1761) or *Bombus terrestris* (Linnaeus, 1758). The presence of rodents in different sub-plots inside the motels provided information about whether any changes to the original Lincoln design (Lincoln University, 2014) were successful, while the presence of rodent and bumblebees provided information about the effects of *M. x giganteus* on motel occupancy of both species. These measurements were taken at four repeated intervals on 16 July, 6 August, 27 August and 2 October 2014.

Bumblebees are most active over the summer period, having been monitored over the June/July UK summer in previous studies investigating their nesting behaviour (Osborne et al., 2008). As this key nesting period (December-February in NZ) was outside the experimental window of this trial, the only data collected from motels was in the form of rodent occupancy, with no evidence of bumblebees recorded.

Statistical analysis of bumblebee motel data was carried out as follows:

- Calculation of the average rodent occupancy rates over all four sample time periods
- The use of three chi-squared goodness of fit tests to determine any significant differences in: 1) total rodent occupancy between *M. x giganteus* and fence line motels, 2) rodent occupancy under different entrance types inside *M. x giganteus*, 3) rodent occupancy under different entrance types along fence lines
- Results presented graphically, showing the proportion of motels/compartments that indicated rodent occupancy, and presented with associated binomial standard errors as opposed to the standard error of the mean as all data recorded was binary

2.3 Functional invertebrate diversity

Invertebrate populations were compared between the vegetation at the base of five developed *M. x giganteus* shelterbelts and vegetation at the base of five adjacent unimproved fence lines.

These populations were measured using a vortis-sampling method similar to that defined by Hawes (2011). This technique obtained one sample using a total of five 5-second sucks at adjacent ground level locations with a vortis machine (16cm diameter at suction point). This produced a total sample area of 105.1cm² per sample.

Replicates were the five *M. x giganteus* shelterbelts with four 105.1cm² samples being collected per replicate. An equal number of samples were collected along adjacent unimproved fence lines (considered as control treatments). The orders of operations (e.g. setting up and assessment) were randomised between the two areas (*M. x giganteus* shelter and unimproved fence line) within each paddock by way of a coin toss. Statistically, the design is a randomised complete block design with the five paddocks being blocks and the two treatments being *M. x giganteus* shelterbelt and adjacent unimproved fence lines (i.e., 10 “plots” with 4 samples per plot). Once invertebrate samples were collected, all sample matter was stored in jars with ethanol (70%) until lab identification of samples could occur.

Lab identification included counting invertebrate numbers per sample using a petri dish and microscope. Invertebrates were classed as natural enemies (predatory) or potential pasture pests (herbivores). Natural enemy classes included Carabid beetles, Rove beetles, Arachnids, Hoverflies, Nabidae and parasitic wasps. Pest classes included Aphids, Thrips, Leaf Hoppers, Psyllidae and Slugs. Other invertebrate groups such as Collembola, although abundant, were considered irrelevant to the trial and as such were not counted.

The presence of natural enemies was deemed to provide ecosystem services while the presence of pests was deemed to provide an ecosystem dis-service.

Statistical analysis of functional invertebrate diversity data was carried out as follows:

- Calculation of the average number of invertebrate pests and natural enemies in each plot from samples (four samples per plot)
- Calculation of standard deviation and standard error for each plot
- The use of an analysis of variance (ANOVA) to indicate any significant differences between the means of each plot
- Results (average number of pests or NE) were expressed graphically with standard error of each plot expressed as error bars

2.4 Trichoderma glasshouse experiment

The endophytic effects produced by different combinations of *T. atroviride* strains on the growth of *M. x giganteus* were explored in this study through a glass house pot trial at the Lincoln University plant nursery.

The trial consisted of a control and five *T. atroviride* treatments consisting of different *T. atroviride* strain mixtures, containing species which have yet to be named formally – titled PR1, PR2, PR5, PR6 and PR7. These six treatments were laid out in a completely randomised block design with each treatment being replicated 20 times – once per block. This led to a total of 120 pots spread over 20 individual blocks of 6 pots, with treatments being randomly allocated within blocks.

Treatments were laid out in 5L pots and kept under glasshouse conditions (16hrs of light, watered once daily) from 22 July until 4 October 2014. Over this period, plants had their height and number of shoots recorded at one month intervals (three times in total), followed by shoot and root dry weights being recorded at the end of the trial. Additionally, one measurement of chlorophyll was undertaken on 3 October 2014. This was undertaken using a SPAD -502 chlorophyll meter, providing a measure of leaf chlorophyll concentration (Markwell et al., 1995).

M. x giganteus plantlets were planted on the 22 July 2014, following inoculation with *T. atroviride* treatments. Inoculation was carried out immediately prior to planting and consisted of weighting (fresh weight) plantlets then soaking the roots in *T. atroviride* mixtures for a 24hr period. Plantlets receiving the control treatments were soaked in water for the same time period as plantlets receiving *T. atroviride* treatments to remove any variation this pre-planting preparation may have caused.

Statistical analysis was carried out by Ivan Valle, the head researcher in this trial – conducting ANOVA, followed by Tukey *Post hoc* tests. Results were expressed graphically along with the standard error of the mean and treatment groupings identified through a Tukey *Post hoc* test.

Chapter 3

Results

The results of each of the four experiments carried out in this study were analysed and presented separately. Results were presented graphically with associated standard errors while averages were presented alongside the standard deviation of the mean (\pm) in text.

3.1 Tracking tunnel rodent population monitoring

Rodent populations were recorded over two sample periods, one month apart. Over this period, a large variation in weather occurred as the season changed from winter to spring, with higher temperatures in the second period. This led to both samples being considered independent, with their results being analysed and presented separately. Results are expressed graphically alongside the standard error of the mean.

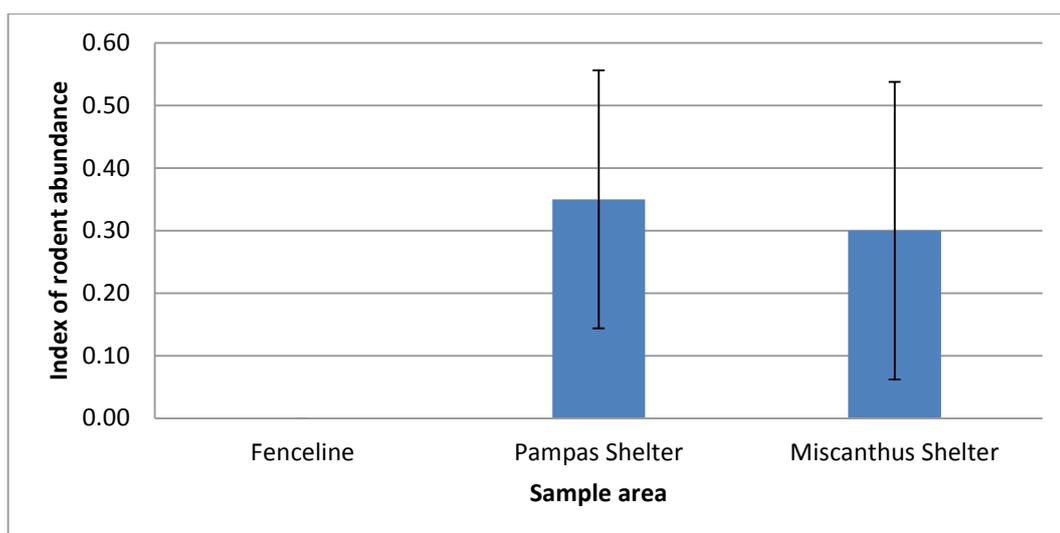


Figure 4: The proportion of tracking tunnels indicating rodent activity in the period – 1 September 2014 (One way ANOVA $F=1.08$, $DF= 2, 11$, $P=0.37$)

In the first sample period (figure 4), no significant difference was identified between sample areas. Although there was no evidence of rodents in tracking tunnel replicates along fence lines, an average of 35% (± 0.41) of tunnels in pampas shelter and 30% (± 0.48) in *M. x giganteus* shelter showed that rodents were present. Large standard errors were identified due to the relatively small sample sizes.

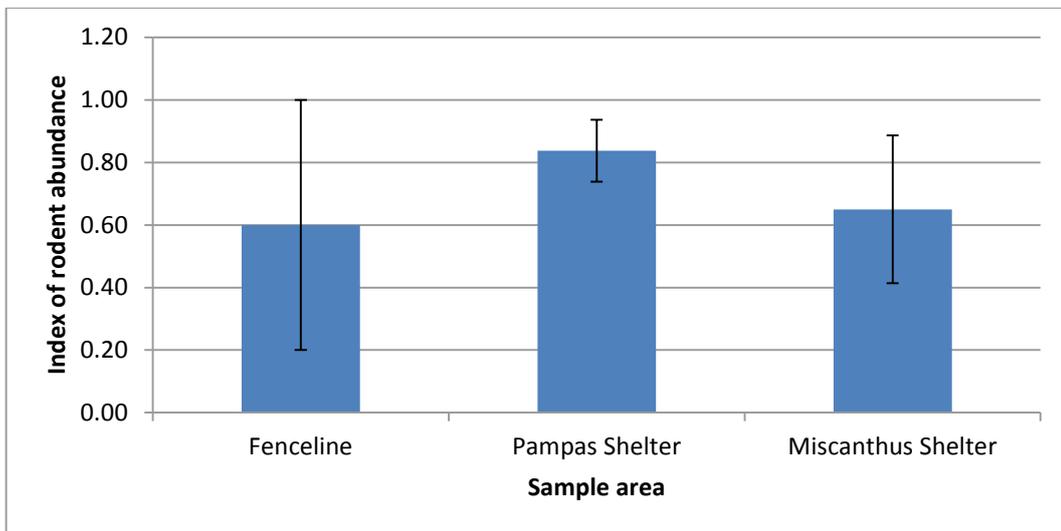


Figure 5: The proportion of tracking tunnels indicating rodent activity in the period – 2 October 2014 (One way ANOVA $F=0.33$, $DF= 2, 9$, $P=0.73$)

The second sample period showed a notable increase in rodent populations compared to the first recording. Despite this there were still no significant differences in rodent abundance between sample areas. Pampas grass again produced the highest rodent abundance with an average tracking rate of 84% (+/- 0.20), compared to 65% (+/-0.47) in *M. x giganteus* and 60% (+/-0.57) along fence lines. Smaller replicates in the second recording as a result of field issues ($n = 9$ compared to $n = 11$) extenuated the issue of large standard errors.

3.2 Bumblebee motel monitoring

Originally these bumblebee motels were deployed with the aim of firstly comparing the preference in bumblebee nesting sites between *M. x giganteus* shelter and that of an unimproved Canterbury dairy farm agro-environment. However, the experimental timeframe occurred outside the key window for bumblebee nesting. This meant that the motels were instead used as another measure of rodent preference between the same sites.

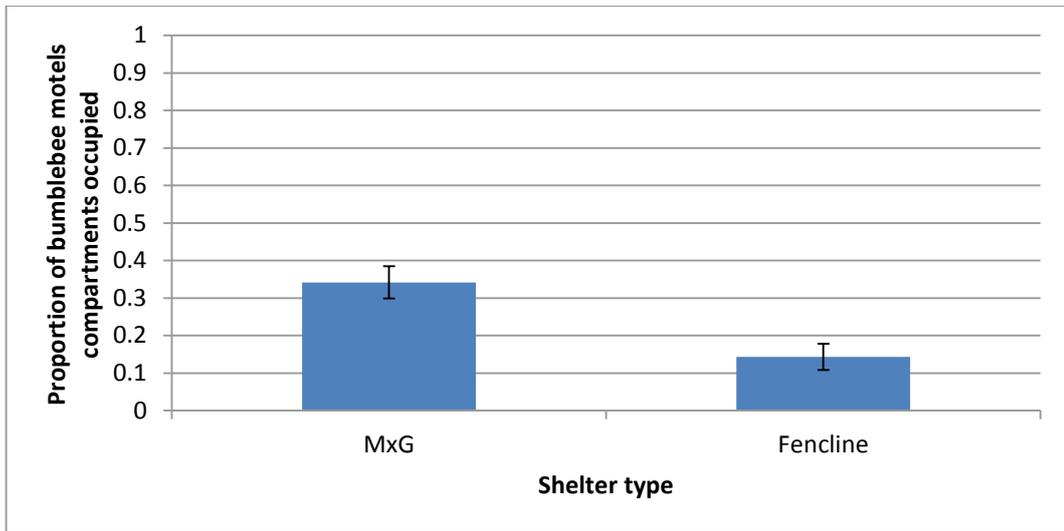


Figure 6: The average proportion of bumblebee motel compartments indicating the presence of rodents inside *M. x giganteus* and adjacent fence lines, expressed with binomial SE (Chi-Sq Goodness of Fit, $P=0.001$, $DF=1$)

Through the use of this monitoring technique, unlike the case with tracking tunnels, a highly significant increase ($P<0.001$) in rodent populations was observed between the *M. x giganteus* shelter and fence lines. Bumblebee motels inside *M. x giganteus* shelter had an average occupancy rate of 34% ($\pm 4\%$) compared to 14% ($\pm 3\%$) along fence lines.

The second level of investigation in this experiment was four different entrance type options, with the aim of mouse-proofing the motels and providing information around potential future improvements to the design. Due to rodent population differences between *M. x giganteus* and fence lines (figure 6), data from both were analysed separately – providing information on entrance type efficacy under high and low rodent population environments.

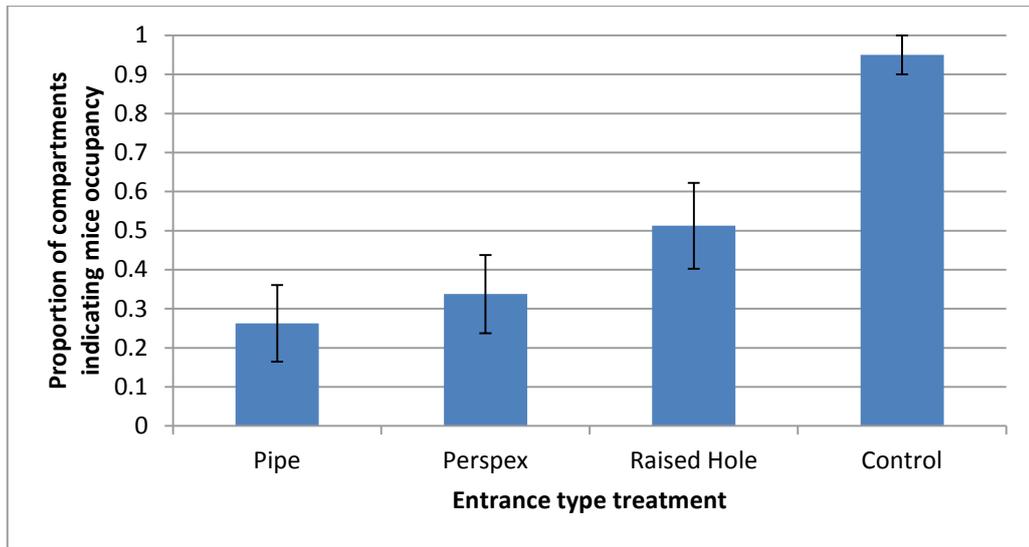


Figure 7: The average proportion of bumblebee motel compartments with different entrance types indicating the presence of rodents inside *M. x giganteus* shelter, expressed with binomial SE (Chi-Sq Goodness of Fit, $P=0.01$, $DF=3$)

When entrance type efficacy was compared inside the *M. x giganteus* shelter, significant ($P<0.01$) differences were observed between treatments (figure 7). Control treatment observed the highest average occupancy (95% \pm 9%), followed by raised whole (51% \pm 11%), perspex (33% \pm 10%) and pipe (25% \pm 9%) treatments.

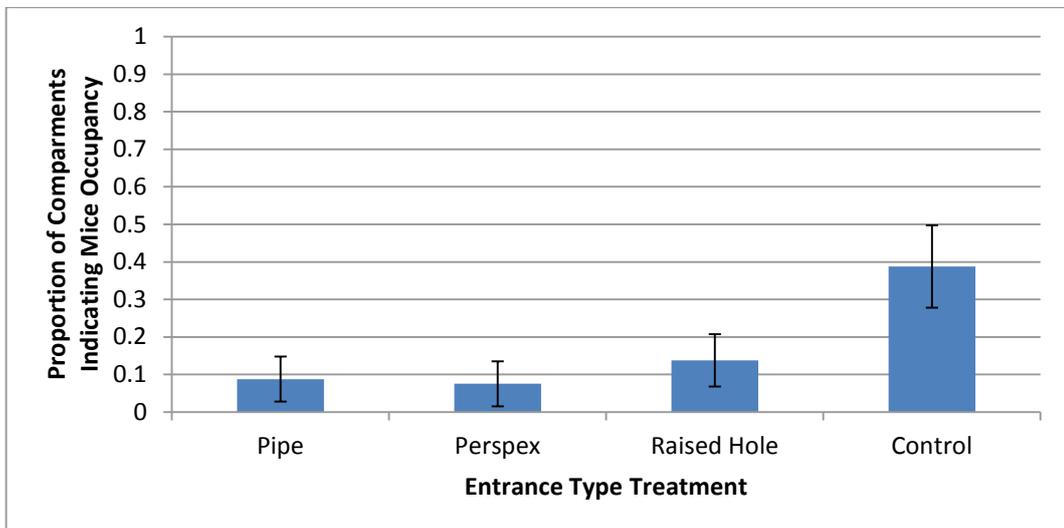


Figure 8: The average proportion of bumblebee motel compartments with different entrance types indicating the presence of rodents along fence lines, expressed with binomial SE (Chi-Sq Goodness of Fit, $P=0.05$, $DF=3$)

When entrance types were compared along fence lines (figure 8), smaller overall rodent abundance lead to smaller differences being observed between treatments, although significant differences were again observed ($P<0.05$). The control treatment was again observed to have the highest

average occupancy rates (38% \pm 11%), followed by raised whole (13% \pm 7%), pipe (9% \pm 6%) and perspex (8% \pm 6%) treatments.

3.3 Functional invertebrate diversity

Functional invertebrate diversity in this trial was compared by measuring the net abundance of groups of invertebrates classed as either pests or natural enemies (NE).

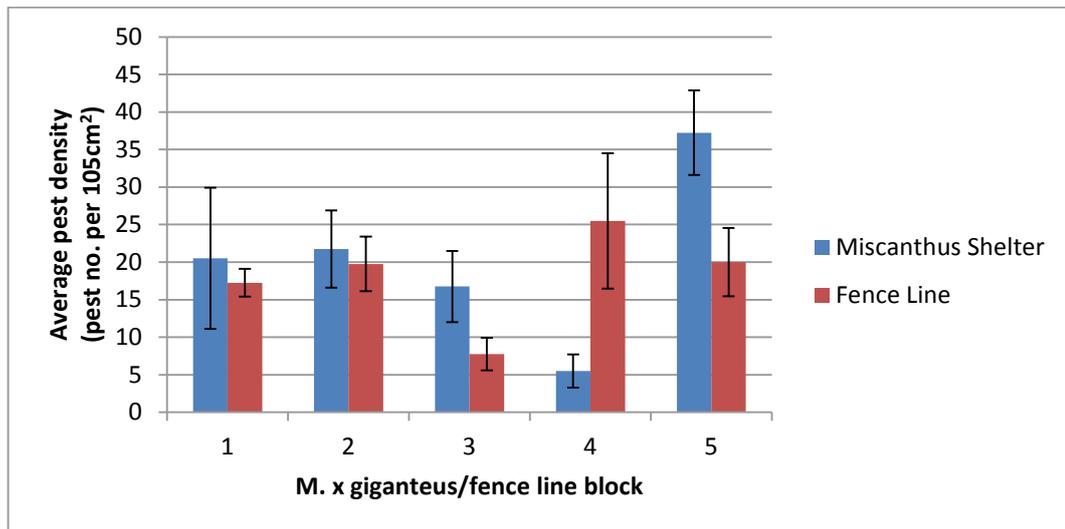


Figure 9: The average density of invertebrate pests (pests per 105cm²) in each plot of *M. x giganteus* and unimproved fence line (One way ANOVA F=0.15, DF= 1,9, P=0.70)

When considering pest densities (figure 9), no significant differences were identified between *M. x giganteus* and fence line treatments. Slightly higher average pest densities were observed in *M. x giganteus* shelter than at the base of fence lines, with an average of 20.35 (\pm 11.41) pests/105cm² compared to 18.05 (\pm 6.50) pests/ 105cm².

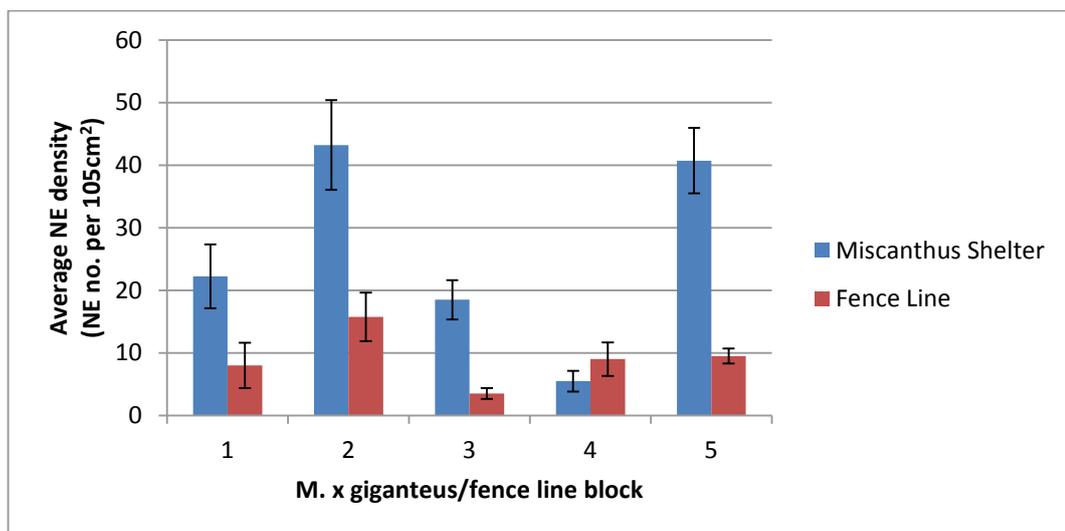


Figure 10: The average density of invertebrate NE (NE per 105cm²) in each plot of *M. x giganteus* and unimproved fence line (One way ANOVA F=5.28, DF= 1, 9, P=0.05)

In contrast to pests, significantly ($P < 0.05$) higher proportions of potential NEs were observed under *M. x giganteus* shelter. The average number of NE was 2.8 times higher under *M. x giganteus* treatments with an average of 26.05 (± 15.86) NE/105cm² compared to 9.15 (± 4.39) NE/105cm².

Time constraints of trial meant repeated measurements were not feasible, allowing only one comparison to take place.

3.4 Trichoderma glasshouse experiment

This experiment compared the growth effects of five different mixtures of *T. atroviride* strains on the height, dry weight, number of shoots and chlorophyll content (SPAD). In terms of height, shoot number and chlorophyll content, three recording intervals were carried out. The final recording interval best indicated the effects of different *T. atroviride* treatments and thus these results will be presented in the report.

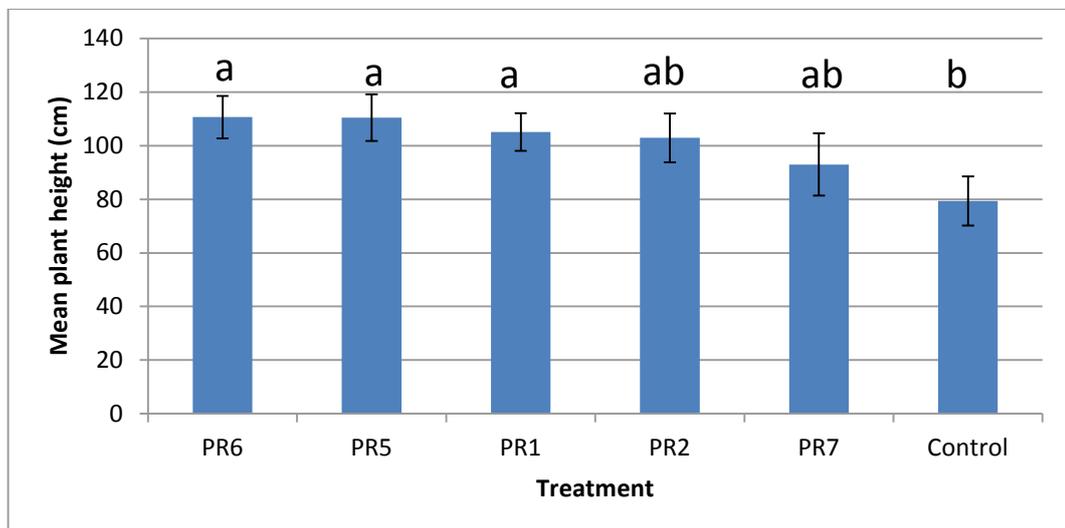


Figure 11: The mean height (cm) of *M. x giganteus* plants under different *T. atroviride* treatments, third recording (One way ANOVA, $P < 0.05$, $DF = 5$)



Figure 12: Examples of height differences between *M. giganteus* seedlings treated with different *T. atroviride* mixtures

Comparison of the average heights of plants under different *T. atroviride* treatments indicated a significant ($P < 0.05$) difference between treatments (figures 11, 12). Treatments PR6 PR5 and PR1 all had significantly higher plant heights compared to the control. PR6 had the highest mean height of $110.6\text{cm} \pm 7.9$, with the control being the lowest ($79\text{ cm} \pm 9.1$).

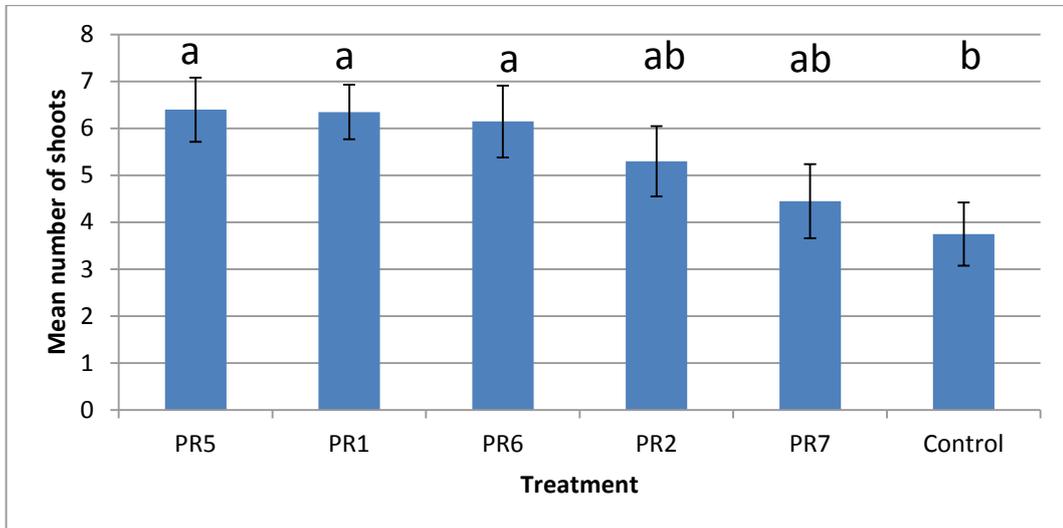


Figure 13: The mean number of shoots of *M. x giganteus* plants under different *T. atroviride* treatments, third recording (One way ANOVA, $P < 0.05$, $DF = 5$)

In terms of shoot numbers, similar results were found to plant height (figure 13). Treatments PR6 PR5 and PR1 all had significantly higher shoot numbers compared to the control. PR5 had the highest mean shoot number with an average of 6.4 ± 0.68 shoots, compared to the control which had the lowest mean shoot number of 3.75 ± 0.67 .

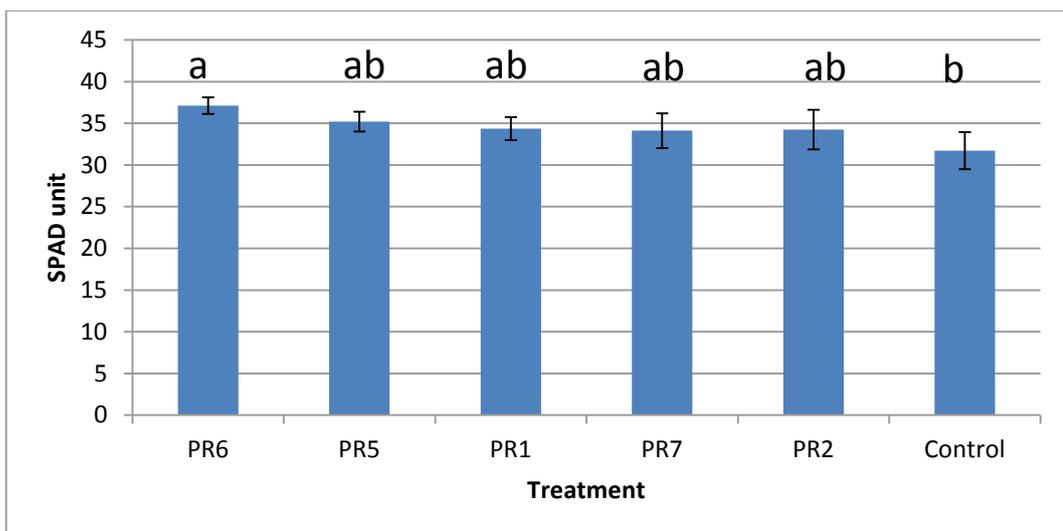


Figure 14: The mean leaf chlorophyll content (SPAD unit) of *M. x giganteus* plants under different *T. atroviride* treatments, third recording (One way ANOVA, $P < 0.05$, $DF = 5$)

Leaf chlorophyll content (figure 14) also proved to be significantly different ($P < 0.05$) between treatments. PR6 had the highest SPAD content with a measure of 37.1 ± 0.99 and was the only treatment significantly higher than the control with a SPAD content of 31.7 ± 2.21 .

No significant differences were observed between treatments in terms of dry weight.

Chapter 4

Discussion

4.1 Tracking tunnel rodent population monitoring

Rodents such as mice and rats are considered to be major agricultural and urban pests worldwide due to their capacity to cause physical damage to agricultural crops, in addition to acting as reservoirs for stock diseases and contaminating food (Stenseth et al., 2003). It is a common perception among farmers that shelterbelts and other riparian plantings act as reservoirs for these pests (Maisonneuve et al., 2001). This belief has led to many farmers actively removing these habitats from the agricultural landscape (Maisonneuve et al., 2001) as is the case on the Canterbury Plains (Jay, 2007). However, in this instance the primary driver has been the need for space to enable implementation of pivot irrigators on developing dairy farms (Goulter, 2010).

These perceptions of riparian habitats are a major impediment to conservation efforts aiming to integrate wildlife habitats into the management of agricultural landscapes (Maisonneuve et al., 2001) – habitats which are considered key wildlife corridors between larger sections of island vegetation (Cummings et al., 1994). Moreover, the removal of this field boundary vegetation reduces the potential for a suite of ecosystem services, such as the biological control of insect pest species (Thies et al., 1999; Landis et al., 2008).

The aim of this experiment was to investigate whether the inclusion of *M. x giganteus* in the agricultural landscape increased the density of these rodent pests, in comparison with a *C. selloana* shelterbelt and an unimproved, fence line with only pastoral species. An increase in the population densities of rodents by *M. x giganteus* would be considered to be an ecosystem disservice and detract from the attractiveness of the species as a shelterbelt option. This is an attribute of *M. x giganteus* with no previous research globally.

The results of both recording intervals indicated no significant differences in rodent populations between *M. x giganteus*, *C. selloana* and unimproved fence lines. Despite this, it should be noted that no rodents were recorded along fence lines in the first recording, while tracking rates of 35% and 30% were identified in *C. selloana* and *M. x giganteus* respectively. It is therefore plausible that a more comprehensive study could potentially find a significant increase in rodent numbers between the fence line and both shelterbelt types, as commonly believed by farmers (Maisonneuve et al., 2001). Similarly, tracking tunnels inside *C. selloana* consistently produced higher average tracking results than those in *M. x giganteus* (e.g. 85% compared to 65% in the second recording). This indicates that *C. selloana* may harbour higher rodent densities than *M. x giganteus*, potentially due

to *M. x giganteus* lacking the dense basal vegetation in which *C. selloana* is characterised (Littlejohn, C., personal communication). Again, this difference in rodent populations could potentially be significant in a more extensive study.

Therefore, although no statistically sound results occurred in this study, the data do indicate potential differences in rodent abundance between shelter types. The lack of significance is likely to have occurred due to large variation between samples resulting from the small number of replicates. This experiment should therefore be considered a pilot study, indicating some potential trends, with *M. x giganteus* harbouring higher rodent densities than an unimproved fence line but lower densities than a *C. selloana* shelterbelt. It must be recommended that future studies should include more shelterbelt replicates in addition to taking more measurements within each shelterbelt.

4.2 Bumblebee motel monitoring

Pollination is an essential ecosystem service providing major economic and social benefits to mankind (Westphal et al., 2003). Unfortunately, the continued adequate provision of this service is reportedly endangered due to global declines in pollinator populations (Daily, 1997). One guild of pollinators which has fallen victim to this population decline is bumblebees, namely the *species B. hortorum* and *B. terrestris*. These are recognised as pollinating a wide range of wild plants and crops in agroecosystems (Corbet et al. 1991), therefore their decline should be of serious concern to all involved in agricultural production.

This decline in bumblebee and overall pollinator populations is thought to be caused in part by simplification of the agricultural landscape through habitat destruction and fragmentation - negative externalities exerted as part of agricultural intensification and conversion to monoculture-like agroecosystems (Tilman et al., 2001). In Canterbury this intensification has mainly occurred in the form of extensive shelterbelt vegetation removal to allow for the implementation of pivot irrigators on dairy farms (Goulter, 2010). Provision of semi-natural habitats in the agricultural environment has been shown to create suitable nesting sites for bumblebees (Svensson et al., 2000). Therefore improving landscape structure through the implementation of *M. x giganteus* shelter belts should theoretically provide shelter and possible nesting sites for these key pollinators, improving their provision of pollination services.

Preferences in bumblebee nesting habitats between the *M. x giganteus* shelter and unimproved Canterbury dairy farm fence lines were carried out through the use of Bumblebee Motels. These were hoped to indicate that improving landscape structure through the addition of *M. x giganteus* would lead to higher nesting rates inside the shelter belt compared to unimproved areas. Unfortunately, the experimental timeframe occurred outside the key window for bumblebee nesting,

late spring through summer (Osborne et al., 2008), meaning that little valuable data surrounding the nesting preferences of bumblebees could be collected.

Bumblebee motels are commonly inhabited by rodents which remove the potential for bumblebees to use them as a nesting site (Littlejohn, C., personal communication, 20/10/2014). This meant that although no information could be gathered about bumblebee nesting site preferences using the motels, they were still able to be used effectively as another measure of rodent preference between the same sites. Additionally, treatments of different entrance type were implemented in an effort to identify any potential future improvements to the motels in regards to making them 'mouse-proof'.

Information gathered surrounding rodent habitat preference (*M. x giganteus* versus fence line), unlike the case with tracking tunnels (section 4.1), indicated a highly significant increase ($P < 0.001$) in rodent populations between the *M. x giganteus* shelter and fence lines. Bumblebee motels inside the *M. x giganteus* shelter had an average rodent occupancy rate of 34% ($\pm 4\%$) compared to 14% ($\pm 3\%$) along fence lines. As previously mentioned, rodents are considered to be major agricultural pests worldwide (Stenseth et al., 2003), with shelter belts and other riparian plantings thought to act as reservoirs for these pests (Maisonneuve et al., 2001). The inclusion of *M. x giganteus* on Canterbury dairy farms could therefore be said to be providing an ecosystem disservice to farmers by increasing on-farm rodent populations.

The second level of study using bumblebee motels was the implementation of entrance type treatments with the aim of collecting information for potential improvements to their design. In both high rodent (*M. x giganteus*) and low rodent (fence line) environments, the compartments assigned the control treatment displayed significantly higher ($P < 0.01$ and $P < 0.05$ respectively) rodent populations than any other entrance type; with an occupancy rate of 95% ($\pm 9\%$) in the high rodent scenario. This initial finding validates the need for this research as motels with such levels of rodent occupancy will be unable to provide information surrounding bumblebee nesting preferences.

Of the treatments implemented, both the perspex and pipe treatment types effectively lowered rodent occupancy rates. Under the *M. x giganteus* shelter, which had higher occupancy rates under all treatments other than fence line motels, occupancy was reduced down to 33% $\pm 10\%$ and 25% $\pm 9\%$ respectively, with no significant differences between occupancy rates under either shelter type. Simply raising the hole was relatively ineffective compared to other treatments, decreasing rodent occupancy to 51% $\pm 11\%$ in the *M. x giganteus* shelter.

It should therefore be concluded that future research using bumblebee motels include either the pipe or perspex treatments in the construction design. As there was no significant difference in occupancy rates between these two treatments the entrance type selection should be based on price

– which in this case would lead to pipe entrances being implemented (the cheaper of the two options). The use of these improvements to the original bumblebee motel design should improve the usefulness of the research tool, allowing for improved future monitoring of bumblebee populations.

4.3 Functional invertebrate diversity

Invertebrate diversity is a factor often overlooked in conventional farm management, however, it can be considered a key aspect in the ability of agroecosystems to function. From a conservation point of view, invertebrate diversity has conservation value in its own right, in addition to providing key food for birds and other high trophic level organisms (Gregory et al., 2005). Invertebrate diversity is therefore a widely accepted indicator of agroecosystem health (Gregory et al., 2005). Nevertheless, for widespread consideration of invertebrate diversity to be adopted on-farm, it must be proved to have functional benefits or provide ‘ecosystem services’. These ecosystem services can be defined as naturally occurring ecosystem functions which can be quantified as directly or indirectly benefitting mankind (Constanza et al., 1997). Whether or not including *M. x giganteus* on Canterbury dairy farms increases functional invertebrate diversity and resulting ecosystem services was the main focus point of this experiment.

Studies considering on-farm biodiversity conservation have been critically scarce for dairy farms worldwide (Fukuda et al., 2011), with the main focus of environmental research on NZ dairy farms being limited to water quality and waste management (Jay, 2007). The expansion of dairy farming on the Canterbury plains has led to a widespread reduction in landscape structure – in the form of extensive shelterbelt vegetation removal to allow for the implementation of pivot irrigators (Goulter, 2010). This simplification of the agricultural landscape in conjunction with increased agrichemical use, has caused rapid declines in invertebrate biodiversity in NZ and worldwide (Tilman et al., 2001; MEA, 2005; Semere et al., 2007). Declining invertebrate biodiversity has been shown to reduce ecosystem service potential such as the biological control of invertebrate pests (Thies et al., 1999; Landis et al., 2008). Additionally, decreased invertebrate diversity on-farm could be said to trigger a trophic cascade with wider ecological implications such as decreased local bird populations of which invertebrates are key food sources (Semere et al., 2007).

A previous study in the UK by Semere et al. (2007) indicated that increasing the structural complexity of landscapes through the growth of *M. x giganteus* substantially increased the number of invertebrate families belonging to Diptera, Hymenoptera, Heteroptera and arboreal Coleoptera. This increase was attributed mainly to the florally diverse habitat of *M. x giganteus* fields in addition to its low input agrochemical management requirements (Semere et al., 2007). Due to entomological knowledge limitations in addition to time constraints, functional invertebrate diversity was carried out in this study by broadly classifying collected invertebrates as predatory or herbivorous in nature.

Predatory invertebrates (natural enemies) were regarded as increasing biological control and therefore providing an ecosystem service while herbivorous invertebrates were expected to impact pasture production, providing an ecosystem disservice.

Although no significant differences were observed in pest populations between *M. x giganteus* and unimproved fence lines, differences ($P < 0.05$) were observed in predator populations. Mean predatory/natural enemy densities increased from 9.15 (± 4.39) NE/105cm² along fence lines to 26.05 (± 15.86) NE/105cm² inside the *M. x giganteus* shelter (figure 10). These results are in line with those produced by Semere et al. (2007), although his study constituted full-paddock *M. x giganteus* growth as opposed to shelterbelt growth as in this study.

Natural enemies are thought to move from reservoirs such as riparian plantings (in this case *M. x giganteus*) into surrounding fields where they are able to predate on artificially heightened pest numbers created by structurally simple agricultural landscapes (Pickett et al., 1998). This biological control is a key ecosystem service which is enhanced by improving landscape structure (Thies et al., 1999; Landis et al., 2008). This research therefore indicates that by including *M. x giganteus* on Canterbury dairy farms, natural enemy populations are increased – raising their potential for biological control as well as other ecosystem services.

4.4 Trichoderma glasshouse experiment

Trichoderma are one in a number of fungi orders which are known to colonise plant roots without causing disease to the plant, instead forming a symbiosis in which both species benefit (Harman, 2011a). This trichoderma-plant interaction was historically thought to benefit the plant solely through disease prevention (Harman et al., 2008). However, recent studies have indicated a suite of benefits resulting from trichoderma colonisation of plant roots:

- Increased growth rates and final yields of plants (Yedida et al., 2001)
- Increased root growth and resulting drought tolerance (Harman et al., 2000)
- Induced systemic resistance to disease (Yedida et al., 2003; Harman et al., 2004)
- Increased nutrient uptake and fertilizer efficiency utilization (Harman et al., 2000; Yedida et al., 2001)
- Increased leaf greenness and photosynthetic rate (Harman and Shores, 2007)

The beneficial effects of many trichoderma species are well documented on a range of plants. However, no studies have yet been published evaluating the growth effects of trichoderma on the

production of *M. x giganteus* (Wratten, S., 2014, personal communication). The effect of different trichoderma strains on plant growth can vary largely between crop varieties (Harman, 2011b). Indeed, most strains of the fungus are relatively ineffective on the majority of common crops (Harman, 2011b), meaning selection of the correct strain for each plant trichoderma is trialled with is essential.

This study examined the effect of five mixtures of different *T. atroviride* strains on *M. x giganteus* seedling growth in terms of plant height, number of shoots, chlorophyll content and dry matter production.

Results from this trial indicate multiple benefits to *M. x giganteus* growth by the inclusion of *T. atroviride*, in line with results from previously mentioned experiments on different plant varieties. When considering plant height, mixtures PR6, PR5 and PR1 significantly increased mean plant heights compared to control (no trichoderma). PR6 had the largest effect on plant height, with a mean increase of 28% compared to the control. As *M. x giganteus* is being grown in this instance in the form of a shelterbelt, this increased height could substantially increase the effectiveness of the species. Once fully established, *M. x giganteus* reaches a final height of around 4m (Jones et al., 2001). If these glasshouse trial results (a 28% increase in height) can be successfully replicated in field, the height of *M. x giganteus* shelterbelts could potentially be increased to 5.12m – drastically increasing the shelter potential of the species.

Comparison of shoot numbers revealed the same three *T. atroviride* mixtures (PR6, PR5 and PR1) producing a significant increase compared to the control. PR5 produced the largest increase in shoot content, increasing the mean number of shoots by 41% compared to the control. Increased plant shoot numbers would lead to an increased density of the shelterbelt – reducing its permeability. The ideal permeability of shelterbelts for a reduction in leeward mean velocity of wind has been calculated at 20% (Raine et al., 1977). As this is the first trial utilizing *M. x giganteus* in the form of a shelterbelt, no data is available concerning its permeability. However, *M. x giganteus* is thought to be a relatively permeable shelterbelt option compared to standard Canterbury Cupressus macrocarpa shelterbelts (Littlejohn, C., personal communication), with a permeability likely greater than the desired 20%. Therefore, increasing shoot numbers through the inclusion of an effective *T. atroviride* mixture could potentially decrease permeability closer to the ideal level – improving the effectiveness of the shelterbelt.

Consideration of chlorophyll content (SPAD value) revealed only one treatment, PR6, producing a significant (15%) increase on the control results. Leaf chlorophyll content is a widely accepted indicator of nutritional stress and the photosynthetic capacity of plants (Filella et al., 1994). As PR6 increased the chlorophyll content of treated plants, it should theoretically have also increased the

photosynthetic rate and resulting growth of the plants. Despite this, when plants were harvested no significant differences in the dry matter yield were observed – indicating no correlation between chlorophyll content and plant growth in this instance. This could potentially have occurred to experimental limitations such as pot size (5L) or the short experimental timeframe (three months).

These findings indicate that PR6 formed the most successful symbiosis with *M. x giganteus*, significantly increasing plant height (28%), shoot number (39%) and chlorophyll content (15%). It should be recommended that this glasshouse experiment be followed by a comprehensive field trial, where these potential relationships can be fully explored in a more realistic setting.

Chapter 5

Conclusions

- *M. giganteus* provides increased on-farm rodent populations compared to an unimproved agricultural landscape, providing a potential ecosystem disservice
- Potential trends indicating *C. selloana* shelterbelts harbour higher rodent populations than *M. giganteus*, although an experiment with higher replicates would be required to prove this conclusively
- The implementation of plastic piping around entrances on bumblebee motels decreased rodent occupancy and improves their potential to be occupied by bumblebees
- *M. giganteus* increases functional invertebrate diversity in terms of heightened natural enemy populations, increasing biological control potential – a key ecosystem service
- *T. atroviride* mixture PR6 effectively forms a symbiosis with *M. giganteus*, increasing plant height by 28%, shoot numbers by 39% and chlorophyll content by 15%

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