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Biofuel plants as refugia for pest biocontrol agents

A Dissertation

submitted in partial fulfilment

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by

Morgan Shields

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Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Science with Honours

Biofuel plants as refugia for pest biocontrol agents

By

Morgan Shields

Agriculture faces multiple global challenges including climate change, food production, fuel security and insect pest management. Agroecology can provide mitigating solutions to these issues such as using *Miscanthus* x *giganteus* (Mxg) shelterbelts on farms. These shelterbelts can provide at least 15 ecosystem services (ES) on commercial dairy farms with centre pivot systems. However, there has been little research that investigates how Mxg shelterbelts could contribute to agricultural functional biodiversity and insect pest management. The former is being degraded by intensive farm management resulting in simplified food webs. This severely inhibits the ES that agroecosystems can provide, such as biological control.

This study investigated what potential generalist soil-surface dwelling biological control agents (BCA) use Mxg shelterbelts as refugia compared to a field margin, using pitfall traps during April (early autumn), August (late winter) and September (early spring) 2015. Furthermore, the effectiveness of these potential BCA at consuming a soil-surface active pest was determined using facsimile prey. Additionally, potential BCA that consumed the prey were confirmed using infrared cameras and Sanger sequencing using specific primers for the CO1 mitochondrial gene. Sanger sequencing was conducted on DNA from slugs, the European harvestman (*Phalangium opilio*), centipedes, predatory beetles and *Dicyrtoma fusca*.

Based on the pitfall trap results, there were distinct potential BCA communities and similar potential BCA richness between Mxg shelterbelts and the field margin. Total facsimile prey consumption was 57 % higher in Mxg shelterbelts. Furthermore, *Phalangium opilio* and slugs such as *Deroceras reticulatum*, were confirmed to consume the facsimile prey using video analysis and DNA sequencing but only in Mxg shelterbelts. These findings suggest slugs could potentially be used as BCA in the presence of Mxg shelterbelts. Additionally, an introduced collembolan species *Dicyrtoma fusca* was found in the field margin on the facsimile prey. This is the first authenticated record of this species in

the southern hemisphere but its role in New Zealand agroecosystems is unknown. As a world first, these results indicate that Mxg shelterbelts are refugia for potential BCA and could be implemented in insect pest management using conservation biological control in agroecosystems. Further research needs to further elucidate potential BCA predation rate and community dynamics over a longer study period and whether potential BCA emigrate from the Mxg shelterbelt into the fields in spring. Additionally, further investigation of slugs as potential biocontrol agents is required and the trade-off between this ES and potential ecosystem dis-services (EDS).

Keywords: agroecology, agroecosystem, conservation biological control, *Miscanthus* x *giganteus* (Mxg) shelterbelts, ecosystem services (ES), ecosystem dis-service (EDS), facsimile prey, *Epiphyas postvittana*, *Deroceras reticulatum*, *Phalangium opilio*, *Dicyrtoma fusca*, biofuel

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I must also thank the team of people who identified my specimens for me. These included: Michael Wilson who identified the slugs; Vikki Smith who identified the spiders; Penny Greenslade who identified *Dicyrtoma fusca*; Peter Johns who identified the centipedes; Rowan Emberson who identified the predatory beetle and Brian Patrick who identified the moths. There was also another team of people who helped me with field work; they included Sophie Heausler, Cat O'Connell, Johnathan Ridden, Lorenz Weston-Salzer, Marie McDonald, Dilani Hettiarachchi, Coralline Housie and others. Lastly, I must thank my family for their encouragement and my fiancée Laura McMillan who also helped me with field work. I will be forever grateful for the support from everyone who helped me.

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Chapter 1

Introduction

1.1 Global agricultural challenges

Agriculture currently faces global challenges such as sufficient food production, pest damage, climate change and dependence on fossil fuels (de Schutter 2010; Gurr et al. 2012; Culliney 2014; Godfray & Garnett 2014; Sandhu et al. 2015; Sparks & Nauen 2015; Tanentzap et al. 2015). One approach to these challenges is using agroecology which is the management of agricultural systems in an ecologically sound and sustainable way (Pywell et al. 2015). Developing solutions to mitigate these worldwide challenges using agroecology is a major focus of researchers (Landis et al. 2000; Littlejohn et al. 2015; Pywell et al. 2015), governments and international agencies such as the Food and Agriculture Organisation of the United Nations (de Schutter 2010). For instance, during December 2015 there is a United Nations Framework Convention on climate change in Paris, where world leaders are discussing the Kyoto Protocol (http://unfccc.int/meetings/paris_nov_2015/meeting/8926.php).

Agroecosystems already cover approximately 5 billion ha worldwide (Sandhu et al. 2015), or around 40 % of non-ice covered land (Ramankutty et al. 2008) and an additional ~1 billion ha of land would need be cleared globally by 2050 to meet the expected 100 % increase in food demand if current trends continue (Tilman et al. 2011). To meet the growing food demand of an increasing human population (Pretty 2013) pesticide use is predicted to have a 400 % increase by 2050 (Tilman et al. 2001). Currently, there are already 586 insect pest species resistant to 325 insecticides (Sparks & Nauen 2015). Furthermore, global insect pest damage already costs an estimated US$ 470 billion per annum (Culliney 2014). Insecticide resistance and insect pest damage will only increase with higher pesticide use as other pest management measures such as biological control become less effective when pesticides are intensively used (Landis et al. 2000; Wratten et al. 2013). Pesticides often kill a large proportion of biological control agents (BCA) (Ali 2014) or change their behaviour which severely impedes the BCA ability to manage pests (Cloyd 2012; Khan et al. 2015). This is exacerbated by farm practises such as the removal of shelterbelts (Littlejohn et al. 2015) and other non-crop vegetation (Bianchi et al. 2006). These aspects are illustrated by biological control of pests contributing to US$ 0 ha/year savings in conventional cropping systems compared to US$ 68–200 ha/year of savings in organic cropping systems (Sandhu et al. 2015). Savings include cash savings to the farmer and economic savings such as reduced environmental costs (Sandhu et al. 2015). Increased pesticide use is also likely to occur in response to higher frequencies of pest outbreaks and invasions which are expected consequences of climate change (Grimm et al. 2013; Meynard et al. 2013).

Climate change is expected to severely threaten food and fuel security with a global mean temperature increase by > 2 °C, changing rainfall patterns and an increased the frequency of severe weather events (Godfray & Garnett 2014). While this may benefit food production in some instances, reduced net yield production is generally predicted (Parry et al. 2009; Knox et al. 2012). Climate change in conjunction with a growing human population, will also increase the demand for goods and services which require land (Lambin & Meyfroidt 2011; Godfray & Garnett 2014). Land use competition is already occurring between food production and biofuel production (Littlejohn et al. 2015). This competition impedes first generation biofuel production and contributes to the current dependency on fossil fuels such as oil, coal and gas which are finite resources and promote climate change (de Schutter 2010).

1.2 Possible solutions

The resulting loss of biodiversity due to the global challenges discussed has caused simplification of ecological food webs with fewer ecosystem services and more ecosystem dis-services (Tscharntke et al. 2005; Cardinale et al. 2012; Gurr et al. 2012). Ecosystem services (ES) are benefits delivered by natural functions and processes (the latter are ecosystem functions) that have value to humanity such as biological control. Conversely, ecosystem dis-service (EDS) are the negative effects on humanity from natural functions and processes and the interactions between these. It is estimated that the human population will reach 9 billion people by 2050 (Sandhu et al. 2015). Providing food and renewable fuel security for this growing population requires sustainable intensification. This involves maintaining or increasing agricultural production by using agroecology and other sustainable methods, primarily by enhancing ES while reducing EDS and damaging anthropogenic inputs (de Schutter 2010; Tilman et al. 2011; Godfray et al. 2014; Sandhu et al. 2015).

1.3 Sustainable intensification: Agroecology in action

There is increasing evidence for the potential value of sustainable intensification by practising agroecology, which can increase net yields in agriculture (de Schutter 2010; Pywell et al. 2015). These methods maintain or increase functional agricultural biodiversity (de Schutter 2010; Gurr et al. 2012) and resources such as nutrients and water (de Schutter 2010). In a review of 40 sustainable intensification projects in 20 African countries, crop yields had a mean increase of 213 % within 3 - 10 years, involving 10.39 million farmers and 12.75 million ha, resulting in a food production increase of 5.79 million tonnes per annum (Pretty et al. 2011). In Kenya, agroecology 'push-pull' strategies are used to manage insect pests. This has doubled maize and milk yields while improving the soil (Khan et al. 2011). These strategies involve crops, for instance maize, that are inter-planted with insect repellent plants such as *Desmodium* spp. which 'push' the insect pests away. These insect pests are also 'pulled' towards trap plants that excrete sticky gum which attracts and traps the insect pests (Khan et al., 2011).

Another method to increase the functional biodiversity of agricultural systems is to insert non-crop vegetation such as refugia and shelterbelts (Landis et al. 2000; Gurr et al. 2012) enhance ES such as biological control (MacLeod et al. 2004; Tschumi et al. 2015). Such ES have been damaged by biodiversity loss and food web simplification in most conventional agricultural systems worldwide (Gurr et al. 2012). 'Beetle banks' have been used to provide overwintering refugia for beneficial polyphagous predators such as spiders and predatory beetles in arable land (MacLeod et al. 2004). Collins et al. (2002) showed 'beetle banks' can reduce aphid pests by 34 %. 'Beetle banks' consist of raised grassy strips of tussock grasses such as *Dactylis glomerata* (L.) where polyphagous predators overwinter and then migrate into the field during spring (MacLeod et al. 2004). 'Beetle banks' also provide ES other than enhanced biological control by increasing agricultural functional biodiversity, such as habitat for taxa of conservation interest (Thomas et al. 2000; Thomas et al. 2001; Gurr et al. 2003; Gurr et al. 2012). 'Beetle banks' are an example of an agroecology method that is multifunctional, which has led to widespread use in Europe (Gurr et al. 2003; Hajek 2004). Agroecology methods need to provide multiple ES simultaneously with clear financial advantages for widespread uptake by farmers to occur. One such recently developed method is *Miscanthus* x *giganteus* (Mxg) (Greef et Deu) biofuel grass providing at least 15 ES when used as shelterbelts on farms with centre pivot irrigator systems (Littlejohn et al. 2015).

Additionally, sustainable intensification using agroecology also creates a cushioning effect against climate change (de Schutter 2010). This is illustrated by reduced yield loss (Eyhorn et al. 2007; Akinnifesi et al. 2010) and reduced top soil loss (Holt-Giménez 2002) during extreme weather events when agroecology methods are implemented (de Schutter 2010). Furthermore, agroecology can reduce the reliance on fossil fuels by producing more biomass and soil organic matter as carbon sinks (de Schutter 2010). An additional benefit of agroecology is that sustainable biofuel use and production can be incorporated into farm management (Littlejohn et al. 2015). The benefits of using sustainable fuel is becoming recognised with US$ 101 billion of global subsidies for renewable energy production (International Energy Agency 2013) such as second generation biofuels like Mxg (Littlejohn et al. 2015).

1.4 *Miscanthus* x *giganteus* (Mxg) shelterbelts

Mxg is a perennial C4, 4 m high, sterile hybrid grass from East Asia that is predominantly used as biofuel or as feedstock in Europe and the USA where it is grown as a first generation biofuel crop that competes for land with food production (Littlejohn et al. 2015). Conversely, in Canterbury, New Zealand, Mxg shelterbelts have been developed on dairy farms, where woody shelterbelts had been previously removed due to the 2 m high clearance required for centre pivot irrigator systems (Littlejohn et al. 2015). Mxg allows the centre pivot irrigator to pass through it without any resistance or damage to the plants. When used as shelterbelts, Mxg biofuel production can occur in conjunction with food

production, therefore biofuel and food production do not need to compete for land (Littlejohn et al. 2015). Furthermore, Mxg shelterbelts around the field edges are unlikely to reduce the overall yield of the field by occupying land. This is because field edges can have at least 38 % lower crop yields than the field centre (Pywell et al. 2015). Shelter and biofuel biomass are only two of the multiple ES that Mxg provides to improve sustainable intensification. These include an 18 % increase in pasture production and increases in farm biodiversity by providing refuges for bumblebees (*Bombus* spp.) and endemic skinks (*Oligosoma* spp.) (Littlejohn et al. 2015). When grown as shelterbelts with centre pivot irrigator systems, 3 – 10 year old Mxg can produce annual yields of 30 t ha-1 yr-1. If used for biofuel, this produces 9000 L of biodiesel. At a replacement cost of US$ 0.89/L, this is equivalent to US$ 8053 ha-1 yr-1 and would be carbon neutral (Littlejohn et al. 2015). Biofuels are categorised by their energy efficiency or net energy ratio (the amount of energy gained/ha) and whether they are also food crops or grown on food producing land. First generation biofuels are grown on food producing land and are also used for food production such as maize (Cobuloglu & Büyüktahtakın 2015; Littlejohn et al. 2015). Second generation biofuels have higher net energy gain and provide ES to the farm environment, but are still predominantly used in conventional crop fields and thereafter compete with food production for land (Cobuloglu & Büyüktahtakın 2015). Mxg is second generation biofuel that has a net energy ratio of 20:1, and is extremely efficient compared to the most other biofuel feedstocks which are commonly used (Littlejohn et al. 2015). For instance sugarcane is the most efficient first generation biofuel which has energy ratios of 3.1 - 9.3:1 (Gasparatos et al. 2013). If Mxg were to be grown worldwide as shelterbelts rather than broad scale in fields, then based on the Littlejohn et al. (2015) findings, global food production could be maintained or increased while drastically increasing biofuel production and greatly reducing the dependency on fossil fuels in the process.

Despite the obvious potential of Mxg to contribute to 'future of farming', it needs to recognised that it could also be susceptible to pests and diseases as Mxg shelterbelts occupy larger areas and from continuous invasion of new organisms due to increased international trade and travel (Goldson et al. 2014; Hosokawa et al. 2014). In this context, in North America and Europe, this plant is attacked by a range of invertebrate herbivores, most of which are serious pests of maize (Spencer & Raghu 2009; Gloyna et al. 2011; Nabity et al. 2011; Prasifka et al. 2012; Ameline et al. 2015). These include the western corn rootworm beetle (*Diabrotica virgifera virgifera* LeConte), which oviposits at the base of Mxg plants (Spencer & Raghu 2009; Gloyna et al. 2011). It is of future importance to anticipate such potential problems elsewhere in the world and to conduct research on non-pesticide approaches to manage such pests in the future. These approaches should ideally not inhibit the ES that Mxg shelterbelts provide to the wider farm environment such as contributing to biodiversity restoration. For example, the European model of 'beetle banks' could be helpful in this context by providing refugia for BCA, which emigrate into the fields in spring (Thomas et al. 1991). This may occur in Mxg

shelterbelts as well. However, even if the BCA do not emigrate from Mxg, they may have a key role in reducing potential pest populations within the Mxg shelterbelts themselves. However, little work to date has been conducted on how Mxg can contribute to insect pest management.

1.5 *Miscanthus* x *giganteus* (Mxg): potential contribution to pest management

As illustrated in Section 1.1., insect pest management is a growing challenge, despite the development of alternative agro-ecological methods (see Section 1.3). This is because farmers either are not aware of the benefits of agroecology methods such as conservation biological control or believe that the initial risk or costs are too high, when they can use the easy alternative of chemical pest management which delivers fast short term results that are obvious, despite farmers' knowing the negative consequences (van Lenteren 2012; Gurr et al. 2012; Forbes et al. 2013). Conservation biological control (CBC), which comprises of better use of existing natural enemies by habitat manipulation and reducing their mortality from pesticides, when managed properly, is often more cost effective than chemical pest management and can be used in integrated pest management (Geiger et al. 2010; Schmitz & Barton 2014; Ottaviano et al. 2015). Furthermore, once set up there are few ongoing costs and the protocols can be tailored to a specific farm or field (Gurr et al. 2012; Tschumi et al. 2015). If Mxg shelterbelts could provide CBC as an ES by providing refugia for BCA, then CBC could be more readily incorporated into farm pest management, reduce pesticide use and add value to the use of Mxg shelterbelts. This is because there is already much potential for these shelterbelts to be used in agriculture worldwide because they can already provide 15 ES, which can reduce on and off farm costs, increase food production and provide income from biofuel (Littlejohn et al. 2015). CBC would be an additional ES that has no further cost associated with it and may reduce pesticide use while restoring agricultural biodiversity and food webs which have been degraded (Geiger et al. 2010; Gurr et al. 2012). This in turn, may reduce pest resistance, negative environment impacts and health hazards associated with pesticide use.

Currently, there has been little research on potential BCA using Mxg as refuges and whether there is potential for CBC enhancement using Mxg. In England, Semere and Slater (2007) surveyed predatory beetles using pitfall traps and arboreal invertebrates using a beating tray in Mxg fields in the summers of 2002-2004. Trapped polyphagous predatory beetles consisted mainly of Carabidae (83 %) (ground beetles) and Staphylinidae (10 %) (rove beetles). Polyphagous predatory arboreal invertebrates included Neuroptera (lacewings), Coccinellidae (ladybirds) and Carabidae (Semere & Slater 2007). This confirmed that known BCA do use Mxg but few taxa were identified to species or genus and this study did not investigate the potential of these potential BCA to control pests. This lack of research leaves large knowledge gaps involving CBC and Mxg that can be further investigated.

1.6 Determining the potential of biological control agents

Determining the potential of BCA can be achieved using video cameras and facsimile prey to represent pests (Merfield et al. 2004; Frank et al. 2007; Sandhu et al. 2010; Sandhu et al. 2015). Sandhu et al., (2010; 2015) used live pea aphids (*Acyrthosiphon pisum* Harris) and frozen blowfly (*Calliphora vicina* R.D.) eggs to determine the economic value of BCA in conventional and organic fields. Merfield et al. (2004) used a combination of blowfly (*Calliphora stygia* F.) eggs and video cameras to measure not only how effective BCA were but also to determine what BCA were contributing to prey consumption in field margins. They found that Acari (mites) and Formicidae (ants) were the most effective BCA of this prey type. Other BCA such as Araneae (spiders) and Opiliones (harvestmen) were also involved in removing the egg prey. Molecular methods involving degraded DNA from gut contents are also becoming common for confirming pest predation (Monzó et al. 2010; Harwood & Obrycki 2013; Varennes et al. 2014; Pérez-Sayas et al. 2015). Taxon specific primers have been used to detect predation of pests such as fruit fly (Monzó et al. 2010), aphids (Harwood & Obrycki 2013) and mites (Pérez-Sayas et al. 2015). Specific primers are useful because they enable fast DNA indentifiaction using Sanger sequencing which is generally more user-friendly and faster than next generation sequencing.

1.7 Project aims and research questions

The aims of this project were to provide an indication of the richness of potential BCA using Mxg shelterbelts as refugia, whether they could contribute to CBC and whether these aspects differed between the field margin and Mxg shelterbelts. This research is a world first because, these aspects have never been investigated before in an Mxg shelterbelt context.

The following research questions were used to address the overall aims:

1. What soil-surface dwelling polyphagous potential BCA use Mxg shelterbelts as refugia compared to the field margin?

2. How effective are these potential BCA at contributing to CBC?

3. What are the potential BCA that are consuming the live facsimile prey?

Approaches used to answer these research questions comprise a hierarchy ranging from counting and identifying potential BCA at two types of refuge sites, facsimile prey consumption rates, infrared video recording, DNA sequencing and principal component analysis. These protocols are developed, explained and discussed in the Method Section 2 below.

Chapter 2

Methods

2.1 Site & plot selection

A commercial dairy farm on Aylesbury Rd, Canterbury, New Zealand, was chosen as a field site because Mxg shelterbelts were already present there and farm management supported Lincoln University conducting experiments on the land (Fig 2.1). Farm coordinates are 43°32'24.61"S 172°16'43.25"E. The central Canterbury Plains, where the field site is located, has a mean annual rainfall of 648 mm, a mean annual temperature of 12.1 °C and a mean wind speed of 15 km/h (NIWA 2013). The soils are stony silt loam (Chertsey silt loam & Lismore silt loam over alluvial shingle) and have a low water holding capacity (< 80 mm) (Hanson 2009). Three mature Mxg shelterbelts (around 4 m tall) (Fig 2.2) were used in the current study. Each Mxg plot was a northwest facing shelterbelt in an 'L' shape with 40 m X 40 m X 7 m dimensions (Fig 2.1 and 2.2). These were located in the corner of different paddocks and > 120 m away from any other plots. The field margin for the control plots was selected because it was the only field margin on the farm of similar width to the Mxg plots (6.5 m wide), was adjacent to paddocks containing Mxg plots and had reasonably consistent vegetation (Fig 2.1 and 2.3). Field margin vegetation consisted of a mixture of tussocks of perennial grass (cocksfoot, *Dactylis glomerata* L.), Bracken fern, *Pteridium sp.* (cf *P. esculentum* Forster), European broom (*Cytisus scoparius* L.) and scattered gorse (*Ulex europaeus* L.). Three field margin plots were selected due to accessibility, > 120m away from Mxg shelterbelts, had vegetation that was > 0.5 high and had similar vegetation (Fig 2.1 and 2.3).

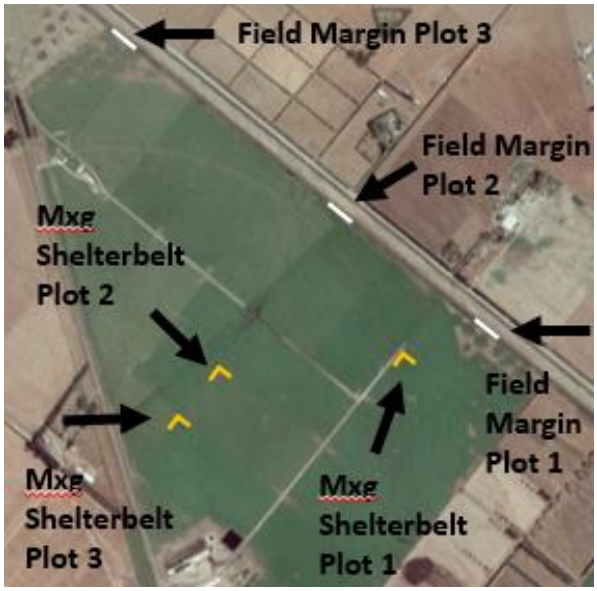

Figure 2.1 Commercial dairy farm field site and plot location. Black arrows point towards labelled *Miscanthus* x *giganteus* (Mxg) (yellow) and field margin (white) study plots. Adapted from Google, CNES/Astrium (2015).

Figure 2.2 *Miscanthus* x *giganteus* (Mxg) shelterbelt plot 3 on a commercial dairy farm, off Aylesbury Rd, Canterbury, New Zealand.

Figure 2.3 Field margin plot 3 on the study farm, off Aylesbury Rd, Canterbury, New Zealand.

2.2 Sample method development

There are many ways to collect potential invertebrate BCA so several sampling techniques which had been used in similar studies were investigated to determine which was the most practical and effective in Mxg shelterbelts and the field margin. All methods investigated were relative sampling methods which allow for site comparisons but are unlikely to represent total species richness or abundance. No sampling method testing occurred in the latter because this has been thoroughly studied in the literature (Thomas et al. 1991, 1992; Landis et al. 2000; MacLeod et al. 2004; Gurr et al. 2012; Bowie et al. 2014)

2.2.1 Beating

Beating foliage and stems above a beating tray had been used by Semere & Slater (2007) in England to determine what arboreal invertebrates occurred in Mxg paddocks in the spring and summer of 2002-2004 compared to field margins and other plant biofuel feedstocks. This method was tested in Mxg shelterbelts at the field site, where three plants were beaten at a 2 m height, every 10 m in each of the three plots on March 21 2015. The potential generalist BCA were collected using an aspirator or 'pooter' (Southwood 1978), then put in labelled plastic tubes and stored in 95 % ethanol. These were later identified to recognisable taxonomic units (RTUs) (Fig 2.4).

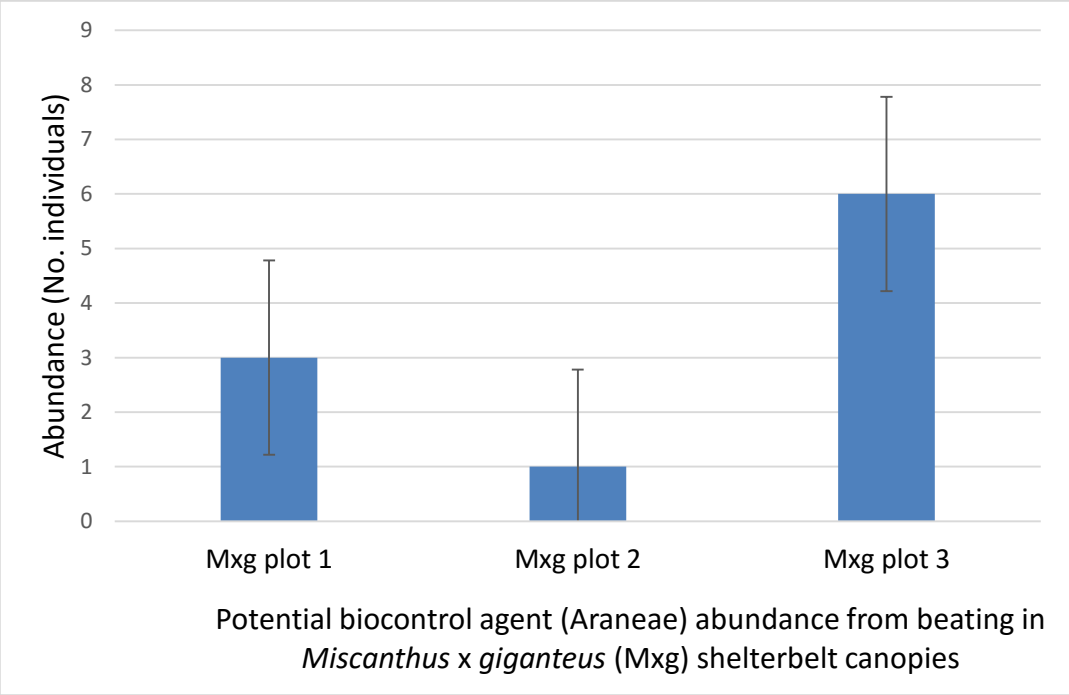

Figure 2.4 Generalist potential biocontrol agent RTUs (Araneae) collected using a beating tray in Mxg shelterbelts on a commercial dairy farm, Canterbury, New Zealand, March 21 2015, (error bars = SEM).

Beating delivered low numbers and diversity of potential BCA in the form of spiders (Araneae) (Fig 2.4). There was also considerably variation between plots (Fig 2.4). Furthermore, this method was considered inadequate because it was difficult to carry out in the dense Mxg canopy, did not work well in wet weather, provides information only on the potential BCA present at the time of collection and would be difficult to replicate in the field margin due to variation in foliage heights.

2.2.2 Suction sampling

This method has been widely used to collect BCA and other invertebrates in field margins and in agricultural fields (Thomas et al. 1991, 1992; McLachlan & Wratten 2003; Greenslade et al. 2013). McLachlan & Wratten (2003) used suction sampling to measure spider diversity and abundance in field

margin shelterbelts and at various distances into pasture paddocks. This method was investigated in Mxg shelterbelt Plot 1 and the adjacent paddock at -3.5 m (centre of the Mxg shelterbelt), 0 m (edge), 0.5 m, 2 m, 5 m, 10 m and 20 m into the paddock on March 19 2015. A Vortis machine (inverted leaf blower) (Macleod et al. 1994) with a 0.05 m2 suction surface area with a removable cup attached, was used for one minute, four times at each distance. Suction positions were at 50 cm intervals at each distance. Invertebrates collected in a cup at each distance were labelled, frozen for 2 h, then sorted and identified to RTUs (Fig 2.5).

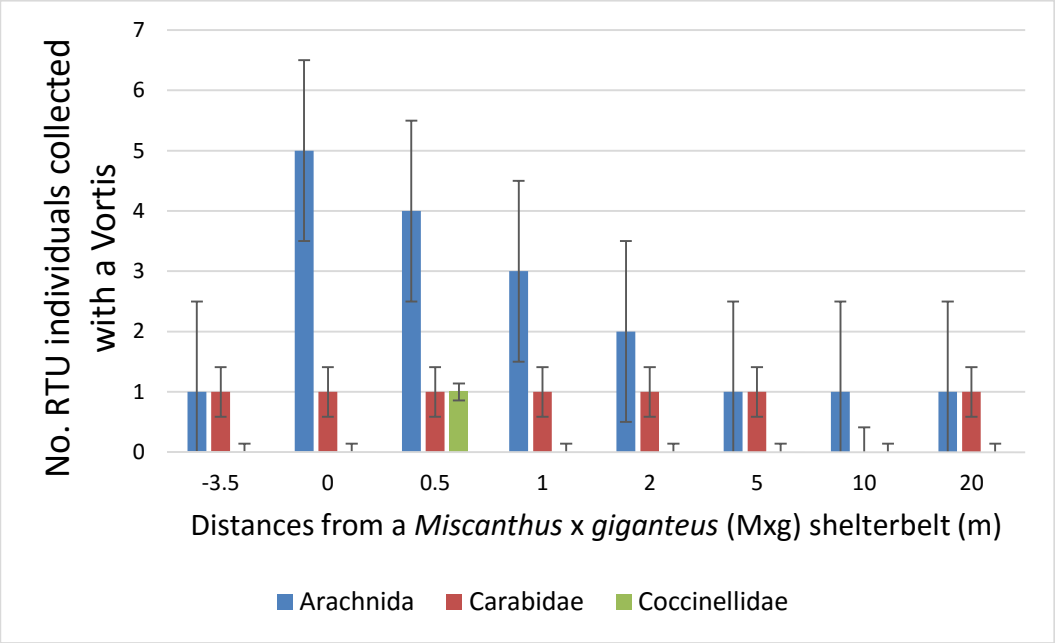

Figure 2.5 Potential biocontrol agent RTUs collected from an Mxg shelterbelt and various distances into a pasture paddock using a Vortis machine on March 19 2015 from a commercial dairy farm, Canterbury, New Zealand, (error bars = SEM).

Suction sampling was slightly more effective than beating for indicating the RTU richness of potential BCA with three RTUs collected (Arachnida, Carabidae and Coccinellidae) (Fig 2.5). This method could be repeated in both the Mxg and field margin plots and allows for the investigation of whether potential BCA migrate from refugia into the paddock. Similarly to beating, however, this method provides information only for the specific time of use. Therefore, in common with many other sampling methods, one-off measurements cannot assess changes in populations and diversity. Another issue with this method is that it can not be used when vegetation is damp, very tall or dense and is therefore inadequate for most days of autumn, winter and spring. Leaf litter sampling using Tulgren funnels was not considered as a viable method to measure potential BCA richness and abundance because there was limited leaf litter in the Mxg shelterbelts and virtually no leaf litter in the field margin and pasture paddocks. This would make replication between the plot types difficult.

2.2.3 Pitfall traps

Pitfall traps are one of the most commonly used methods for collecting soil surface dwelling invertebrates (Southwood 1978; Seldon & Beggs 2010) and have been previously used to determine what beneficial predatory beetles occur in Mxg fields in England (Semere & Slater 2007) and beneficial arthropod abundance in agricultural fields in Canterbury, New Zealand (Greenslade et al., 2013; Bowie et al. 2014). Here, this method was investigated in the context of potential BCA occurring in Mxg shelterbelts in a similar manner to the suction sampling, with pitfall traps at distances of -3.5 m (centre of the Mxg shelterbelt), 0 m (edge of shelterbelt), 0.5 m, and 5 m into the pasture paddock, along a transect from the February 21 - March 7 2015 using Mxg Plot 1. Each pitfall trap consisted of a metal or plastic sleeve inserted into the ground, fitted with an 80 mm diameter removable pitfall cup flush with the soil surface. 100 mL of mono-propylene glycol was added as a preservative with a drop of dishwashing detergent to break the surface tension and a steel roof 1-3 cm above the soil surface was added to prevent access to vertebrates and keep out rain. Collected potential BCA were labelled in vials, stored in 95 % ethanol and later identified to RTUs.

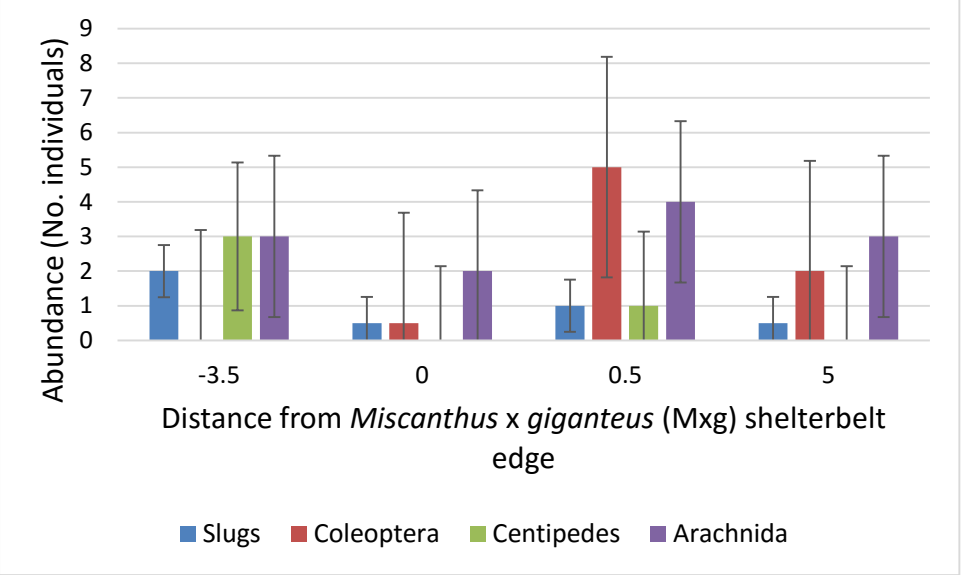

Figure 2.6 Potential biocontrol agent RTUs collected using pitfall traps February 21– March 7 2015 along a transect from a Mxg shelterbelt into a pasture paddock on a commercial dairy farm, Canterbury, New Zealand (error bars = SEM).

Pitfall trapping yielded similar potential biocontrol agent RTU richness to suction sampling with predatory Coleoptera (Carabidae and Staphylinidae), centipedes and Arachnida (Araneae and Acari) been detected. This method was initially very labour intensive but indicated the richness and relative abundance of RTUs for a longer period time, providing a more complete assessment of the presence of potential biocontrol agents than other methods assessed. This method can also has the advantage that it can be used in both Mxg and field margin plots regardless of weather conditions and vegetation

architecture. However, it was realised that determining whether potential biocontrol agents migrate between the Mxg and the field compared to those in the field margin, was beyond the scope of this Honours project, partly because Mxg acting as a refugium in that way is most likely to occur in winter (see Thomas et al. 1991, 1992).

2.2.4 Development of using baited pitfall traps within Mxg shelterbelts and the field margin

Based on the above, it was determined that pitfall traps would be used to investigate what potential generalist biocontrol agents use Mxg shelterbelts compared to the field margin. Furthermore, pitfall traps can be used in conjunction with prey baits that measure the predation rate of generalist invertebrate predators (Seldon & Beggs 2010). Using a facsimile prey to represent an insect pest provides the opportunity to indicate how much potential biocontrol agents contribute to pest predation in Mxg shelterbelts, compared to the field margin. To test baited pitfall traps, nine pitfall traps, 5 m apart were set up using the protocol mentioned above (see 2.2.3) in an 'L' shape in the three Mxg shelterbelt plots and in straight transects in the three field margin plots (Fig 2.7).

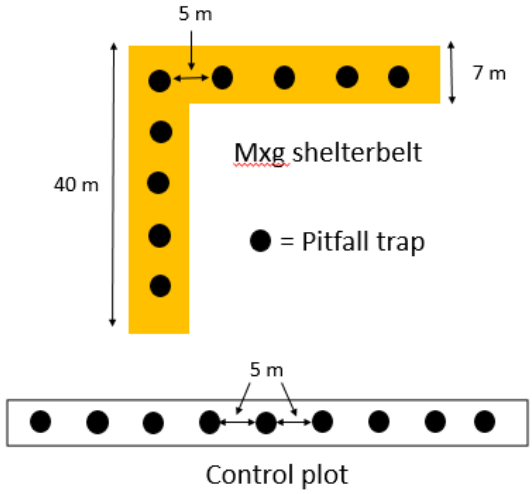

Figure 2.7 Pitfall trap spacing in *Miscanthus* x *giganteus* (Mxg) shelterbelt and field margin plots.

Around each pitfall trap, four facsimile prey batches were positioned in a North, South, East, West manner (Fig 2.8), each consisting of a light brown apple moth (*Epiphyas postvittana* Walker) (LBAM) egg batch containing 50 – 200 eggs and outlined with pencil on wax paper, which were held to the soil surface by wooden cocktail sticks (Fig 2.8). The prey baits and pitfalls were left for three trap nights (17-20 March 2015) before being removed. Trapped potential biocontrol agents were stored at -4 °C in individual labelled vials with 95 % ethanol and identified to RTUs. The facsimile prey consumption rate was determined by eye sight because either all of a facsimile prey was consumed or none of it was.

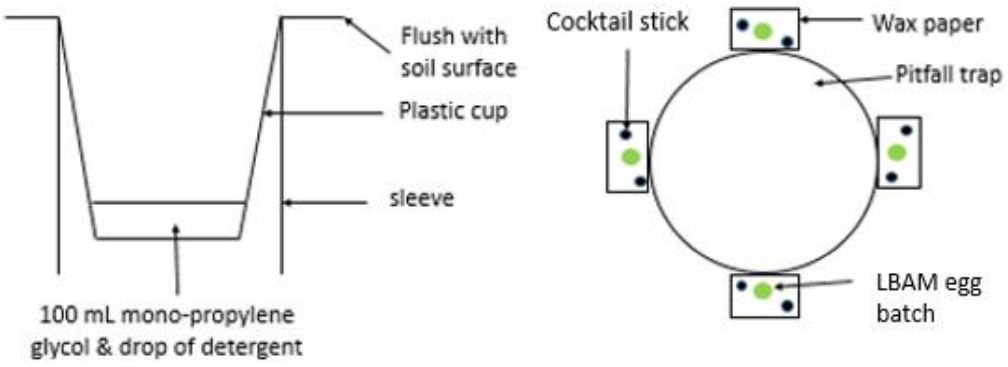

Figure 2.8 Pitfall trap set up with preservative and facsimilie prey (LBAM, *Epiphyas postvittana* egg batches) for baited pitfall sampling of potential surface dwelling biocontrol agents.

Live LBAM egg batches were used as the facsimile prey because they were available all year round from Plant and Food Research, Auckland. They can be stored live at 10 °C for around 3 weeks and take 9 days to hatch at 20 °C, therefore they will not hatch while in the field for 48 hrs. LBAM adults already occur in the field site vicinity, indicated by light trapping in March 2015. In contrast, actively moving pests could not be introduced to the commercial dairy farm as facsimile prey due to the risk of establishment and the difficulty measuring the predation of these pests. In addition, LBAM larvae do not feed on Mxg. This was tested by placing egg batches and first larvae on young Mxg plants (around 6 months old) within mesh enclosures and on artificial diet (Singh 1984) as a control in controlled temperature (CT) rooms with a 16:8 hr light dark cycle at 20 °C for 2 weeks. No LBAM larvae survived on the Mxg and there was no indication that feeding occurred.

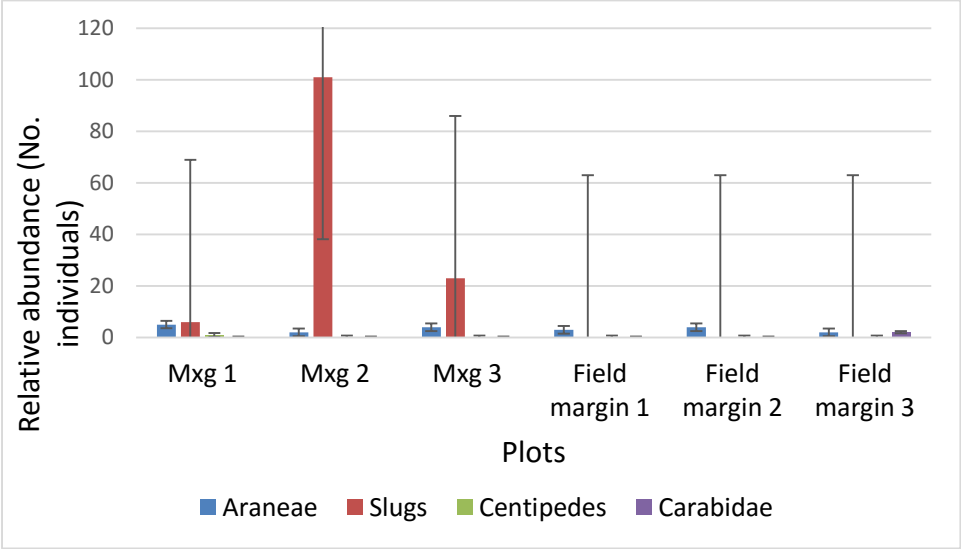

Figure 2.9 Relative abundance of potential biocontrol agent RTUs within Mxg and field margin plots, caught in pitfall traps baited with LBAM (*Epiphyas postvittana*) egg batches over three trap nights 17-20 March 2015, on a commercial dairy farm, Canterbury, New Zealand (error bars = LSD 5%).

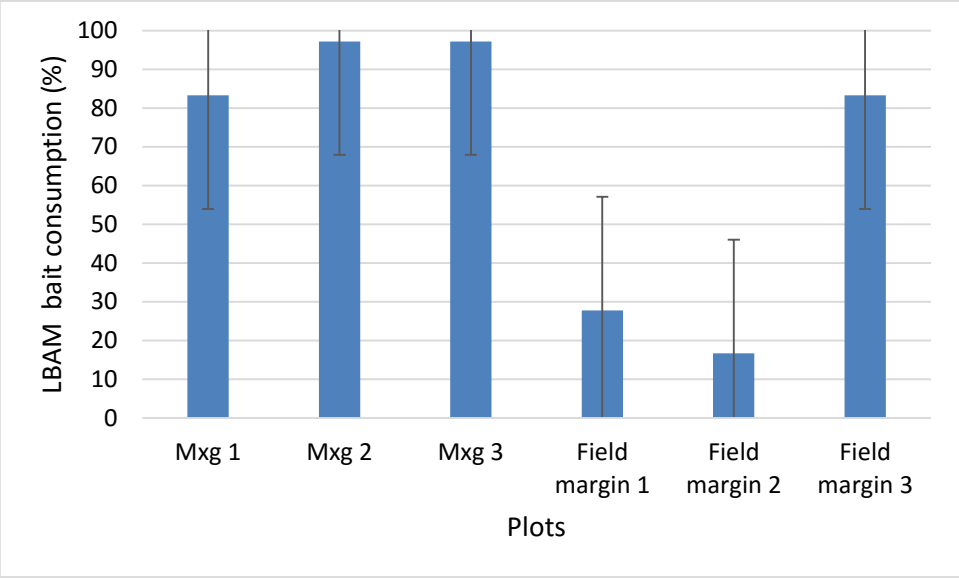

Figure 2.10 LBAM (*Epiphyas postvittana*) consumption around pitfall traps by potential generalist biocontrol agents within Mxg and field margin plots over three trap nights 17-20 March 2015, on a commercial dairy farm, Canterbury, New Zealand (error bars = LSD 5%).

Pitfall trapping with facsimile prey for three trap nights caught a similar RTU richness of potential biocontrol agents as non-baited traps for 14 trap nights and provided an indication of predatory activity (Fig 2.9 and 2.10). However, there was a large variation in relative abundance and prey consumption rate between plots and within plot type (Fig 2.9 and 2.10). This variation may be due to plots having different microclimates within them, the location of the different plots, small plot sample size and lack of replication in this trial. Increasing the plot sample size to reduce the potential effect of different microclimates and plot position was not possible because there were no other mature Mxg shelterbelts available. However increased replication is possible and may reduce the variation found within plot type (Fig 2.9 and 2.10). Setting up and collecting all the pitfall traps in all the plots at the same time was highly labour intensive and would not be achievable on a regular basis. Measuring all the plots at the same time was also risky. This is because environmental conditions such as temperature and moisture are likely to strongly influence the activity of potential BCA and these conditions are highly variable.

Potential BCA may search for prey on the stems and foliage of Mxg and in the field margin, therefore prey consumption was investigated on the stems of Mxg plants at the field site at 0 m, 0.1 m, 0.05 m and 1 m heights up the Mxg plant. This was achieved by gluing the wax paper containing the LBAM egg batches onto the stems with spray-on ADOS adhesive glue and left for three nights (19-22 March 2015). However, there was no LBAM consumption at any stem height. This and the inconclusive results from

the beating investigation (Fig 2.4) indicated that soil surface potential BCA predominantly use Mxg shelterbelts as refugia.

2.3 Developing video techniques to investigate facsimile prey consumption

Infrared video cameras have been used to determine what BCA are consuming pests using prey in previous research (Merfield et al. 2004; Frank et al. 2007). This method was investigated in Mxg shelterbelts at the field site using Sanyo HD4600 CCTV cameras with external IR illuminators, on tripods, with a battery pack containing four 12 V batteries. Two of these cameras continuously recorded activity around LBAM prey on wax paper held to the soil surface by tooth picks (Fig 2.8), for 13 hrs (1700-0600), one in Mxg Plot 2 and one in Mxg Plot 3 on the night of March 19 2015. The resulting images indicated that slugs can consume the facsimile prey, either by scraping the wax paper clean with their radulae or removing part of the wax paper, leaving a hole where the LBAM batch once was. These videos also suggested that mice could occasionally be implicated in consuming LBAM prey as one was recorded on one occasion. However prey consumption by mice could be easily determined by the LBAM prey and cocktail sticks being removed or broken up. Furthermore, European harvestmen (*Phalangium opilio* L.) investigation of the LBAM prey was observed, although no prey consumption occurred during this recording period.

2.4 Determining facsimile prey consumption by potential biocontrol agents under lab conditions

LBAM prey consumption by *P. opilio* which were observed using cameras and Lycosidae spiders which were commonly caught in the pitfall traps were investigated under laboratory conditions using four infrared cameras (see Section 2.3). Ten individuals of *P. opilio* (3 - 5 mm body length) and Lycosidae spiders (5 - 10 mm body length) were collected alive in empty pitfall traps that were checked daily, and were starved in individual containers with damp tissue paper for 4 days at 10 °C. *P. opilio* individuals were then placed in 85 mm diameter X 25 mm high Petri dishes, each with a single LBAM egg batch outlined with a pencil. This also occurred with the Lycosidae spiders but with 85 mm diameter X 15 mm high Petri dishes. These dishes and four control ones containing only outlined LBAM prey were left in a CT room at 15 °C with a 16:8 hr light/dark cycle for 48 hrs. LBAM prey batches were outlined in pencil to indicate where they occurred on the wax paper after prey consumption occurred. The area of these prey batches was also measured prior to use at 12.5 X magnification with an Olympus SC100 camera attached to an Olympus SZX12 stereo microscope to accurately measure partial prey consumption (Fig 2.11). There was 100 % LBAM prey consumption by *P. opilio* indicating that they could consume LBAM prey in the field. *P. opilio* predation indications were either complete removal of the LBAM prey from the wax paper or remaining remnants of damaged transparent egg cases. There was no LBAM prey consumption by Lycosidae spiders which is not surprising considering they hunt

moving prey. These factors suggest that spiders in this family do not contribute to LBAM prey consumption in the field.

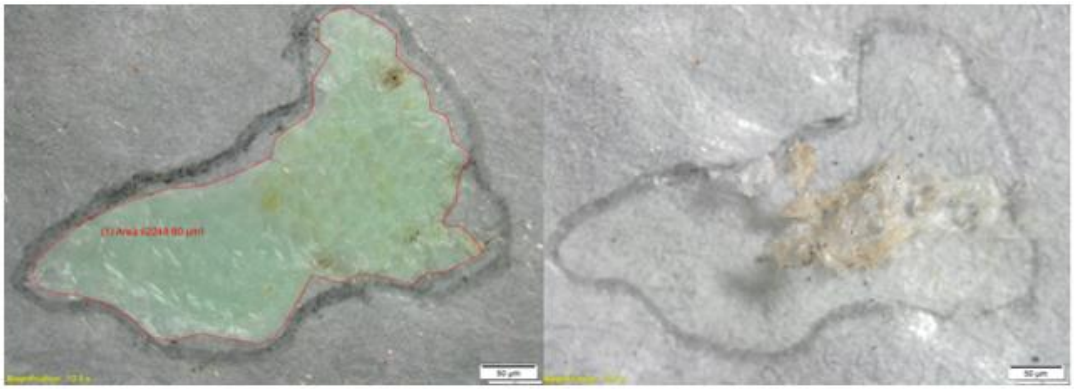

Figure 2.11 Before and after pencil outlined prey consumption by a starved European harvestman under lab conditions after 48 hrs. Photos taken at 12.5 X magnification, scale length is 50 µm.

To determine if identified specimens of potential BCA collected from pitfall traps from the Mxg shelterbelt and field margin plots had consumed the LBAM prey before becoming trapped, DNA gut content analysis was investigated. This involved the testing of the *Epiphyas*-specific primer set EPOS3 (EPOS3-F: 5'-AGCAGGTATAGTAGGAACATCCC-3', 23 base pairs, EPOS3-R: 5'-AAACTGTTCATCCTGTACCAGCT-3', 23 base pairs) developed by L. Ward at Plant and Food Research, Auckland. These primers amplify a 311 base pair sequence of the mitochondrial CO1 gene. LBAM is the only Epiphyas species to currently occur in New Zealand. However, there are many other Tortricidae genera that do occur here. Therefore, this primer was tested for positive DNA amplification on related species and available out-group taxa (Table 2.1). These other moths were collected using a light trap at the field site during March 2015. These and other out-group taxa had their DNA extracted, amplified and run through gel electrophoresis (Table 2.1) to test for any amplification of false positive results using the protocols below.

2.4.1 DNA extractions and amplification

A Zymo Research Tissue and Insect DNA miniprep kit was used for DNA extractions, following the Varennes et al. (2014) DNA extraction protocol modifications. The following Polymerase Chain Reaction (PCR) protocol was used per extracted DNA template; 12.5 µL of GoTaq Green Master Mix 2X; 1 µL of EPOS3-F primer; 1 µL of EPOS3-R primer; 1 µL of Bovine Serum Albumin (BSA); 6.5 µL autoclaved PCR water; 3 µL of extracted DNA template. The DNA template in these 25 µL PCR products was then amplified in the PCR machine with the following settings; one cycle at 94 °C for 2 minutes; 40 cycles of 94 °C for 45 seconds, 58 °C for 45 seconds, 72 °C for 2 minutes; 1 cycle of 72 °C for 7

minutes then 4 °C until PCR products are removed. PCR products were then put through gel electrophoresis to determine if any DNA had been amplified. Electrophoresis consisted of molecular grade agarose (1.5 % solution) dissolved in SYBR Safe DNA Gel stain to form a gel, using combs to form wells. 5 µL of each PCR product was inserted into individual wells. Gel electrophoresis ran at 100 V for 30 minutes.

Table 2.1 Taxa used to test the Epiphyas specific EPOS3 primers with gel electrophoresis results.

Species	Family	Higher taxa rank	Identified by:	Positive DNA amplification
***Epiphyas postvittana* (LBAM)**	Tortricidae	Lepidoptera	Morgan Shields	Yes
Merophyas sp.	Tortricidae	Lepidoptera	Brian Patrick	No
Merophyas leucaniana	Tortricidae	Lepidoptera	Brian Patrick	No
Tmetolophota propria	Tortricidae	Lepidoptera	Brian Patrick	No
Capua semiferana	Tortricidae	Lepidoptera	Brian Patrick	No
Graphania ustistriga	Noctuidae	Lepidoptera	Brian Patrick	No
Graphania morose	Noctuidae	Lepidoptera	Brian Patrick	No
Species unknown	Geometridae	Lepidoptera	Brian Patrick	No
Species unknown	Cambidae	Lepidoptera	Brian Patrick	No
Phalangium opilio	Phalangiidae	Opiliones	Morgan Shields	No
Scaptomyza flava	Drosophilidae	Diptera	Ryan Rayl	Weak band, contamination
Dicyrtoma fusca	Dicyrtomidae	Collembola	Penelope Greenslade	No
Lampona cylindrata	Lamponidae	Araneae	Cor Vink	No

The only strong amplification of DNA using EPOS3 primers was from LBAM adults caught at the same time as the other moth species (Table 2.1). No other DNA was amplified except for that of *Scaptomyza flava* (Fallen). Another PCR and gel electrophoresis which only involved *S. flava* DNA was carried out to test for contamination. This confirmed that the *S. flava* DNA extraction itself was contaminated with *Epiphyas* DNA, which could have occurred when all the DNA extractions in Table 2.1 were carried out at the same time. In subsequent trials there was no DNA amplification using EPOS3 primers and DNA extractions involving S. flava (Table 2.2). These results suggest EPOS3 primers have a relatively low risk of false positives.

2.4.2 Primer testing on degraded DNA from Arthropod gut contents

The EPOS3 primers were then tested on the gut DNA of laboratory fed *P. opilio* using the collection and lab feeding protocol in Section 2.4 in a no-choice test with the following modifications: cameras were not used; five *P. opilio* were fed LBAM and five *P. opilio* were each fed ten adults of the leaf miner fly *S. flava* which had been stored in a freezer for 2 h. Individuals that had consumed LBAM prey or *S. flava* were stored in a - 4°C freezer in individually labelled vials with 95 % ethanol. PCR products that produced positive bands in gel electrophoresis were sequenced using Sanger sequencing. Successful DNA sequences were aligned and trimmed using the computer program MEGA 6.06. Sequences were then blasted on the GenBank data base to confirm that LBAM DNA had been sequenced.

Table 2.2 EPOS3 primer trial with degraded LBAM DNA

Lab fed taxa	Visible signs of prey consumption	DNA amplification	*Epiphyas* sequence verification on Genbank?
European harvestman fed with LBAM	Yes, 3 of 5	Yes 2 of 3	Yes, 100 % match
European harvestmen fed with *Scaptomyza flava*	Yes, 5 of 5	No	No

This investigation confirmed that EPOS3 primers can amplify degraded LBAM DNA from the LBAM prey consumed by a potential BCA and determined that the DNA extraction and amplification protocol works for degraded LBAM DNA (Table 2.2).

2.5 Determining what soil-surface dwelling generalist potential biocontrol agents use Mxg shelterbelts as refugia compared to the field margin.

The protocol in the Section 2.2.4 was altered to determine what soil surface dwelling generalist potential BCA use Mxg shelterbelts compared to the field margin. This included the following modifications: seven pitfall traps per plot; each trapping period consisted of two trap nights; when not in use, pitfall traps were covered with a labelled lid to reduce the risk of trapping endemic skinks (*Oligosoma spp.*). Furthermore, one Mxg plot and one field margin plot were selected with a random number generator for each trapping period. The plots previously used in the same month were excluded from random selection for the next trapping period. This allowed all three plots of each type to be randomly chosen and measured within the same month in a completely randomised design. Each trapping period occurred between 3 - 7 days after the previous one within the same month. Trapping months included April (early autumn), August (late winter) and September (early spring). These months were selected based on equipment, prey and time constraints and to determine if there were seasonal differences in potential BCA richness and relative abundance. Emigration out of the Mxg and

field margin plots into the paddocks could not be measured with the above method because the required emigration data were not collected. Pitfall trapped potential BCA were later identified by specialists in their fields (see Acknowledgements).

2.6 Predation rates in the field

LBAM prey were used following the protocol in Section 2.2.4 with the trapping period modifications described in Section 2.5. The consumption percentage of the LBAM facsimile prey was ascertained using the protocol used in Section 2.4 with the following modification: LBAM prey area was not measured before use in the field due to practicality constraints. However, the pencil outline around the LBAM prey was conducted more precisely then previously to provide accurate results.

2.7 Infrared video analysis

The cameras used in Section 2.3 recorded predator activity around the prey used in the protocol of Section 2.7 on the first trap night of each trapping period. Two cameras were used per plot during each trapping period, each recording around a pitfall trap selected by a random number generator for 13 hrs from 1800-0700. Once one pitfall trap had been randomly chosen, those on either side of that trap were excluded from the selection of traps that the second camera could be positioned at within the same plot. This was aimed to reduce any biases associated with the positon of the pitfall traps. The camera batteries were charged for three days prior to field use which limited when trapping periods could occur. Video recordings were stored on a 16 GB SD card then transferred to a computer where they were converted from one minute videos in an M4V file format to a continuous recordings in a WMV file format and analysed. The RTU identification of potential BCA that consumed LBAM prey, the duration, the time of activity and any behaviours involving prey consumption was recorded. Video data were needed to be used in conjunction with pitfall traps and DNA methods because pitfall traps do not work well for some taxa and there were not enough cameras to only use video data to determine which potential BCA use Mxg shelterbelts as refugia compared to the field margin. But video data confirm what BCA RTUs are consuming the facsimile prey if molecular methods do not work. However, only molecular methods can determine what species-level identified individuals have consumed the facsimile prey.

2.8 DNA verification of facsimile prey consumption by *Dicyrtoma fusca* (Collembola)

Dicyrtoma fusca (Lucas) had consistently been observed on LBAM prey within the field margin plots during August and September. Based on this observation, *D. fusca* was tested for LBAM prey consumption following the protocol in Section 2.5.2 with the following modifications: ten 50 mm diameter Petri dishes were used with five *D. fusca* per dish; five dishes contained *D. fusca* with LBAM

prey and five contained ten diamond backed moth (*Plutella xylostella* L.) eggs as a control; each dish contained damp filter paper. This trial was conducted in a 10 °C incubator with a 16:8 hr light/dark cycle.

2.9 DNA analysis of gut contents

A sample of specimens from potential BCA that had been collected during the April, August and September periods was tested for DNA verification of LBAM consumption using the protocols described in Section 2.5.1. The following modification was used: each specimen larger than 5 mm was dissected and the internal organ tissue (it was difficult to selectively remove the gut) removed to be used in the DNA extraction. This reduced the total amount of tissue used, lowered the risk of DNA extraction and PCR inhibitors being present, false positives occurring and contamination. PCR products that produced positive bands in gel electrophoresis were sequenced using Sanger sequencing. Successful sequences were compared to the GenBank data base to confirm that LBAM DNA had been sequenced. This confirmed that the potential BCA that were collected from pitfall traps had consumed the LBAM prey. Araneae (spiders) and most *D. fusca* specimens collected from the pitfall traps were excluded from DNA analysis of gut contents due to funding and time constraints.

2.10 Statistical Analysis

Principal component analysis (PCA) was used to compare RTU community composition between Mxg and field margin plot types on the computer program PC-ord version 6.0 (McCune & Mefford 1999). This analysis was considered appropriate because it allowed the comparison of community comparison between refugia types (Mxg shelterbelt and the field margin) using the abundance matrix for all 20 RTUs in 37 pitfall traps in six plots. Statistical analysis was conducted on total relative abundance of potential BCA RTUs, endeminsm, predation rate (%) and seasonal variation using untransformed data with t-tests on Microsoft Excel 2013. This test was used because there was a small sample size and allowed comparison between refugia type (Mxg shelterbelt and field margin) without pseudo-replication over time. Transformation of data was unnecessary in the current study as it would have little impact on the results based on the data set used (Dave Saville, pers. comm. Saville Statistical Consulting, November 2015). RTUs which could not be classified as endemic, native or exotic due to taxonomic limitations were excluded from endemism analysis. *D. fusca* was also excluded from the relative abundance and endemism analysis due to the proportion of specimens collected from each pitfall trap not being recorded and the uncertainty of whether it is predatory. Error bars in figures represent two standard errors (SE) (one on either side of the mean).

Chapter 3

Results

3.1 Potential biocontrol agent richness, community composition and relative abundance

There were 20 potential BCA RTUs identified (160 individuals) with eight RTUs found only in Mxg shelterbelts (79 individuals) and eight found only in the field margin (19 individuals) from April, August and September trapping periods (Table 3.1). Principal components analysis (PCA) resulted in three components each of which explained greater than 10% of the variance in species composition among pitfall traps; these three components together explained 37.2% of the variance (variance explained: PC1 = 14.5%, PC2 = 12%, PC3 = 10.7%). Plot scores for the first two components showed the greatest distinction between the two refugia types (Fig 3.1); clustering of the pitfall traps in the two refugia types in the PCA diagram shows that the invertebrate composition of Mxg shelterbelts was different to that of the field margin (Fig 3.1). The RTU community composition within Mxg shelterbelts had considerably less variation (one main cluster) than the scatterred RTU community composition of the field margin (Fig 3.1). This was indicated by the pitfall trap and RTU clusters and the length of RTU lines which represent the magnitude of the effect of each species in explaining compositional variation among pitfall traps (Fig 3.1 and 3.2). Different groups of RTUs were observed in different refugia types. For instance, the RTUs of slugs, *P. opilio* and Aleocharinae were observed only in Mxg shelterbelts, whereas most spider RTUs were observed only in the field margin (Fig 3.2).

Statistically, there were no seasonal or within refugia-type differences of potential BCA RTU richness or abundance between Mxg shelterbelt and field margin plots with the exception of the centipede *Lamyctes emarginatus* (Newport). This species was trapped only in Mxg shelterbelts with a mean relative abundance of 1 ± 0 (95 % CI; $t = \infty$, $p = 0.000$, d.f. = 2). The most abundant potential BCA were slugs, particularly *Derocerus reticulatum* (Muller) (53 individuals) and spiders, predominantly *Anoteropsis hilaris* (Koch) (26 individuals): however, there was large variation between plots for almost all RTUs identified with RTU taxonomic groups (slugs, *P. opilio*, centipedes, spiders (Araneae) and predatory beetles) p values > 0.05. Endemism of total potential BCA varied between Mxg shelterbelt and field margin plots (Fig 3.3). Mxg shelterbelts had more native (which are not endemic) potential BCA with a mean of 1 ± 0 (95 % CI; $t = \infty$, $p = 0.000$, d.f. = 2) and higher exotic potential BCA richness with a mean of 4 ± 2.618 (95 % CI; $t = 3.889$, $p = 0.018$, d.f. = 4) than the field margin (Table 3.1 and Fig 3.3). There was no statistical difference between the richness of endemic potential BCA in Mxg shelterbelts and the field margin.

Table 3.1 Species list and endemism of identified potential biocontrol agent RTUs from Mxg shelterbelt and field margin plots, collected from baited pitfall traps during April, August and September 2015. RTUs found only in Mxg shelterbelt plots are in yellow, those found only in the field margin plots are in light blue and those found in both Mxg and field margin plots are in white. Endemic RTUs are in green, native RTUs are in orange and exotic RTUs are in grey.

Taxa	RTUs	Status	Identified by:
Slugs	*Derocerus reticulatum*	Exotic	Michael Wilson (AgResearch)
	Derocerus panormitanum	Exotic	
	Arion hortensis (agg)	Exotic	
	Arion intermedius	Exotic	
European harvestman	*Phalangium opilio*	Exotic	Morgan Shields (Lincoln University)
Spiders (Araneae)	*Anoteropsis hilaris*	Endemic	Vikki Smith (Lincoln University)
	Supunna picta	Exotic	
	Pakeha sp.	Endemic	
	Dolomedes sp.	Endemic	
	Uliodon sp.	Endemic	
	Gnaphosidae	-	
	Araneidae	-	
	Linyphiidae	-	
	Mysmenidae	-	
	Anyphaeindae	Endemic	
	Theridiosomatidae	-	
Centipedes	*Lamyctes emarginatus*	Native	Peter Johns (Canterbury Museum)
	Lithobius microps	Exotic	
Beetles	*Metaglymma moniliferum*	Endemic	Rowan Emberson (Lincoln University)
	Aleocharinae	-	

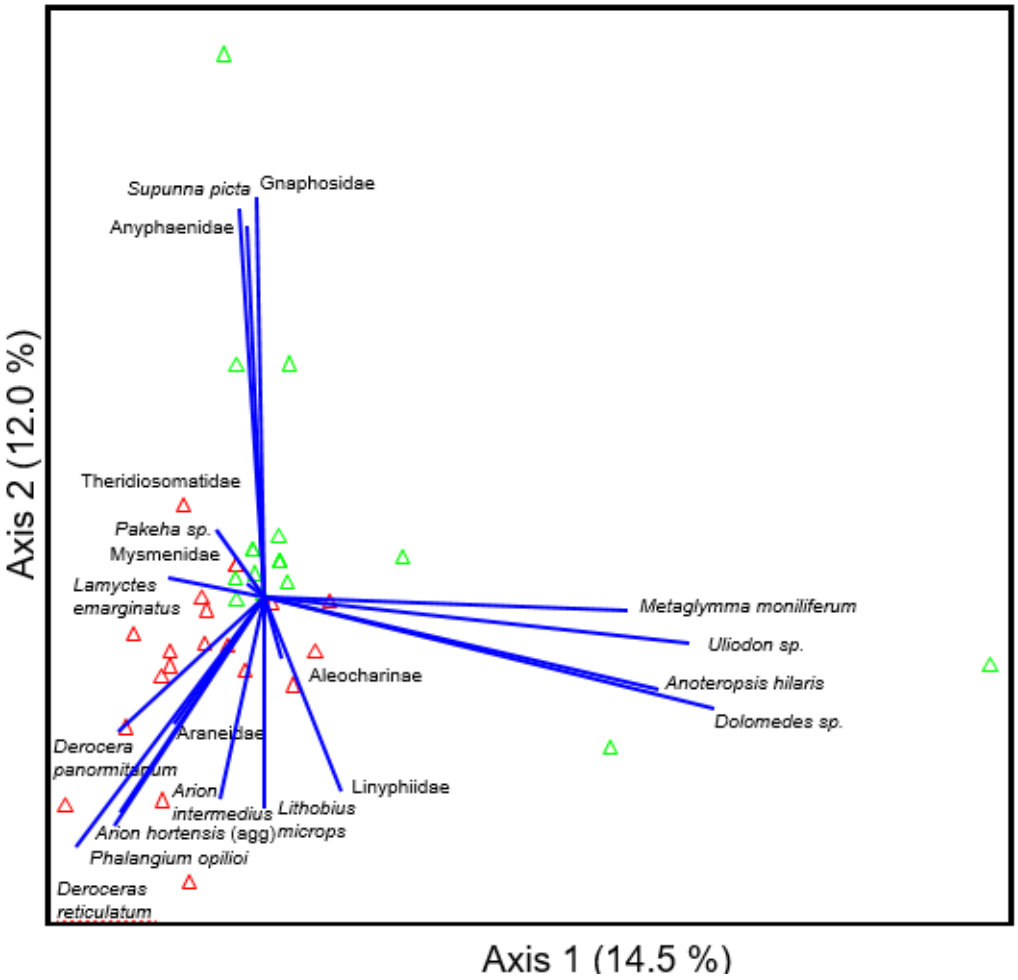

Figure 3.1 Principal component analysis of potential biocontrol RTU community composition between two types of refugia on a commercial dairy farm, Canterbury, New Zealand, 2015. Labelled blue lines represent the effect of each RTU on compositional variation among plots. Triangles represent refugia types: *Miscanthus* x *giganteus* shelterbelts = red ; field margin = green. PC1 (axis 1) and PC2 (axis 2) explained 14.5% and 12.0% variance for 37 pitfall traps.

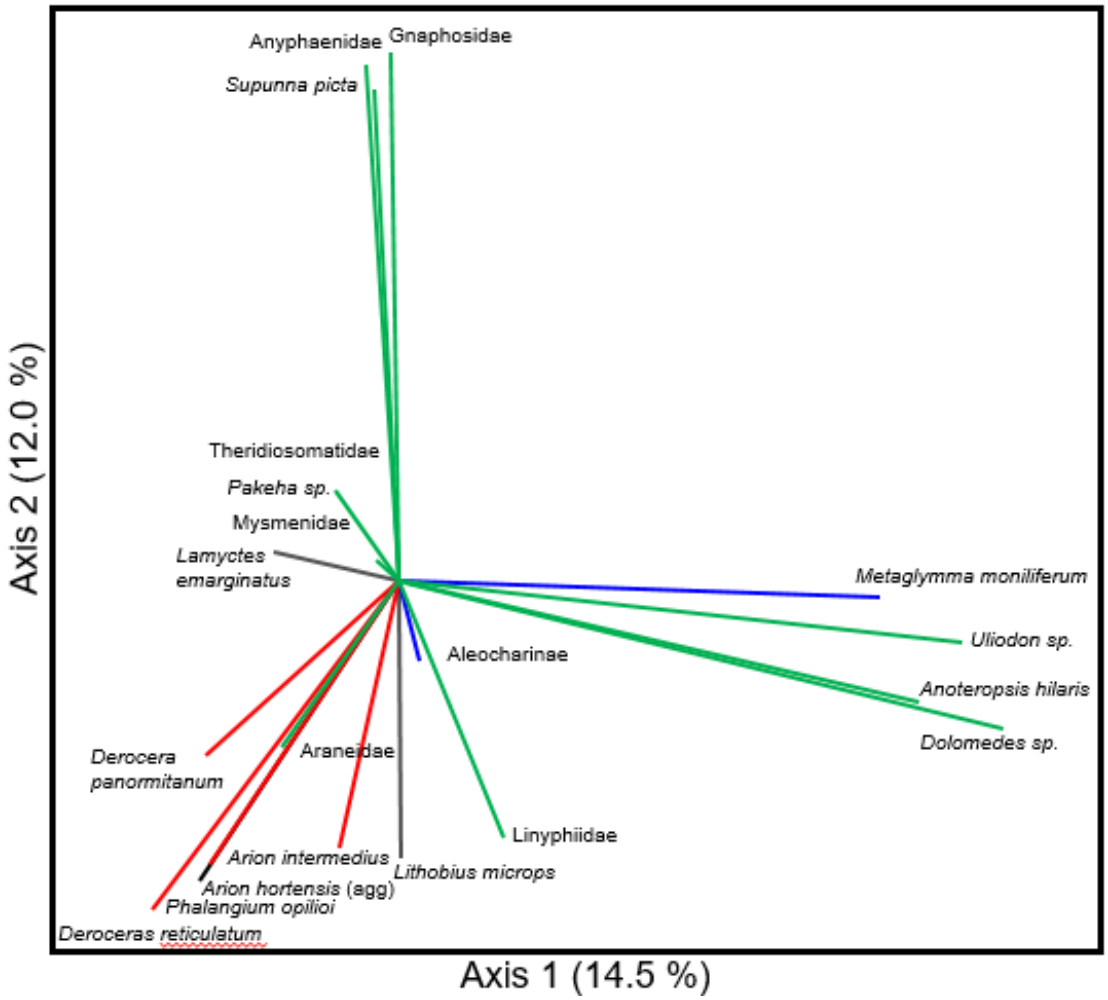

Figure 3.2 Principal component analysis of potential biocontrol RTU community composition between *Miscantus* x *giganteus* shelterbelts and the field margin on a commercial dairy farm, Canterbury, New Zealand, 2015. Labelled lines represent the effect of each RTU on compositional variation among plots and their colours represent taxonomic groupings: red = slug RTUs, black = European harvestman, green = spiders, grey = centipedes, blue = predatory beetles. PC1 (axis 1) and PC2 (axis 2) explained 14.5% and 12.0% variance for 37 pitfall traps.

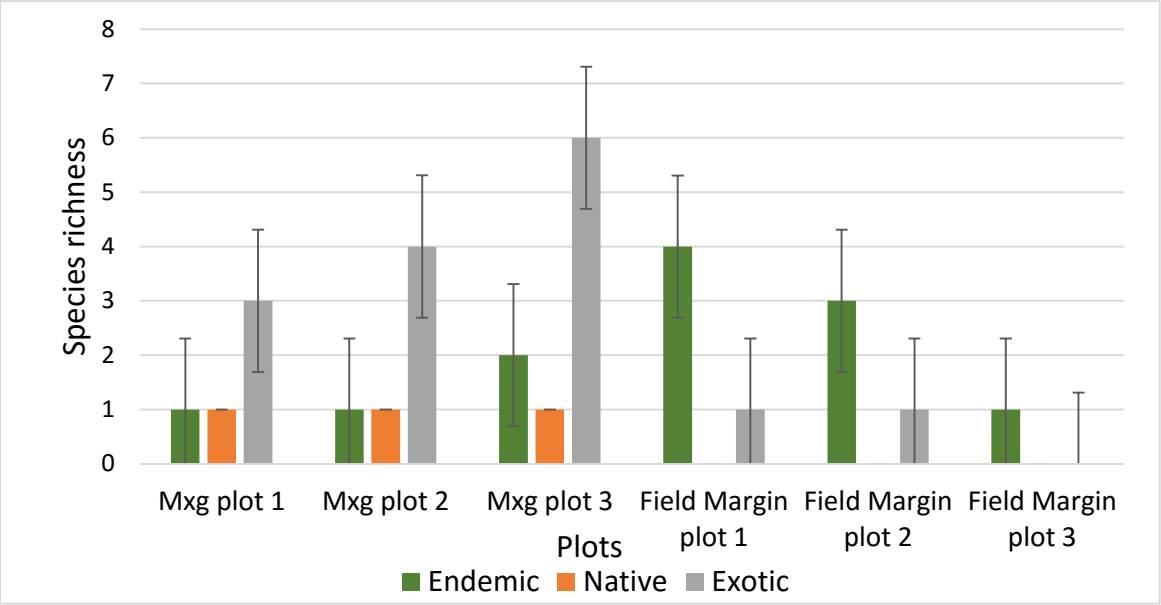

Figure 3.3 Total richness of endemism of potential biocontrol agent RTUs found in *Miscanthus* x *giganteus* (Mxg) shelterbelt and field margin plots on a commercial dairy farm, Canterbury, New Zealand from April, August and September 2015. Error bars are shown as SE. t (native) = ∞, mean 1 ± 0 (95 % CI); p = 0.000: t (exotic) = 3.889, mean 4 ± 2.618 (95 % CI); p = 0.018: t (endemic) = NS.

3.2 Facsimile prey consumption

Total facsimile prey consumption was a mean of 57 % ± 37 % higher (95 % CI; t = 4.240, p = 0.013, d.f. = 4) in the Mxg shelterbelts compared to the field margin (Fig 3.4). There were seasonal differences of consumption between the Mxg shelterbelts and the field margin (Fig 3.4 and Table 3.2). Prey consumption was a mean 58 % ± 38 % higher in April (95 % CI; t = 4.252, p = 0.013, d.f. = 4) and a mean 59 % ± 45 % higher in September (95 % CI; t = 3.655, p = 0.022, d.f. = 4) in the Mxg shelterbelts compared to the field margin (Fig 3.4 and Table 3.2). There was no statistical difference between prey consumption in the Mxg shelterbelts and field margin during August. There were no other seasonal differences of prey consumption within or between the refugia plot types (Mxg shelterbelt and field margin) (Fig 3.4 and Table 3.2).

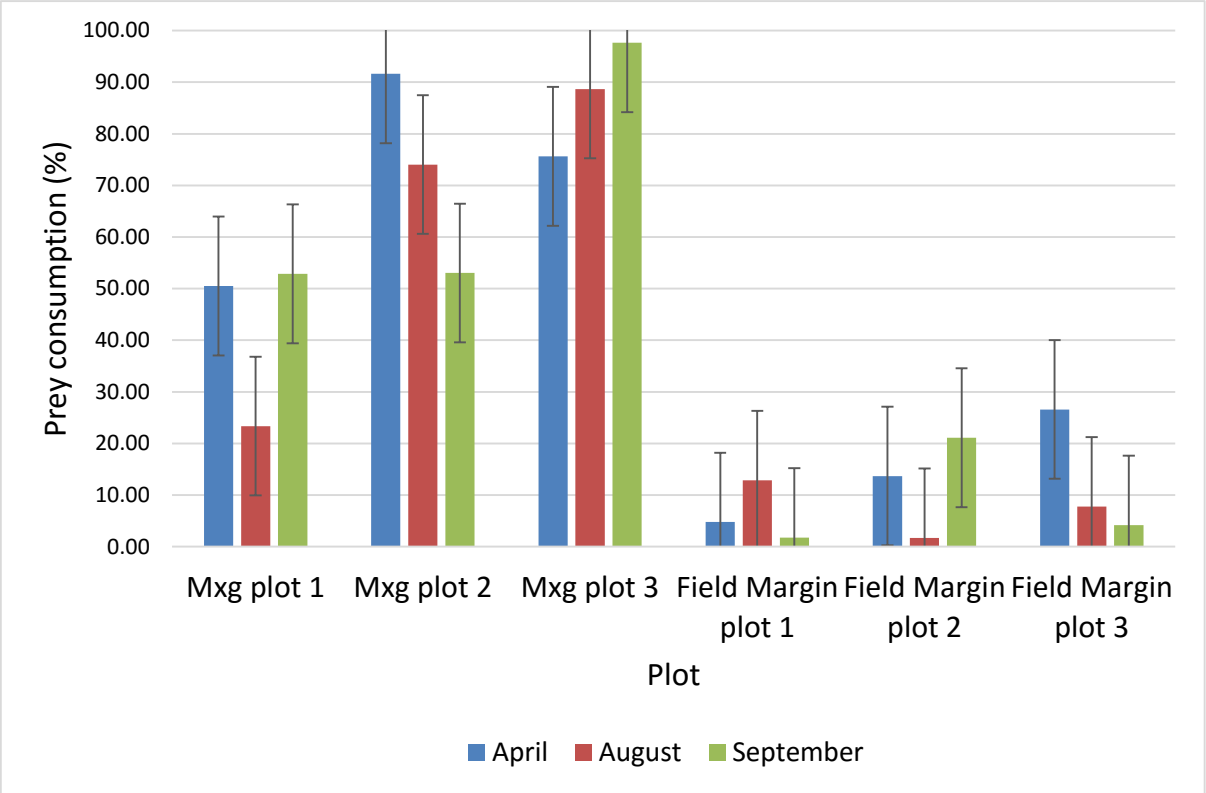

Figure 3.4 Facsimile prey consumption (LBAM) in Mxg shelterbelts compared to the field margin during 2015 on a commercial dairy farm, Canterbury, New Zealand. Error bars are shown as SE. t (total Mxg for all dates) = 4.240, mean 57 % ± 37 % higher (95 % CI); p = 0.013: t (April Mxg) 4.252, mean 58 % ± 38 % higher (95 % CI); p = 0.013: t (August) = 2.722, NS; p = 0.053: t (September Mxg) = 3.655, mean 58 % ± 45 % higher (95 % CI); p = 0.022.

Table 3.2 Predation rate (%) of facsimile prey (live *Epiphyas postvittana* egg batches) in *Miscanthus* x *giganteus* (Mxg) shelterbelts and the field margin on a commercial dairy farm during April, August and September 2015, Canterbury, New Zealand.

Refuge plot	April mean predation rate (%)	April prey predation rate Std (%)	August mean predation rate (%)	August predation rate Std (%)	September mean predation rate (%)	September predation rate Std (%)
Mxg Plot 1	50.51	49.44	23.36	29.38	52.87	29.52
Mxg Plot 2	91.63	24.03	74.03	42.57	53.02	49.01
Mxg Plot 3	75.62	40.89	88.67	28.14	97.66	18.90
Field Margin Plot 1	4.75	11.97	12.85	30.51	1.74	5.25
Field Margin Plot 2	13.68	32.33	1.68	3.28	21.10	41.63
Field Margin Plot 3	26.58	19.32	7.79	20.07	4.17	8.88

3.3 Video analysis of facsimile prey consumption

There were two RTUs that were observed feeding on the facsimile prey around pitfall traps in a total recording time of 60 h 34 mins in Mxg shelterbelts. A total of 17 slug individuals were observed consuming the facsimile prey (seven individuals within Mxg Plot 2 and 10 individuals within Mxg Plot 3) (Fig 3.5). 11 slug individuals were recorded in close proximity to the baited pitfall traps but they did not consume the prey. A total of four *P. opilio* individuals were also observed consuming the prey (three individuals in Mxg Plot 2 and one individual in Mxg Plot 3) (Fig 3.6). There were five *P. opilio* individuals which were recorded within close proximity to the baited pitfall traps but they did not consume the facsimilie prey. No prey consumption was observed in the field margin in a total recording time of 40 h 29 mins.

Behavioural interactions between slugs and *P. opilio* were observed. *P. opilio* individuals were observed deterring slugs feeding on the facsimilie prey on two occasions by what apears to be prodding of the slug with its legs and mouthparts until the slug moved on, after which *P. opilio* fed on the remaining LBAM prey (Fig 3.7). This behaviour occurred over 43.24 minutes at 18:57-19:40 pm and 50.35 minutes at 22:27-23:18 pm. The opposite behaviour was also observed on one occasion where a slug deterred a *P. opilio* individual that was consuming some LBAM prey by sneaking underneath the *P. opilio*. This covered the prey with the slug's body which prevented *P. opilio* from feeding, the latter

then moved away. This behaviour's duration was 12.42 mins at 19:07-19:20 pm. Almost all recording was limited to 1800 - 2400 due to technical issues and both slugs and *P. opilio* were active in Mxg shelterbelts during this time.

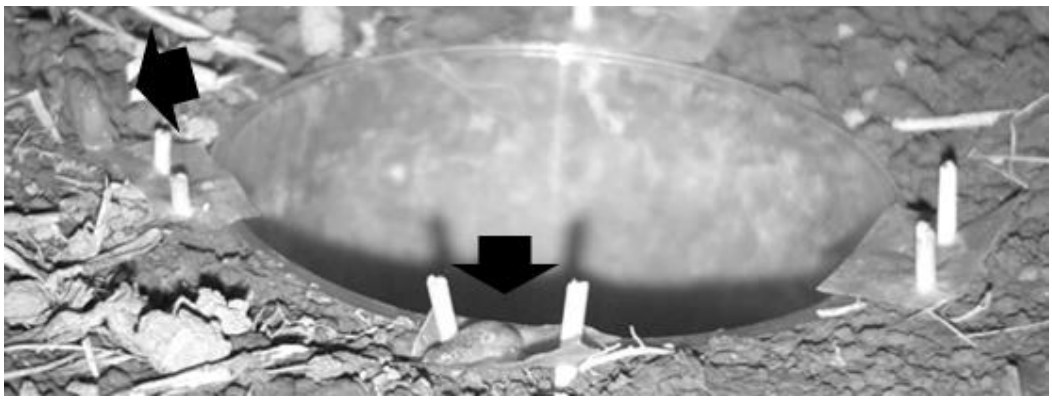

Figure 3.5 Image taken from infrared video recordings of a baited pitfall trap in a *Miscanthus* x *giganteus* (Mxg) shelterbelt on a commercial dairy farm, Canterbury, New Zealand, 2015. The black arrows indicate slugs feeding or about to feed on a facsimile prey (LBAM, *Epiphyas postvittana*).

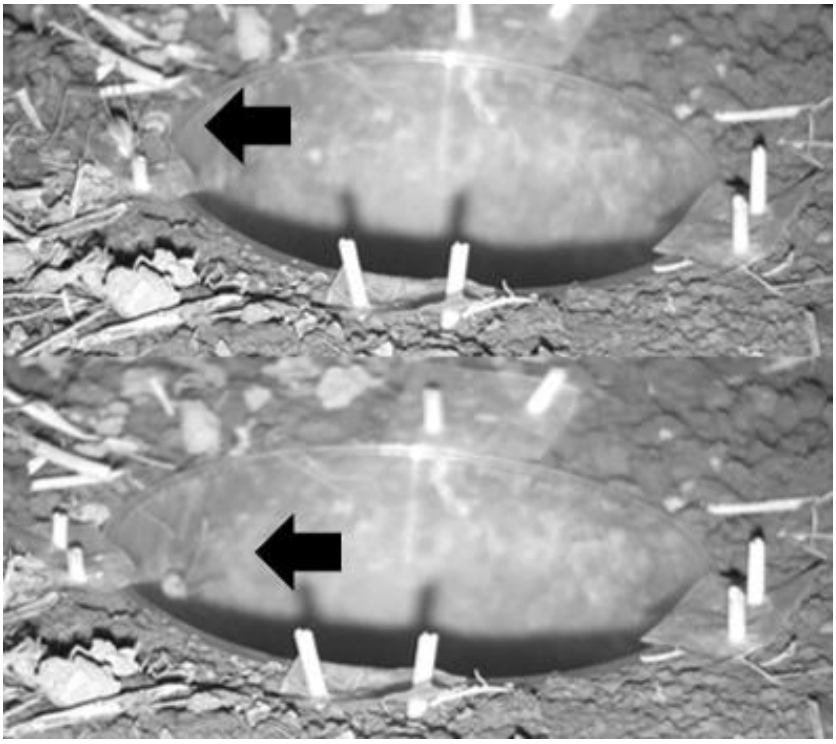

Figure 3.6 Images taken from infrared video recordings of a baited pitfall trap in a *Miscanthus* x *giganteus* (Mxg) shelterbelt on a commercial dairy farm, Canterbury, New Zealand, 2015. Black arrows indicate a European harvestman (*Phalangium opilio*) feeding on a facsimile prey (LBAM, *Epiphyas postvittana*) (top) then falling into the pitfall trap (bottom).

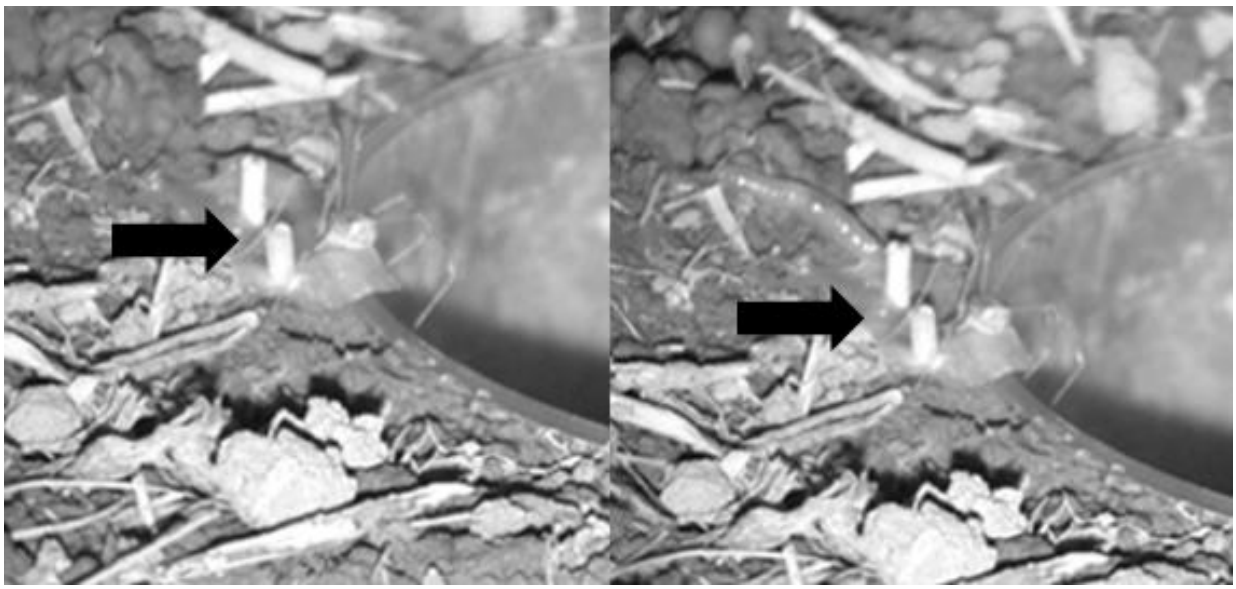

Figure 3.7 Images taken from infrared video recordings of a baited pitfall trap in a *Miscanthus* x *giganteus* (Mxg) shelterbelt on a commercial dairy farm, Canterbury, New Zealand, 2015. Black arrows indicate a European harvestman (*Phalangium opilio*) detering a slug from live facsimile prey (*Epiphyas postvittana*) by what appears to be 'proding' of the slug with its mothparts and legs (left). The European harvestman than consumes the prey (right).

3.4 New record of a potentially predatory collembolan in a field margin

A potentially predatory Collembola species was consistently observed on the facsimile prey (LBAM egg batches) around pitfall traps in the field margin plots (Fig 3.8) during August and September 2015. This was identified as *Dicyrtoma fusca* (Fig 3.9) by Australasian Collembola specialist, Penelope Greenslade. This is the first authenticated record of *D. fusca* in the southern hemisphere. Based on the above field observation, this would also be the first record of this species potentially being predatory. However, there was no indication of LBAM prey or P. xylostella egg consumption when this was tested for in *D. fusca* laboratory trial (Section 2.9). Furthermore, there was no indication of nocturnal LBAM prey consumption by Collembola when infrared video cameras were used (Section 2.8 and 3.4).

Figure 3.8 *Dicyrtoma fusca* potentially consuming live LBAM (Epiphyas postvittana) egg batches around a pitfall trap in the field margin of a commercial dairy farm, Canterbury, New Zealand, August 18 2015.

Figure 3.9 *Dicyrtoma fusca* at 40 X magnification. Photo: Andrew Murray.

3.5 DNA analysis of facsimile prey consumption

There were a total of 66 DNA extractions, PCRs and electrophoreses carried out on potential BCA collected from the baited pitfall traps in Mxg shelterbelts and the field margin (see Sections 2.6, 2.7, 3.1 and 3.2) to confirm which RTUs consumed the facsimile prey (see Section 2.10). Of these 66 RTU DNA extractions, four were successfully amplified indicating that they contained *Epiphyas* DNA, these were later sequenced. All sequences had a 100 % match to Epiphyas DNA on Genbank, three were from the internal tissue of the slug *D. reticulatum* and one was from the internal tissue of a *P. opilio* all of which were from Mxg shelterbelts (Table 3.3 and Fig 3.10).

Table 3.3 Results from DNA extraction, amplification, gel electrophoresis and sequencing of internal DNA from potential biocontrol agents (BCA) collected from pitfall traps baited with LBAM (*Epiphyas postvittana*) egg batches in *Miscanthus* x *giganteus* shelterbelts and the field margin during April, August and September 2015 on a commerical dairy farm, Canterbury, New Zealand.

Taxa	RTUs	No. DNA extractions	DNA amplified in PCR	Gel electrophoresis bands	No. of individuals in which *Epiphyas* DNA was sequenced
Slugs	*Derocerus reticulatum*	35	20	3	3
	Derocerus panormitanum	3	3	0	0
	Arion hortensis (agg)	1	1	0	0
	Arion intermedius	1	1	0	0
European harvestman	*Phalangium opilio*	11	11	1	1
Centipedes	*Lamyctes emarginatus*	3	3	0	0
	Lithobius microps	9	9	0	0
Beetles	*Metaglymma moniliferum*	2	2	0	0
	Aleocharinae	1	1	0	0
Total	9	66	51	4	4

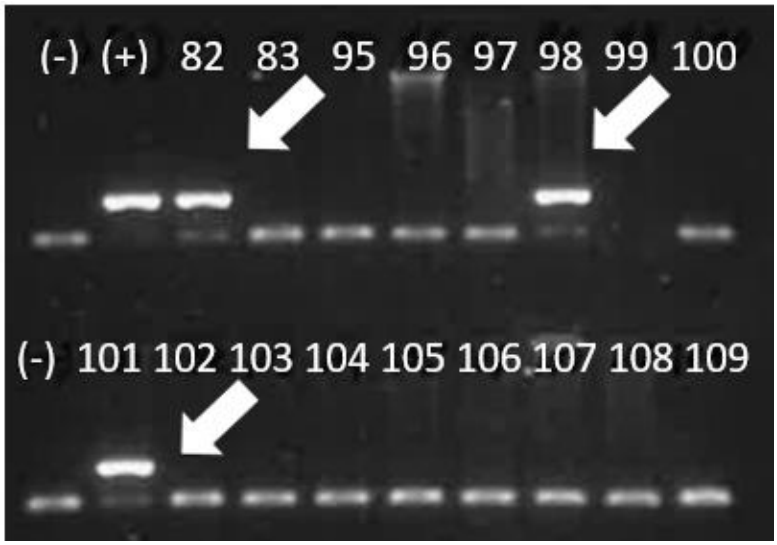

Figure 3.10 Gel electrophoresis results of potential biocontrol agents having consumed facsimile prey in *Miscanthus* x *giganteus* (Mxg) shelterbelts and in the field margin (Negative control, positive control and sample units: 82-83, 95-109 in white) on a commercial dairy farm in Canterbury, New Zealand, 2015. The white arrows indicate facsimile prey (LBAM, *Epiphyas postvittana*) consumption (*Epiphyas* DNA detection): 82 is DNA extracted from a European harvestman (*Phalangium opilio*); 98 and 101 are DNA extracted from two slug individuals (*Derocerus reticulatum*); samples 82, 98 and 101 were all collected from baited pitfall traps in Mxg shelterbelts.

Chapter 4

Discussion

4.1 Richness and composition of the potential biological control agents (BCA) associated with Miscanthus x giganteus (Mxg)

The current study determined that potential soil-surface dwelling BCA do occur in Mxg shelterbelts (Table 3.1) and that there were distinct differences in BCA community composition between Mxg shelterbelts and the field margin (Table 3.1, Figs 3.1 and 3.2). Each refugia type (Mxg shelterbelt and field margin) had eight potential BCA RTUs found only in that specific refugium type. These differences were not statistically significant. However, this is likely to be due to large variation within refugia types and a small sample size. Despite this, these findings are interesting ecologically, notably *Phalangium opilio*, *Lamyctes emarginatus*, *Pakeha sp.* and Aleocharinae which were found only in Mxg shelterbelts (Table 3.1). These taxa represent harvestmen, centipedes, spiders and staphylinid beetles, all of which are implicated in biological control (Sivasubramaniam et al. 1997; Merfield et al. 2004; Bowie et al. 2014). This indicates that Mxg shelterbelts can deliver the ES of providing refugia for BCA. Therefore, if these shelterbelts were to be widely used in agriculture, then the subsequent CBC may reduce the need for pesticides while potentially restoring farm biodiversity and providing multiple other ES. This would help mitigate the global agricultural challenges of sustainable insect pest management, food and fuel security with a changing climate. Furthermore, there was no statistical difference between refugia type in the number of endemic RTUs they harboured. However, Mxg shelterbelts had more exotic and native RTUs (Table 3.1 and Fig 3.3). This suggests that if this type of shelterbelt were to replace the non-crop vegetation in the field margin, refugium potential BCA RTU endemism and richness would be maintained.

An unexpected finding was that four European slug species were also found only in Mxg shelterbelt refugium with *Derocerus reticulatum* being the most abundant RTU in this study (53 individuals). Furthermore, there is evidence that suggests these slugs could contribute to biological control (see Section 4.3). The utilisation of Mxg shelterbelts but not the field margin by slugs may be due to the former receiving water from a centre pivot irrigator, whereas the field margin has no irrigation. Irrigation would create a moist microhabitat within the already sheltered habitat of the Mxg shelterbelts, which slugs are more likely to prefer (Speiser & Hochstrasser 1998). Slugs may also not occur in the field margin because slug predators such as carabid beetles including *Metaglymma moniliferum* (Bates) are found there (van Toor 2006). Carabid beetles can be used to manage slug pests in CBC (Renkema et al. 2014; Giffard et al. 2015).

The occurrence of RTUs in different refugia is likely to be related to habitat preference; for instance *P. opilio* prefers open vegetation (Martens 1978) such as the pasture paddocks, which surround the Mxg shelterbelts (Fig 2.1). This is indicated by these shelterbelts potential BCA community composition only having one main ordination cluster and relatively low variation. This is because there is only one dominant vegetation type (Mxg) which is consistent over all the Mxg shelterbelts (Fig 3.1 and 3.2). In contrast, the potential BCA community composition in the field margin had more variation and much more variation amoung pitfall traps (Fig 3.1 and 3.2). This can be explained by the field margin having a more diverse plant community (see Section 2.1). These findings suggest that CBC could be enhanced by inserting beneficial plant species into Mxg shelterbelts which would increase the likelihood of additional BCA using Mxg shelterbelts. These plants would need to be low growing and shade tolerant to allow Mxg harvesting, and provide floral resources to BCA such as *Phacelia tanacetifolia* Benth., buckwheat (*Fagopyrum esculentum* Moench) and alyssum (*Lobularia maritima* L.) (Tschumi et al. 2015). Low growing New Zealand endemic plants could also be incorporated into Mxg shelterbelts such as *Acaena inermis* 'purpurea' (Hook), which can be provide multiple ES under vineyards (Shields et al. in press). This combination of floral strips and Mxg would not only provide the ES of shelter for BCA but also the ES of food resources for BCA and beneficial bees. These additional ES would improve the multifunctional array of ES provided by Mxg shelterbelts which together can increase farm production, produce biofuel and now may potentially be used in insect pest management.

4.2 Predation rate of potential BCA

There was substantially higher facsimile prey consumption in Mxg shelterbelts (67 %) compared to the field margin (10 %). Prey consumption was particularly high in Mxg shelterbelts during April (early autumn) (73 %) and September (early spring) (68 %) (Fig 3.4 and Table 3.2). This indicates that the potential BCA community using Mxg would be more effective than that in the field margin at managing insect pests. Such pests would either oviposit near the soil surface or be active on it within Mxg. An example is the pest *D. virgifera virgifera* in North America and Europe (Spencer & Raghu 2009; Gloyna et al. 2011) or foliage pests falling onto the ground such as aphids (Winder et al. 1988; Östman et al. 2003). The lower predation rate in the field margin could be because spider RTUs were the predominant potential BCA found there, with the most abundant spider RTU being *Anoteropsis hilaris* (Koch) (14 individuals). This species is from the family Lycosidae (wolf spiders) which are active hunters that search only for moving prey (Persons & Uetz 1997; Hils & Hembree 2015). Therefore, it is unlikely that *A. hilaris* would consume the live LBAM egg prey. This was investigated under laboratory conditions with Lycosidae spiders in a no-choice test, resulting in no LBAM egg consumption (see Section 2.4). These results further support the use of Mxg shelterbelts in agriculture because not only does it contain the same potential BCA RTU richness and endemism to the field margin (Table 3.1) but the BCA within Mxg also have a higher likelihood of managing future insect pests. Therefore, if farmers

used Mxg shelterbelts around the edges of their fields, CBC would increase in conjunction with the other 15 ES that Mxg shelterbelts provides (Littlejohn et al. 2015) which is unlikely to cause overall yield loss from the shelterbelts occupying filed margin land (Pywell et al. 2015). However, this could cause a loss of endemic species that do not occur in Mxg.

4.3 Confirmation of potential BCA consuming the facsimile prey

Video and DNA analysis confirmed that at least two potential BCA were consuming the facsimile bait in Mxg shelterbelts; these were the European harvestman *P. opilio* and the European *slug D. reticulatum* (see Section 3.3 and 3.5). It is unclear what BCA consumed the facsimile prey in the field margin as no prey consumption was observed with video cameras (Section 3.3) or detected with DNA methods (Section 3.5). *P. opilio* is known to contribute to CBC, with observations from video cameras (Newton & Yeargan 2001; Merfield et al. 2004; Frank et al. 2007). Furthermore, *P. opilio* occurs in surveys of beneficial predatory arthropods in agricultural land (Merfield et al. 2004; Bowie et al. 2014). In contrast, the observation of slugs consuming live LBAM eggs in the field from video analysis and DNA confirmation of *D. reticulatum* consuming this prey is new to science. Although LBAM egg batches do not naturally occur in the abundance or specific locations used in this study, LBAM does occur at the field site and probably feeds on the broom and gorse found there (Suckling et al. 1998). Therefore, it is possible for the interaction of *D. reticulatum* consuming LBAM eggs to occur naturally.

Furthermore, the idea of using slugs, particularly *D. reticulatum*, as BCA is also likely to be new to science as these animals are usually considered pests (Douglas et al. 2015). However, they may contribute to other ES (Goble 2007) as they can be important food source for wildlife in Europe and North America such as hedgehogs, birds and reptiles (Yalden 1976; Platt et al. 2009; Vickery et al. 2009). These results suggest that *D. reticulatum* and other slugs prefer Mxg shelterbelts over the field margin (Table 3.1) and that these slugs may reduce the populations of soil-surface dwelling pests by consuming the pest's eggs. The current findings also indicate that Mxg shelterbelts should only be used with centre pivot irrigators when slugs are not a considerable pest. This is because a pest slug population could grow due to Mxg shelterbelts providing favourable habitat.

Additionally, the video analysis captured an interspecific interaction between slugs and *P. opilio* where potential competition for food resources occurred in the field. On three occasions interference competition was observed between slugs and *P. opilio* when these invertebrates were feeding on the facsimile prey (Section 3.3 and Fig 3.7). This is likely to occur naturally when there are limited food resources during autumn to early spring and may reduce the potential effectiveness of these BCA at managing pests. However, further investigation is required to determine how often this interaction occurs and what effect it has on the pest management.

4.4 New record of the potentially predatory collembola *Dicyrtoma fusca* in New Zealand

The use of LBAM as the facsimile prey revealed a new record of Collembola species in New Zealand. This species was consistently observed on the LBAM egg batches in the field margin during August and September 2015 (see Section 3.4) and was later morphologically identified as *D. fusca* by Penelope Greenslade in Australia (Federation University of Ballarat). This is the first morphologically determined record of *D. fusca* in the southern hemisphere. *D. fusca* commonly occurs in Europe and has been found in moist habitats up to 1500 m in fields, forest and caves (Bretfeld 1999). It is likely that *D. fusca* was accidently introduced in to New Zealand with European soil or plants.

The observation of *D. fusca* on the facsimile prey suggested that it may be consuming the LBAM eggs. If so, then this would also be the first record of *D. fusca* being predatory, but there was no indication of LBAM prey consumption by Collembola from nocturnal video analysis or from the no-choice experiment and DNA analysis (Section 3.4). However, no-choice tests and DNA analysis of laboratory and baited field collected specimens would need to be repeated on a larger scale to be more conclusive, which was beyond the scope of this research.

There is little is known about New Zealand Collembola (Greenslade et al. 2013) with no study conducted on the family Dicyrtomidae (of which *D. fusca* belongs to) within New Zealand for over 50 years. This is illustrated by Greenslade et al. (2013) discovering eight new records of Collembola species in agricultural land including three new species. Despite this poor understanding, Collembola play a major role in ecosystem functions such as plant decomposition (Rusek 1998) involving carbon and nitrogen mineralisation (Schröter et al. 2003). These ecosystem functions are essential for making nutrients available for plant growth and are therefore crucial in agroecosystems (Greenslade et al. 2013), especially in the context of global agricultural challenges. However, the relative economic importance of Collembola has not been quantified (Greenslade et al. 2013) which impedes the acknowledgement of the ES in which Collembola provide. This is of increasing importance because the agro-ecological food webs that Collembola occupy are being degraded by intensive agriculture (de Vries et al. 2013), yet the value of their ecosystem functions are largely unknown.

4.5 Limitations of this study's research approaches

The current study had several limitations, predominantly the time of year in which it could occur and the sample sizes that could be used. Additionally, the pitfall traps were unlikely to capture the total

richness of potential soil-surface dwelling BCA in the Mxg shelterbelts and the field margin due to using a total of only 18 trap nights. This number was restricted by the use of a facsimile prey which could not be left in the field for long intervals due to the risk of the eggs hatching. Another factor was the use of DNA analysis, which limited the time that specimens could be left in the pitfall traps. The video analysis was impeded by the limited equipment available and technical difficulties involved with that equipment. Furthermore, funding and time constraints prevented DNA analysis of the spider RTUs and most of pitfall trapped *D. fusca*. In addition 21 pitfall trapped slug specimens were accidently lost by Michael Wilson (AgResearch, Hamilton) after identification took place. These lost but identified specimens were still included in the dataset with the exception of gut analysis. Despite these limitations, the study was still successful by achieving its aims while providing information novel to science.

4.6 Future research

Future research on Mxg shelterbelts providing refuges for BCA needs to involve a 12 month survey to determine what potential BCA use Mxg shelterbelts throughout the year. This could be done in conjunction with an investigation of whether potential BCA emigrate from Mxg into the field/paddock after winter. Furthermore, this could be accompanied by facsimile prey, potentially with different types, with 24 h video analysis using multiple new high definition infrared cameras. This would provide a clearer picture of potential BCA abundance, behaviour and prey consumption. Additionally or instead of video analysis, next-generation sequencing could be used to determine what prey the potential BCA had been consuming. These methods could also apply to *D. fusca*, although targeted field collection is advised to collect *D. fusca* individuals if facsimile prey is used rather than using a generalised pitfall method.

4.7 Conclusions

Global agricultural challenges such as changes in the needs of insect pest management can be mitigated using agroecology methods such as Mxg shelterbelts with CBC. Mxg shelterbelts are used by potential BCA as refugia and potential BCA in Mxg shelterbelts are likely to be more effective at managing future insect pests than those found within the field margin. Both refugia types have similar potential BCA richness and level of endemism. *D. reticulatum* slugs have the potential to be used as BCA in the presence of Mxg shelterbelts and *D. fusca*, which could potentially be predatory, was found in the field margin, which is a new record for the southern hemisphere. These findings are new to science and support the implementation of Mxg shelterbelts in agricultural land which could contribute to the sustainable intensification (Littlejohn et al. 2015) of agroecosystems.

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