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Operation COBRA: Developing a new protocol for measuring spider biodiversity in New Zealand pastures

A thesis submitted in partial fulfilment of the requirements of Master of Science

at
Lincoln University

by
Kate Mary Curtis

Lincoln University
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Abstract of a Thesis submitted in partial fulfilment of the requirements for Masters in Science

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By
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Research in conservation biology, ecology, and agroecology requires accurate knowledge of species distributions. Arthropods are often ignored or under-sampled in biodiversity and conservation assessments due to the large amount of effort caused by their large diversity, small size, and lack of taxonomic guides. To assess these groups accurately, rapid biodiversity assessment programmes have been established. These programmes are based on sampling protocols that are required to be standardised to allow for comparison and optimised to obtain the maximum possible data with the minimum amount of effort. This research project aims to develop a new protocol for measuring spider biodiversity in pastures in New Zealand. A COBRA (Conservation Oriented Biodiversity Rapid Assessment) protocol consists of an intense sampling of an ecosystem by using the optimal combination of sampling methods. This study intensively sampled dairy and cropping pastures in the Canterbury area, and used ground sampling, suction sampling, sweeping, and pitfall traps to assess spider biodiversity. The minimum amount of effort and combination of methods required to record different levels of taxonomic and functional diversity was calculated. A total of 2767 spiders were caught, which included 1384 adults (50%) representing 12 families and 28 species. Pitfall traps collected 92% of the estimated number of species for all sampling methods. Ground hand collection collected 80.7% and suction sampling collected 22.5% of the estimated number of species. Sweeping collected the lowest estimated number of species with 17.8%. Therefore, of the four sampling methods used pitfall traps and ground
hand collection were far more efficient at collecting spider species. Pitfall traps collected 12 unique species. Ground hand collection collected four unique species. Sweeping collected one unique species and suction sampling collected no unique species. Pitfall traps and ground sampling are the best methods to use to quickly assess spider diversity in pasture. Sweeping and suction sampling have limitations. Neither method can be used if the pasture is wet and suction sampling is not cost-efficient. Night sampling yielded more species compared to day sampling. Using a standardised protocol in pasture allows biodiversity to be measured over time to accurately assess whether it has increased or decreased as farming strategies change. For monitoring, ultimately, these tools will be used for assessing biodiversity on farms.

**Keywords:** agriculture, Conservation Oriented Biodiversity Rapid Assessment, ground sampling, New Zealand, optimised, pastures, pitfall traps, spiders, standardised, suction sampling.
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Chapter 1: Introduction

1.1 Why study arthropods?

Arthropods are one of the most diverse phyla on Earth and are found in almost every habitat (Cardoso, 2009; Hawksworth & Bull, 2007; Starr et al., 2014). Assessing arthropod richness, abundance, composition, geographical patterns and their roles in ecosystems is often difficult due to the great effort and resources required to collect or generate data on them (Cardoso, 2009; Ramos et al., 2001), which can be a constraint in conservation biology, ecology, agroecology, and biogeography (Ramos et al., 2001; Curr et al., 2004; Blanchet et al., 2015). Arthropods, although being largely ignored in conservation studies, play an essential role in ecosystems, as ecological indicators, and can be used in identifying conservation priority sites (Franklin, 1993; Predavec et al., 2016; Campbell et al., 2017).

Ecosystem services are defined as the profits and benefits humans receive from ecosystems (Dempsey and Robertson, 2012; Wratten et al., 2013). There are four types of ecosystem services: supporting (e.g. nutrient cycle and water), regulating (e.g. erosion prevention and water purification), provisioning (e.g. food production) and cultural (e.g. spiritual and aesthetics) (Wratten et al., 2013; Potts et al., 2016; Maron et al., 2017). Agroecosystems are provisioned by humans to optimise food, fuel and fibre (Zhang et al., 2007). Ecosystem dis-services, including herbivory by pests and undesired species competing for water and nutrients also occur in agriculture, which decreases production and increases production costs (Zhang et al., 2007).

The Canterbury Plains have seen much change in their ecosystems, which were previously a variety of forests, shrublands and grasslands (Ecroyd and Brockerhoff, 2005). Pre-European vegetation on the Canterbury Plains has been replaced with large areas of agricultural land and only 1% of the native biodiversity remains (Wratten et al., 2013). Maintaining native biodiversity is of high importance as it contributes to a healthy and sustainable environment. It is achieved by restoration, planting large areas with native plants and the appropriate urban/land management polices (Meurk and Hall, 2006; Curtis et al., 2016). The agriculture
landscape in New Zealand is highly modified and there is a great need for pollination. Honeybees are not the only insects that pollinate crops, particularly in New Zealand where hoverflies and other fly species also pollinate (Power and Stout, 2011). A study completed by Rader et al., (2009) compared how efficient New Zealand hoverflies species were with the western honeybee Apis mellifera. The results showed that hoverflies are equally efficient as honeybees for most pollination, although in high mass cropping, they are not as effective as the western honeybee. However, due to the decline of honeybee populations by the impacts of Varroa destructor (Iwasaki et al., 2015) and colony collapse disorder (Frazer et al., 2015) these hoverflies are still very beneficial in agricultural landscapes.

Like invertebrates, spiders are useful in agriculture and provide crucial ecosystem services (Fukuda et al., 2011; Hogg and Daane, 2011; Marc and Canard, 1997). Spiders are polyphagous predators whose prey includes insects and other spiders (Marc and Canard, 1997; Hogg and Daane, 2011). It has been suggested that, because of polyphagy, spiders are not efficient in controlling pests (Debach and Rosen, 1991). Additionally, spiders also feed on other spiders and parasitoids that are beneficial fauna (Hallander, 1970; Traugott et al., 2012). There have been few studies that investigate the biocontrol potential of spiders in pastures. In agroecosystems spider species abundance is generally quite high but is often dominated by only a few species (Agnew and Smith, 1989; Weibull et al., 2003; Isaia et al., 2010; Michalko and Pekar, 2015). Nevertheless, spiders can be present even when pest species are absent, preying on alternative prey and as well as possessing adoptions for times of deprivation (Michalko and Pekar, 2015; Greenstone and Bennett, 1980; Harwood et al., 2004). Therefore, when a pest enters an agroecosystem, spiders may already be present (Symondson et al., 2002; Michalko and Pekar, 2015). There are studies that show particular assemblages of spider species can reduce crop damage from pest insects in orchards and crops (Hoefler et al., 2006; Michalko and Pekar, 2015). Philidippus clarus a jumping spider has been used as a biocontrol agent in greenhouses to examine whether the presence of this species reduces pest numbers and crop damage (Hoefler et al., 2006). Results showed that after one-week pest numbers reduced and plant damage was less with Philidippus clarus present. Philodromus species (crab spiders) have been studied with regards to the effect of natural prey in orchards (Michalko and Pekar, 2015). Results suggest that spiders have potential as biocontrol agents.
in fruit orchards as there was a decrease in pest numbers and this species of spider is reluctant to prey on beneficial fauna.

Because arthropods are highly diverse, sampling requires an efficient and well-structured approach to maximise the always limited resources (New, 1999a). Furthermore, as awareness of the impact of human activities on ecosystems and organisms increases it becomes more important to have standardised protocols for evaluating and monitoring biodiversity (Cardoso, 2009; Whitmore et al., 2002).

1.2 Ad hoc sampling vs. optimised sampling

The most common approach for sampling arthropods is ad hoc sampling (non-optimised and site specific). Optimised and standardised protocols, such as COBRA (Conservation Oriented Biodiversity Rapid Assessment) are not common (Cardoso et al., 2009a). Ad hoc sampling is based on expert judgement of the collectors, where it is assumed the best combination of sampling methods that will provide the maximum information about sites in a minimum amount of time (Cardoso et al., 2009a). This approach is often used for compiling species lists (Gordon & Newton, 2006; Roberts et al., 2007) for well-known taxa, such as birds (Droege et al., 1998), but because the sampling events are seldom designed in the same way, ad hoc sampling does not allow for reliable or repeatable comparisons (Cardoso, 2009). The COBRA protocol is a relatively new approach to sampling highly diverse arthropods, such as spiders (Cardoso, 2009). This protocol is designed to collect the maximum number of species, in a minimum amount of time, combining a variety of sampling methods (optimised) while being applicable to multiple sites (standardised).

Sampling efficiency – expert judgement vs. optimised sampling

Ad hoc sampling may be less efficient (Malumbres-Olarte et al., 2017) in regard to the number of species collected over time (person-days) than the COBRA approach (Figure 1). This may occur because ad hoc sampling often uses methods that overlap with other or uses that are less efficient at collecting many species (Cardoso et al., 2009a). By contrast, COBRA protocols avoid these issues by combining samples from different methods. In other words, a COBRA protocol will be composed of the samples from different methods that provide the
largest number of species possible to obtain an accurate estimate of diversity using those methods.

Figure 1: "Randomized accumulation curves and respective confidence limits for species richness obtained by the ad-hoc and COBRA approaches. Curves are drawn either with person-days or individuals as measures of effort.” (Cardoso et al., 2009a).

1.3 Standardised sampling

A common problem with numbers of samples is that they will only represent a tiny fraction of the community (Cardoso et al., 2009a). When sampling is very small, comparison of communities is difficult. The benefit of standardised sampling is that, even if species lists are incomplete, comparisons of communities are possible (Duelli et al., 1999; Stork et al., 1996; Jones & Eggleton, 2000).

An advantage of standardised sampling is that not only does the data represent species richness, but it also provides a measure of species relative abundance (Cardoso et al, 2009a). Therefore, standardised sampling can be used to answer ecological and biogeographical questions as well as being applied to conservation biology. Standardised protocols are not widely available, and this hinders consideration of arthropods in conservation plans (New, 1999b; Cardoso et al., 2008a). Emblematic groups, such as the
New Zealand kaka *Nestor meridionalis* (Gmelin, 1788), have been relied on for worldwide conservation efforts (Cardoso et al., 2011a; Recio et al., 2016). This has been established by the confidence of using the umbrella species concept where one species is chosen with the knowledge that protecting that species also protects other species within a specific ecological community (Cardoso et al., 2011a). Conservation managers and scientists still use umbrella species widely, however, this concept has been challenged and has been shown to be unfounded (Muñoz, 2007; Cabeza et al., 2008; Martín et al., 2010; Cardoso et al., 2011a). Furthermore, in some cases, invertebrates are often the initial group to become extinct in response to habitat loss and disruption (Cardoso et al., 2010). Invertebrates also face 1,000 times less funding than each mammal species (Cardoso et al., 2011b). They have a low public conservation profile and most people only consider them as pests (Berenbaum, 2008). All these factors impact on the strategy and scientific recommendations that all contribute to the absence of interest in invertebrate conservation (Barua et al., 2012). Standardised protocols follow certain criteria and steps, but it remains uncertain if they can be applied to invertebrate communities (Malumbres-Olarte et al., 2017).

There are four shortcomings of invertebrate research (Cardoso et al., 2011b). The Linnean shortfall is that many species are yet to be identified and described and the knowledge of many species on Earth is very poor. The Wallacean shortfall is the lack of knowledge of the distribution and scale of many species. The Prestonian shortfall is the lack of data in time and space for comparative species. The Hutchinsonian shortfall is that the effect of habitat changes due to sensitivity, functional roles and the diverse ways of life of many species on Earth is often unknown (Cardoso et al., 2011b). However, there are some exceptions where standardised protocols have been established for carabid beetles (Niemela et al., 2000), ants (Agosti and Alonso, 2000) and butterflies (Pollard and Yates, 1993). This approach can be less efficient than non-optimised sampling (Gordon & Newton, 2006) and has led to the development of field protocols that are both standardised and optimised (Cardoso, 2009).

The ALL protocol (the Ants of the Leaf Litter) is designed to be a standardised protocol for sampling ground-dwelling ants (Agosti and Alonso, 2000). The ALL protocol uses two collection methods, mini-Winkler extractors and pitfalls traps, to collect the largest number of ground-dwelling ants. It also allows for other methods to be included in the protocol,
depending on the study aims. These include inspecting dead wood, scraping soil, and handling collected specimens. The protocol is run for 48 hours to achieve the standard sample, however, sampling can be left longer if desired. The ALL protocol data is useful because it allows comparison of global patterns.

Pollard and Yates (1995) developed a butterfly monitoring scheme in the 1960s and 1970s. Capture-mark-recapture is one of the most common methods of estimating population size (Morton, 1982; Pollard and Yates 1995; Tufto et al., 2012). This method, however, is labour intensive and takes time. For a good protocol to work, the methods need to be simple to use and time efficient (Yates and Pollard, 1995). Pollard and Yates (1995) monitoring method is a compromise between the expected outcome and what can be attained in the field. Transect counts are chosen as the method, and although they only provide the population size index, they are used to measure the change in abundance over time (Pollard and Yates, 1995). Butterflies can occur in large populations of up to tens of thousands (Pollard and Yates 1995). For large populations the transect method works best, as the capture-mark-recapture method is limited as it is impossible to capture and mark enough individuals to give a representation of a very large population.

The first standardised and optimised protocols – termed COBRA – were proposed by Cardoso (2009) and were designed to sample in temperate forests and meadows. COBRA protocols consist of a very intense sampling of an ecosystem by using the optimal combination of sampling methods (Cardoso, 2009; Cardoso et al, 2008b; Cardoso et al, 2009b). This approach provides a more reliable estimation of the true number of species existing in each site at the time of sampling than ad hoc sampling (Cardoso et al, 2009a).

Cardoso (2009) developed a standardised and optimised protocol for those surveying Iberian spiders. The two objectives of this study were; (1) to expand the inventories of arthropods by providing guidelines and statistical methods to standardise and optimise these inventories and (2) to provide a standardised and optimised protocol that followed these guidelines and methods. The protocol included the level of effort to obtain the greatest amount of information possible, using a combination of sampling methods efficiently across all sites, and by adding an optimization algorithm to the data. Sampling
was always carried out in May and June, where spider abundance is at its highest. Each sampling site was one hectare and had the following sampling methods: pitfall traps, sweeping, ground searching, foliage beating, and aerial searching. Sixty-four samples were used for each sampling method and pitfall traps were left in the field for two weeks at a time, each sampling unit was a person-hour of fieldwork. Day and night sampling were distinct statistically and sampling effort was divided equally across each method combination. From the results, Cardoso (2009) proposed six explicit steps that a standardised and optimised protocol should meet.

1. Suitability: The sampling methods and effort should suit the selected organism. Pitfall traps would not be suitable for an orbweaver as they predominately build webs in trees and are seldom found on the ground. The sampling method chosen should allow for the collection of a sizeable amount of the known community.

2. Efficiency: The sampling methods should provide the maximum information with the least amount of given effort. Often this requirement is disregarded by researchers for more habitual and familiar methods.

3. Feasibility: The sampling methods and efforts that are used can be modified to accommodate differing amount of resources (human, financial, time). Often researchers do not adjust to the different resources that have led to poor quality catalogues.

4. Flexibility: Different people will have access to different resources, and the methods and effort used needs to be flexible. Other protocols only recommend one mix of methods and a number of samples; therefore, it does not offer flexibility.

5. Transparency: The design process must be transparent because if the protocol design works, different collectors will be able to replicate the protocol.

6. Accountability: The protocol results should support a posteriori evaluation and review. Often the quantity of data collected is limited and the reliability cannot be adequately evaluated.

*Comparability — site-specificity vs. standardisation*

If *ad hoc* sampling is performed by an experienced collector who knows the sampling site and when a sampling method is most productive, it can provide a quick accumulation of species (Cardoso, 2009; Cardoso et al., 2009a). However, this sampling is site-specific and
has many limitations; (1) experienced collectors can be biased towards certain taxa; (2) a collector can miss a portion of a community in a site because they are not aware that these species are present, particularly areas with small/cryptic species; and (3) experienced collectors are few in number and often less experienced collectors are used. A COBRA protocol, however, is less likely to have these issues as it is standardised for a given ecosystem with a specific number of samples per method.

**Species accumulation curves**

Species accumulation curves account for the total species numbers during the data collection (Gotelli and Colwell, 2001). As most species are collected from early sampling and fewer additional species are collected as sampling continues, the curve of a cumulative species number graph at first rises steeply and then flattens off, as additional species are collected, until it reaches asymptote (Gotelli and Colwell, 2001). This can provide lower-bound estimates from asymptotic non-parametric estimators for taxon-rich groups, such as arthropods. The non-parametric estimators used in the study are; Jackknife 1, Jackknife 2, Chao 1 and Chao 2. The advantage and efficacy of the Jackknife is due to it measuring the occurrence or non-occurrence of species in an area rather than measuring the richness of the species (Sarmah, 2017). To calculate the abundance of rare species, Chao is used to collect the maximum data about the number of non-occurrence of species in an area. These data are created by considering the quantity of species found in the area and the theory of singletons and doubletons (Sarmah, 2017).

1.4 Justification for using spiders and pastures

Spiders are very diverse and have 48,321 known species that live in every terrestrial ecosystem on Earth, except for Antarctica (World Spider Catalog, 2019). Spiders are generalist predators (Riechert and Lawrence, 1997) and are good indicators of overall biodiversity in an area (Marc et al, 1999; Churchill, 1998; Pearce & Venier, 2006). Spiders respond strongly to habitat changes (Zieche & Roth, 2008) either by physical changes to their environment (Greenstone, 1984; Malumbres-Olarte, 2013) or changes in prey populations (Marc et al, 1999). In agro-ecosystems, spiders are the most abundant predators (Topping and Lovei, 1997; Vink et al., 2004; Wise, 1993). Agroecosystems with
high levels of disturbance have decreased spider diversity and abundance (Topping and Lovei, 1997). There have been several studies across the world that have experimented with exhaustive sampling methods that are based on semi-quantitative methods for spiders, e.g. Bolivia (Coddington et al., 1991), the USA (Dobyns, 1997; Toti et al., 2000), Tanzania (Sorensen et al., 2002) and Peru (Silva and Coddington, 1996). These studies were not completely standardised or optimised for a number of variables (Cardoso et al., 2008a). In the Mediterranean, there have been several studies by Cardoso and colleagues (e.g. Cardoso et al., 2008a; Cardoso et al., 2008b; Cardoso et al., 2009b) and in Tanzania (Malumbres-Olarte et al., 2017) that have standardised and optimised protocol for sampling spiders

Most exotic pastures in New Zealand are occupied by exotic species of spiders (Vink et al., 2005). Martin (1983) sampled pastures in Nelson and identified 45 spider species. *Tenuiphantes tenuis* (Blackwall, 1852) is one of the most common found in New Zealand pastures (Vink et al., 2004). This is an exotic species and most likely originated from Europe (Millidge, 1988). Linyphiidae appears to be the more dominant family found in pastures (Clark et al., 2004). Martin (1983) found *Erigone wiltoni* Locket, 1973 was also an abundant species in Nelson pastures. Species from this family are minute spiders that produce sheet/dwarf webs often just above the ground surface (Paquin et al., 2010). Curtis et al. (2017) found *Haplinis fucatinia* (Urquhart, 1894) was the most abundant linyphiid (Mynogleninae) in the pasture at the Lincoln University Demonstration Dairy Farm. *Anoteropsis hilaris* (L. Koch, 1877) is the most dominant species of Lycosidae (wolf spiders) that are found in pastures (Vink et al., 2004). This species has an iridescent layer behind their retinas in four eyes (Doucet and Meadows, 2009). This trait is useful to the collector when sampling at night as the spider’s eyes will reflect like cats’ eyes when torch light reflects off them making it easier to see them in the pasture.

There are more than 14.3 million hectares of primary production land in use in New Zealand (Statistics New Zealand, 2012), and it is important, particularly for monitoring, to have a reliable, efficient, and accurate sampling protocol for arthropods of this habitat. A protocol will enable farmers to assess biodiversity on their farms and to evaluate how their farming practices impact the biodiversity. If this protocol is used successfully in the agricultural
industry it could be a good reference for other industries that have an impact on the environment, e.g. quarries, forestry, and aquaculture.

1.5 Sampling spiders

There are many effective methods for sampling spiders in different habitats. Certain sampling methods are more efficient in particular habitats (refer to chapter 2.2). Resources should not be used where they are not required when using standardised sampling. For sampling exotic pastures in New Zealand pitfall traps, ground sampling, sweeping and suction sampling have been chosen as suitable sampling methods for this particular habitat (Wise 1993; McLachlan 2000; McLachlan & Wratten 2003)

1.5.1 Pitfall traps

Pitfall traps are commonly used to monitor ground-dwelling arthropods (Moeed & Meads, 1985; Norbury et al., 2009) and are used to collect entomological data on arthropod density and activity (Uetz & Unzicker, 1976 as cited in Green, 2000). Pitfall traps can be used in almost all habitats and are suitable for pastures. There are downsides to using pitfall traps as they can overestimate or underestimate different taxa (Lang, 2000; Topping and Sunderland, 1992). A mix of abiotic and biotic factors can affect pitfall traps including surrounding vegetation, spatial resistance/animal movement capacity, temperature, humidity, species interactions and reproductive behavior can influence spider activity (Dinter, 1955; Greenslade, 1964; Malumbres-Olarte, 2010). Pitfall traps are suitable for catching ground dwelling spiders although catching other types of spiders, like orb weavers, are less likely. There will always be limitations with different methods used to sample arthropods, but pitfalls have proven to provide valuable information (Topping and Sunderland, 1992; Uetz and Unzicker, 1976; Malumbres-Olarte, 2010).

1.5.2 Ground Sampling

Ground sampling involves direct searching for spiders (Sutherland, 2006). This method is straight-forward, inexpensive and only relies on the collector’s eye and an aspirator and/or vial. It is more efficient in open vegetation, including: tussock grasslands, dunes and
quarries, where other direct methods like sweeping would be less efficient (Sutherland, 2006). Also, ground sampling can access areas where direct methods cannot, such as looking under logs or stones or in pasture using a knife to get at the level of the roots and placing the soil and roots into a beating tray to look for cryptic species (Sutherland, 2006). Ground sampling can be standardised by counting numbers per unit effort or per unit of vegetation in this area (Sutherland, 2006).

1.5.3 Sweeping
Sweep nets are used in long and short vegetation areas, including grasses or dwarf-shrubs, and are used to collect arthropods, including spiders, flies, small beetles, and true bugs (Sutherland, 2006). Sweeping is a cheap, quick and efficient way of collecting huge numbers of invertebrates (Sutherland, 2006). It is not recommended that sweeping take place on damp vegetation or in strong winds as it renders this method impractical at collecting invertebrates (Sutherland, 2006). The depth, speed, and angle of the net being pulled up from the vegetation can influence the number of invertebrates being caught (Sutherland, 2006). For example, jumping spiders are more likely to avoid slow approaching nets compared to fast approaching nets. Sweeping is not quantitative but can be standardised (Pruess et al., 1977; Sutherland, 2006).

1.5.4 Suction sampling
Suction samplers are often used in entomological research to gather quantitative data in agroecosystems (Samu et al., 1997; Bell et al., 2000; Brook et al., 2008). The effectiveness of sampling spiders using a suction sampler can vary as it can overestimate or underestimate certain populations of spiders (Samu et al., 1997). Suction samplers are not always widely available and are heavy and expensive (Bell et al., 2000). However, they are a quick and useful method for sampling spiders (Samu et al., 1997; Malumbres Olarte, 2010).

1.6 The COBRA protocol
A COBRA protocol was developed based on the data collected in meadows in the Paül de Arzila Nature Reserve in Portugal (Cardoso et al., 2009a). We do not know if the COBRA
A protocol developed for Portuguese meadows will work for New Zealand pastures as the protocols were designed to be applicable to Mediterranean or temperate meadows. There is currently no efficient method for sampling spiders in New Zealand agricultural pastures accurately and the development of a new COBRA protocol for these pastures would be beneficial. There are many different types of pastures in New Zealand, ranging from exotic pastures to native tussock grasslands (Table 1). Developing a COBRA protocol that can be used in exotic pastures within New Zealand could be a good reference for future research. I will develop the COBRA protocol by focusing on pastures as they are simplified ecosystems that are likely to have relatively few species and provide a useful test case study.

**Table 1: Grasses common in exotic pastures and native tussocks in New Zealand.**

<table>
<thead>
<tr>
<th>Exotic Pastures</th>
<th>Native Tussocks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scientific name</strong></td>
<td><strong>Common name</strong></td>
</tr>
<tr>
<td><em>Lolium perenne</em></td>
<td>Perennial ryegrass</td>
</tr>
<tr>
<td><em>Trifolium repens</em></td>
<td>White clover</td>
</tr>
<tr>
<td><em>Chichorium intybus</em></td>
<td>Chicory</td>
</tr>
<tr>
<td><em>Plantago lanceolata</em></td>
<td>Ribwort plantain</td>
</tr>
<tr>
<td><em>Medicago sativa</em></td>
<td>Lucerne</td>
</tr>
<tr>
<td><em>Phleum pratense</em></td>
<td>Timothy</td>
</tr>
</tbody>
</table>

Developing a COBRA protocol will enable planning and prioritising for the way land could be used, as well as to monitor any damage caused by different farming activities. It will serve to develop conservation or monitoring plans. COBRA-generated data gives a repeatable, consistent way to monitor biodiversity over time to gauge impacts from changes in practice. The data obtained from using this COBRA protocol will help understand how to use the land in a way that lessens the impact on ecosystems. Fertiliser and insecticide applications, irrigation, disturbance of soil, and stocking levels are all practices that are used in agriculture that can affect biodiversity.
Farmers are increasingly likely to be required to account for the effects of their farming management practices (Norton, 2009; Bellamy et al., 2016; Landis, 2016). Ultimately, COBRA may be a tool that farmers, or consultants that the farmers are contracting, could use to assess biodiversity on farms.
1.7 Aim and Objectives

My aim is to develop a protocol for sampling spiders efficiently in exotic pastures.

Objectives

1. Developing a new COBRA protocol for exotic pastures in New Zealand.

2. To estimate the minimum amount of time/resources/money to efficiently and accurately sample exotic pastures.

2.1 Study sites

I collected data from ten sites that varied from 0.6 hectares – 8.3 hectares. Each site was in the Canterbury region and covered different pastures/communities. Plots were at least 100 metres from the edge of a paddock to reduce edge effects (Horvath et al., 2002; Rodrigues et al., 2014). Six plots were on dairy farms, and the other four plots were on sheep/multiple crop farms (Figure 2 and Figure 3). Sampling was carried out from February to May. The COBRA protocol is standardised and should be applicable to many sites within the same type of habitat (Cardoso, 2009). The greater the differences in the (community) data used to develop the protocol, the more the protocol is generalisable and widely applicable.

Figure 2: Aerial map of all three farms. Farm 1; Lincoln University Demonstration Dairy Farm, Farm 2; Lincoln University Research Dairy Farm, Farm 3; Iversen Fields.
2.1.1 Pilot study at Lincoln University Demonstration Dairy Farm

The Lincoln University Demonstration Dairy Farm (-43.6420, 172.441) is approximately 2 km from Lincoln and covers 186 ha of land with 160.1 ha of productive land. The farm was originally a sheep farm and was converted to a dairy farm in 2001. The farm is divided into two main blocks: north and south with Ellesmere Junction Road running between the two main blocks. The average rainfall per year is 666 mm and is supplemented with another 450 mm/year from irrigation to maintain the average evapo-transpiration rate of 870 mm/year. The pasture consists of Ronsyn/Impact ryegrass (Lolium perenne), Aran sustain white clover (Trifolium repens) and a small area of timothy (Phleum pratense). The Lincoln University Demonstration Dairy Farm integrated approximately 6000 native plants into different sections on the farm in 2008. The main planting sites were the four corners and four corridors in the north block.

The Lincoln University Demonstration Dairy Farm was used for a pilot study to assess each sampling method. Two sites were used, one month apart, to test the robustness of results from different tests (Figure 3). N1 was the first site used and was 8.3 hectares. Pitfall traps were placed at least 100 m from the fence line in a diagonal transect line and put into four groups of four, each one metre apart. Each set of grouped pitfall traps were three metres apart (Figure 7). Sweeping was performed by laying down 30 m transect lines and sweeping down one side, at least five metres from the transect line, and then returning along the other side. Sweeping was performed continuously for five minutes and resulted in moving back and forth two or three times along the transect line. The sweep net was emptied onto a beating tray where samples were collected with an aspirator. The spiders were placed from the aspirator into a vial of 70% ethanol and labelled. Sweeping was performed for three hours at these sites as dew on the ground made it impossible to sweep for five hours. Sweeping cannot be done while the ground is wet. This also happened during the day when irrigation was active. Other sites were chosen that did not have irrigation on during sampling. Ground sampling was also carried out along the 30 m transect lines. The search areas were within five metres of a transect line. Each area was searched for ten minutes. There were five transect lines taking a total of five hours to sample. Samples were labelled and placed into vials with ethanol. Suction sampling was completed in a 1 m² area. This plot
was chosen at random by throwing a piece of scrunch ed up paper and sampling the area where it landed. Within the plot the suction sampler was placed in different points and sampled for 30 seconds to compile three minutes in total. Ethanol was placed into the plastic cups (69 mm in diameter) and were labelled.
Figure 3: Map of two sample sites at Lincoln University Demonstration Dairy Farm, Canterbury, New Zealand. The two sampling sites N1 and N7 are highlighted in yellow. The green highlights the areas where the native plants have been planted.
2.1.2 Lincoln University Research Dairy Farm

The Lincoln University Research Dairy Farm (-43.639 172.456) is approximately 1.5 km from Lincoln University. It is used for trialing concepts, conducting research with potential commercial applications, and demonstration of the best practice dairy techniques. Some of the research includes the health, nutrition and welfare of the stock that are fed on high quality pastures; the efficiency of nitrogen and water on pastures; using supplements on pastures that reduce environmental issues but also enhances milk production; environmental impacts caused by dairying; and different grazing management practices (Helen Hague, pers. comm. 2017). Much of the research is carried out by post-graduate students from Lincoln University. Large areas of the pasture consist of perennial ryegrass \((Lolium perenne)\) and white clover \((Trifolium repens)\). There are other areas of diverse pasture that contain chicory \((Chichorium intybus)\), plantain \((Plantago lanceolate)\), and lucerne \((Medicago sativa)\). The farm is 56 hectares and has 250 Friesian cross Jersey cows. The irrigation average is 105.1 millilitres per month.

Four sites were used on this farm (Figure 4). Site one was E1b and E1c combined (0.75 ha) were combined because the area of any one alone was too small to meet the requirement. E4 was site two and measured 0.89 hectares. E5 was site three measuring 1.08 hectares. F6 was site four, was 3.08 hectares, and was a diverse pasture. The sampling protocol was a similar design to that used at the Lincoln University Demonstration Dairy Farm. The only difference was the sweeping protocol, as at the Demonstration Dairy farm sweeping could only be performed for three hours before the pasture became wet either by dew on the grass or the irrigation system activating in the paddock. I was able to choose sites that did not have irrigation on while I was sampling. Sweeping was done by laying down 30 metres transect lines and sweeping down one side, at least ten to twenty metres from the transect line, and then returning along the other side. There were a total of five transect lines taking a total of five hours to sample.
Figure 4: Map of four sample sites at Lincoln University Research Dairy Farm, Canterbury, New Zealand. The four sample sites are highlighted in yellow.
2.1.3 Lincoln University Field Service Centre Iverson Field

Iversen field (-43.647, 172.466) is 15.3 hectares and is located south west of Lincoln University. The area is used for multiple crops, including wheat, barley, peas, beans, oil seed rape, forage brassicas and vegetables. The pasture is put into rest blocks after intensive cropping has been done. These areas are left in pasture for up to three years depending on the experiments and research requirements. It also improves the soil structure. The pasture is grazed by hoggets. The pasture is planted with Arrow ryegrass (*L. perenne*) at 20 kg/ha and white clover (*T. repens*) at 4 kg/ha. The irrigation average per month is 100 ml.

Four sites were used (Figure 5). I13 was site one and was six hectares. I14 was site two and was 1.1 hectares. I1 was site three and was five hectares. I5 was site four and was 1.2 hectares. Two sites I13 and I14 were used again a year later. The sampling protocol was the same design used in the Lincoln University Research Dairy Farm.

![Figure 5: Map of four sample sites at Lincoln University Field Service Centre Iversen Field, Canterbury, New Zealand.](image-url)
2.2 Sampling Procedure

Common sampling methods for spiders are pitfall traps, emergence traps, sweep netting, suction sampling, leaf litter extraction, ground searching, and beating (Churchill and Arthur, 1999; Sutherland, 2006). Not all of these sampling methods are appropriate for pastures, with leaf litter extraction and beating techniques not used. Pasture grasses are short and do not have a compact structure as some native grasses do (Malumbres Olarte, 2010) and that is a reason why foliage beating would not be suitable as a sampling method for exotic pastures. Leaf litter extraction is used mainly in forests as there is more depth in the litter (Stevenson and Dindal, 1982) compared to exotic pasture (Curtis et al., 2017). Emergence traps collect insects as they emerge from a substrate, like soil, and have been known to catch spiders, but are less efficient than other traps (Malumbres Olarte, 2010). Suction sampling is often used for sampling spiders in agroecosystems (McLachlan and Wratten, 2003; Vink et al., 2004) but are often difficult for the public to access and can be expensive (Sutherland, 2006). Ground sampling, sweeping, and pitfall traps are the most appropriate for pastures and are inexpensive to use.

Table 2: Experimental design of the new COBRA protocol in New Zealand pastures.

<table>
<thead>
<tr>
<th>Per (1 ha) plot</th>
<th>Existing meadow COBRA (samples)</th>
<th>Data for new pasture COBRA in New Zealand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground sampling day</td>
<td>0</td>
<td>5 (hours)</td>
</tr>
<tr>
<td>Ground sampling night</td>
<td>4 (hours)</td>
<td>5 (hours)</td>
</tr>
<tr>
<td>Sweeping day</td>
<td>4 (hours)</td>
<td>5 (hours)</td>
</tr>
<tr>
<td>Sweeping night</td>
<td>4 (hours)</td>
<td>5 (hours)</td>
</tr>
<tr>
<td>Pitfall traps</td>
<td>12 traps</td>
<td>4 traps</td>
</tr>
<tr>
<td>Suction sampling</td>
<td>Was not used</td>
<td>9 (mins)</td>
</tr>
<tr>
<td>Total (samples/hours)</td>
<td>24 (hours)</td>
<td>36 (hours)</td>
</tr>
</tbody>
</table>
Table 2 shows the number of samples per (1 ha) plot, for the protocol. Each sample (except the pitfall traps) represents an hour of collecting effort. This excludes related activities such as repairs to gear or transferring specimens to storage vials. Sampling was done in daylight between 9am – 3pm and darkness from 8pm – 2am for each sampling method at all sites. There were four pitfall trap sites, with each site composed of four pitfall traps. Spiders collected were sorted and identified to species level using the taxonomic literature. Six sites in cropping pasture and six sites in dairy pasture were sampled. The data collected were used to i) identify the optimal sampling protocol (combination of sampling method) for different levels of effort, ii) validate lower intensity sampling in other pastures and make it easier to apply generally, and iii) to investigate whether there is a difference in biodiversity in cropping and dairy pastures.

2.3 Pitfall trap protocol

There were 16 pitfall traps used (Figure 6) and they were placed in groups of four at each site. In the four groups, each pitfall trap was one m apart and three m from other groups (Figure 7). The pitfall traps were 100 m away from the fence line. Plastic cups, 69 mm in diameter, were placed into the ground and were filled one third Monopropylene Glycol and labelled with a liquid-resistant pen. A metal roof was placed over each pitfall trap (Figure 6), which follows the Department of Conservation’s guidelines for invertebrate pitfall traps (Sherley and Stringer, 2016). The traps were left in the field for seven days. Initially the traps were going to be left in for two weeks, however, it was limited to a week, as the sites were part of working farms. For this study, animals were off-site as livestock will often interfere with pitfall traps (Sutherland, 2006).
Figure 6: Pitfall trap; sampling cup with antifreeze and a label; metal roof on top of the sampling cup.

Figure 7: Pitfall trap layout.
2.4 **Ground sampling protocol**

The transect lines were 30 metres long. Ground sampling was performed in six 10-minute periods and were five metres distant from the transect line. This was repeated another four times on different transect lines to reach the five hours of sampling. Each site had a total of 30 samples. Ground searching involved crawling through the pasture with an aspirator and searching through the grass at root level (Figure 8). Occasionally, small areas (approx. 10 cm across and 2 cm deep) of thick pasture were dug up and searched on a beating sheet. The labelling and placing specimens into vials were done at 5-minute intervals. All labels for each sampling method were pre-made, as it made labelling faster.

![Ground sampling procedure](image1.jpg)

**Figure 8:** Ground sampling procedure (left) shows typically what is seen at ground level and (right) shows researcher using pooter to collect samples.
2.5 Sweeping protocol

The transect lines were 30 metres long. Continuous sweeping was done for twelve, five-minute periods and were within ten to twenty metres distance from the transect line (Figure 9). Each site had a total of 60 samples. There was a total of five transect lines. It was not possible for some of the sampling to be completed as dew had formed on the grass during some of the sampling times. There were four pasture sites where only three hours of sweeping was done due to this issue. There was also a two-minute maximum collecting period to remove spiders found in the net, excluding labelling. The sweep net was emptied onto a beating tray or sheet and was collected using an aspirator and/or hand collected in vials. Specimens were put in vials with 70% ethanol and labelled. Sweeping was timed with a stopwatch for accuracy.

![Sweep net and sweep net procedure](image)

Figure 9: Sweep net and sweep net procedure.

2.6 Suction sampler protocol

The suction sampler had a sampling pipe diameter of 16.4 cm and was built from a modified leaf blower. To avoid bias, the area was chosen by throwing a piece of paper and sampling the area where it landed. This was done by walking roughly into the centre of the pasture. To avoid bias my eyes were closed and I spun around 5 times and threw it into the direction that was straight in front of me at the end of the spinning. It was not done on a windy day. Suction sampling was carried out in a square 1 m². In the transect square the sampling pipe was placed firmly on the ground and held for 30 seconds. A pitfall trap cup was placed onto the other end of the pipe to collect the spiders. The suction sampler was lifted and placed
onto another patch of ground in the transect square and held for another 30 seconds. This was repeated until it reached three minutes (Figure 10). Each site had three samples totaling nine minutes of sampling. Samples were transferred into labelled 69 mm diameter plastic cups containing 70% ethanol.

2.7 Data analysis

The software package Genstat 19th Edition (VSN International, 2019) was used to calculate randomised species accumulation curves for observed species richness and Slope S and to statistically determine if the randomised curves reached asymptote, and whether the curve was still increasing or decreasing at the end of the sampling procedure. The final section of the curve was calculated as Slope S = 1/(n_s - n_s+1) where n_s = total quantity of individuals, i.e. conforming to the overall richness of value S, and n_s+1 = number of individuals conforming to the curve at a point where the last single species was deducted or added to the value of S, i.e. conforming to the value richness of S + 1 (Cardoso et al., 2008b). All curves are sample based, and individuals are rescaled. Four species richness estimators (Jackknife 1, Jackknife 2, Chao 1, and Chao 2), observed species richness. Singletons and doubletons were calculated for each site and sampling method with 100 sample order randomizations in R (studio). Sampling completeness is the number of observed species
divided by the estimates (Chao 1) number of species. It has been suggested in previous studies that Chao 1 is the most accurate and is the reason why the value of completeness can be evaluated to different studies (Cardoso et al., 2008b). To assess the Methods and Dairy and Cropping Interaction, the data transformed by square rooting the data in excel to fit where possible a normal distribution curve. These data were summarised by using log 10 as there were many zeros present. This involved ignoring the individual species and focusing on the methods and the vegetation types. In Minitab a Johnson’s transformation was used to meet the assumptions of a normal distribution curve. An ANOVA was run with the summarised data on Minitab. A multiple comparison Sidaks was used as it has a conservative estimation.
Chapter 3: Results

3.1 Collection methods
A total of 2767 spiders were caught, which included a total of 1384 adults (50%) representing 12 families and 28 species (Table 4 and 7). Pitfall traps caught the highest total of 1500 individuals, that included 820 adults (Table 3). Ground sampling caught a total of 1093 individuals, including a total of 522 adults (Table 3). Suction sampling caught a total of 89 spiders with 36 of them being adults (Table 3). Sweeping caught the least number of spiders with a total of 85 that included only 6 adults (Table 3).

Table 3: Number of individual spiders caught in each different sampling method.

<table>
<thead>
<tr>
<th>Sampling Method</th>
<th>Adults</th>
<th>Juveniles</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pitfall Traps</td>
<td>820</td>
<td>680</td>
<td>1500</td>
</tr>
<tr>
<td>Ground Sampling</td>
<td>522</td>
<td>571</td>
<td>1093</td>
</tr>
<tr>
<td>Suction Sampling</td>
<td>36</td>
<td>49</td>
<td>85</td>
</tr>
<tr>
<td>Sweeping</td>
<td>6</td>
<td>83</td>
<td>89</td>
</tr>
</tbody>
</table>

3.2 Richness estimators
The number of species present at each site was estimated using four different richness estimators (Jackknife 1, Jackknife 2, Chao 1 and Chao 2) (Table 4). Sampling method was one of the most important factors influencing the result producing significant differences between methods ($F_3, 40 = 13.22, p = <0.01$).

3.2.1 Pitfall traps
Among the non-parametric estimators, the accumulation curves of Chao 1 and Chao 2 reached asymptote (Table 4). Chao 1 (26 species) and Chao 2 (27 species) produced the lowest estimation of the number of species found in pitfall traps. Jackknife 1 (29 species) and Jackknife 2 (30 species) were higher and the accumulation curves did not reach asymptote. Sample completeness of 92% was estimated using Chao 1. This means that 92% of the estimated sum of species at the site was collected. Three species were singletons (11.1%) and two species were doubletons (7.6%) (Figure 15).
3.2.2 Ground Sampling

The accumulation curves of Chao 1, Chao 2, and Jackknife 2 reached asymptote (Table 4). Both Chao 1 and Jackknife 2 generated the lowest estimate of 21 species. The estimate of Jackknife 1 (22 species) was higher, but the accumulation curve did not reach asymptote. Sample completeness of 80.7% was estimated using Chao 1. One species was a singleton (4.7%) and two species were doubletons (9.5%) (Figure 16).

3.2.3 Sweeping

Among the non-parametric estimators, the accumulation curves of Chao 1 and Chao 2 reached asymptote (Table 4). An estimate of 5 species for Chao 1 and 4.5 species for Chao 2 produced the lowest estimates. The estimates of Jackknife 1 (6 species) and Jackknife 2 (8 species) were higher, but the accumulation curves did not reach asymptote. Sample completeness of 17.8% was estimated using Chao 1. Two species were singletons (40% of the total species collected using this sampling method) and there were no doubletons (Figure 17).

3.2.4 Suction sampling

Among the non-parametric estimators, the accumulation curves of Chao 1 and Chao 2 reached asymptote (Table 4). The estimates of 6 species by Chao 1 and Chao 2 were the lowest estimators. The estimators of Jackknife 1 and Jackknife 2 (7 species) were higher, but the accumulation curves did not reach asymptote. Sample completeness of 22.5% of the estimated species collected at the site was found using Chao 1. One species was a singleton (15.8%) and one species was a doubleton (15.8%) (Figure 18).
Table 4: Quantitative results and species estimates from the sampling methods used in the COBRA protocol.

<table>
<thead>
<tr>
<th></th>
<th>Pitfalls</th>
<th>Ground Sampling</th>
<th>Suction Sampling</th>
<th>Sweeping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individuals</td>
<td>1500</td>
<td>1093</td>
<td>85</td>
<td>89</td>
</tr>
<tr>
<td>Species</td>
<td>26</td>
<td>21</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Singletons</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Doubletons</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Jackknife 1</td>
<td>29</td>
<td>22</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Jackknife 2</td>
<td>30</td>
<td>21</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Chao 1</td>
<td>27</td>
<td>21</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Chao 2</td>
<td>26</td>
<td>21.5</td>
<td>6</td>
<td>4.5</td>
</tr>
<tr>
<td>Slope S</td>
<td>26.9</td>
<td>21.7</td>
<td>6.3</td>
<td>5.01</td>
</tr>
</tbody>
</table>

Pitfall traps asymptote at 26.9 species (Figure 11). Ground sampling asymptotes at 21.7 species (Figure 12). Suction sampling asymptotes at 6.3 species (Figure 13). Sweeping asymptotes at 5.01 species (Figure 14).

Figure 11: Species accumulation curve of observed species collected by pitfall traps. Error bars indicate the variation around the estimate for the number of species.
Figure 12: Species accumulation curves of observed species caught by ground sampling. Error bars indicate the variation around the estimate for the number of species.

Figure 13: Species accumulation curve of observed species collected by suction sampling. Error bars indicate the variation around the estimate for the number of species.
Figure 14: Species accumulation curve of observed species caught by sweeping. Error bars indicate the variation around the estimate for the number of species.
Figure 15: Randomised accumulation curves of observed species richness, singletons, doubletons and other estimators for pitfall traps.
Figure 16: Randomised accumulation curves of observed species richness, singletons, doubletons and other estimators for ground sampling.
Figure 17: Randomised accumulation curves of observed species richness, singletons, doubletons, and other estimators for sweeping.
Figure 18: Randomised accumulation curves of observed species richness, singletons, doubletons, and other estimators for suction sampling.
3.3 Time of day

Table 5 shows the species caught from ground sampling, sweeping, and suction sampling that were carried out during the day and the night. These data do not include pitfall traps. The data had too many zero occurrences to perform an ANOVA. A total of seven species were caught during the day and eleven species during the night. *Novakiella trituberculosa* (Roewer, 1942) was the only species that was caught during the day time that wasn’t collected during the night.

Table 5: Percentage of individuals found during the day and the night to one decimal place.

<table>
<thead>
<tr>
<th>Species</th>
<th>Day</th>
<th>Night</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anoteropsis hilaris</td>
<td>8.3%</td>
<td>8.3%</td>
</tr>
<tr>
<td>Araeoncus humilis</td>
<td>8.3%</td>
<td>8.3%</td>
</tr>
<tr>
<td>Badumna longinqua</td>
<td>0</td>
<td>4.1%</td>
</tr>
<tr>
<td>Cryptachaea veruculata</td>
<td>0</td>
<td>8.3%</td>
</tr>
<tr>
<td>Cyclosa fuliginata</td>
<td>8.3%</td>
<td>8.3%</td>
</tr>
<tr>
<td>Erigone wiltoni</td>
<td>16.7%</td>
<td>8.3%</td>
</tr>
<tr>
<td>Eriophora pustulosa</td>
<td>16.7%</td>
<td>16.7%</td>
</tr>
<tr>
<td>Haplinis mundenia</td>
<td>0</td>
<td>8.3%</td>
</tr>
<tr>
<td>Holoplatys oppressus</td>
<td>0</td>
<td>4.1%</td>
</tr>
<tr>
<td>Hypoblemum sp.</td>
<td>0</td>
<td>4.1%</td>
</tr>
<tr>
<td>Novakiella trituberculosa</td>
<td>8.3%</td>
<td>0</td>
</tr>
<tr>
<td>Taphiassa punctata</td>
<td>0</td>
<td>4.1%</td>
</tr>
<tr>
<td>Tenuiphantes tenuis</td>
<td>33.3%</td>
<td>16.7%</td>
</tr>
</tbody>
</table>
3.4 Dairy and Cropping interaction with spiders

![Interval Plot of counts](image)

*Individual standard deviations are used to calculate the intervals.*

**Figure 19**: The interaction between vegetation types: Cropping (C) and Dairy (D) with sampling method: Ground sampling (A), Pitfall traps (P), Suction sampling (Su), and Sweeping (Sw).

There was no significant between vegetation type and methods of capture ($F_{3, 40} = 0.81, p = 0.424$). There was a significant difference between methods of capture ($F_{3, 40} = 13.22, p < 0.01$). There was a significant difference between vegetation types of dairy pasture and cropping pasture ($F_{1, 40} = 5.60, p = 0.023$) (Appendix A). Using Sidak’s multiple comparisons at 5% ALPHA, A (ground sampling) and P (pitfall traps) were not significantly different. Su (suction sampling) and Sw (sweeping) displayed no significant difference. Between A & P and Su and Sw there was a significant difference between groups (Appendix A). There was a significant difference between the two pasture types: dairy and cropping (Figure 19; Appendix A).
3.5 Unique species

Pitfall traps collected 12 unique species. Ground hand collection collected 4 unique species (Table 6). Sweeping collected 1 unique species and suction sampling collected no unique species (Table 6).

Table 6: Unique species captured by specific sampling methods (x) exotic and (n) native.

<table>
<thead>
<tr>
<th>Pitfall traps</th>
<th>Ground sampling</th>
<th>Sweeping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anzacia gemmea (x)</td>
<td>Badumna longinqua (x)</td>
<td>Novakiella trituberculosa (x)</td>
</tr>
<tr>
<td>Cantuaria dendyi (n)</td>
<td>Holoplatys appressus (n)</td>
<td></td>
</tr>
<tr>
<td>Diploplecta communis (n)</td>
<td>Hypoblemum scutulatum (x)</td>
<td></td>
</tr>
<tr>
<td>Erigone prominens (x)</td>
<td>Taphiassa punctata (n)</td>
<td></td>
</tr>
<tr>
<td>Haplinis fucatina (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haplinis titan (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemicloea rogenhoferi (x)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lampona cylindrata (x)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laetesia germana (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microctenonyx subitaneus (x)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nyssus coloripes (x)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ostearius melanopygius (x)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 7: A guide to all the spider species caught in this study.

<table>
<thead>
<tr>
<th>Species/Common name</th>
<th>Family</th>
<th>Key features</th>
<th>Female genitalia</th>
<th>Male genitalia</th>
<th>Key references</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Taphiassa punctata</em></td>
<td>Anapidae</td>
<td>Minute size (0.57-0.67). Abdominal scutes reddish-brown. Carapace is coarsely punctate, see below.</td>
<td><img src="image1" alt="Female genitalia" /></td>
<td><img src="image2" alt="Male genitalia" /></td>
<td>Forster (1959) pp 301-302. Rix and Harvey (2010) pp 80, 143, 271. Paquin et al., (2010) p 106.</td>
</tr>
<tr>
<td><em>Cyclosa fuliginata</em></td>
<td>Araneae</td>
<td>Medium size (3.5-5 mm). Both eyes are strongly recurved. Highly variable colour pattern on the abdomen.</td>
<td><img src="image3" alt="Female genitalia" /></td>
<td><img src="image4" alt="Male genitalia" /></td>
<td>Dondale (1966) pp 1162, 1164, 1167. Paquin et al., (2010) p 106.</td>
</tr>
</tbody>
</table>
| **Eriophora pustulosa**  
Knobbled Orbweaver | **Araneae** | **Description** | **References** |
|-------------------|-------------|-----------------|---------------|

![Photo taken by: Mark Tutty](image)

| **Novakiella trituberculosa**  
Orbweaver | **Araneae** | **Description** | **References** |
|-------------------|-------------|-----------------|---------------|

![Photo taken by: Mark Tutty](image)
| **Nyssus coloripes**  
Spotted Ground Swift Spider | Corinnidae | Medium size (9 - 10 mm). Black and white pattern on abdomen and cephalothorax. Front pair of legs have an orange colouration. Eyes are in a compact group with posterior row strongly recurved. |  | Raven (2015) pp 131-134.  
| --- | --- | --- | --- | --- |
| **Badumna longinqua**  
<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Description</th>
<th>Sources</th>
</tr>
</thead>
</table>
| **Hemicloea rogenhoferi**  
Flattened Bark Spider | **Gnaphosidae** | Large size (16 mm). Legs in the laterigrade position. It has a distinctively flattened body and an elongated oval abdomen. | **Paquin et al., (2010) pp 18, 76, 77, 106.** |
|----------------------|----------------|-------------------------------------------------------------------------------------------------|-----------------------------------------------|

| **Cantuaria dendyi**  
Trapdoor Spider | **Idiopidae** | Large size (12 -18 mm). Carapace orange brown. The fovea is deep and straight. Males have tibial processes on leg one. | **Forster (1968) pp 16 -17.**  
**Paquin et al., (2010) p 106.** |
| **Lampona cylindrata**  
| Forster (1979) p 71. |

| **Araeoncus humilis**  
| Sheet-web Spider | Linyphiidae | Minute size (1.45-1.80 mm). Abdomen grey to black. The anterior part of the cephalothorax is elevated and truncated. | Internal genitalia | Millidge (1988) pp 61 – 62.  
| Blackwall (1841) pp 636 – 637.  
| **Diploplecta communis**  
Sheet-web Spider | Linyphiidae | Small size (1.5 - 2 mm). Spider eyes have a black eyeshadow appearance. Variable pattern of black markings on the abdomen. Legs are long with spines on the tibiae & metatarsi. | Millidge (1988) p 45.  
<table>
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<tbody>
<tr>
<td><img src="image1" alt="Diploplecta communis" /></td>
<td><img src="image2" alt="Diploplecta communis" /></td>
<td><img src="image3" alt="Diploplecta communis" /></td>
<td><img src="image4" alt="Diploplecta communis" /></td>
</tr>
</tbody>
</table>

| **Erigone promiens**  
Dwarf Spider | Linyphiidae | Small size (0.83 - 1.95 mm). Chelicerae in the male have 6 curved and pointed teeth on the anterolateral face. | Millidge (1988) pp 63, 65, 66.  
Locket (1973) pp 159, 161.  
<table>
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<tbody>
<tr>
<td><img src="image5" alt="Erigone promiens" /></td>
<td><img src="image6" alt="Erigone promiens" /></td>
<td><img src="image7" alt="Erigone promiens" /></td>
<td><img src="image8" alt="Erigone promiens" /></td>
</tr>
</tbody>
</table>
| **Erigone wiltoni**  
Dwarf Spider | **Linyphiidae** | Small size (1.4-2.1 mm). Carapace orange-brown to deep chestnut brown. Chelicerae in the male have 4 to 5 curved and pointed teeth on the anterior-lateral face. | **Millidge (1988) pp 64 – 66.**  
**Locket (1973) pp 162 – 163.**  
**Paquin et al., (2010) p 106.** |
| --- | --- | --- | --- |
| **Haplinis fucatina**  
Sheet-web Spider | **Linyphiidae** | Small size (1.75 – 2.48 mm). Cephalothorax dark brown. Legs annulated. Tibia one black with a yellow basal band. | **Blest (1979) pp 119 – 121.**  
**Paquin et al., (2010) p 106.** |
| **Haplinis mundenia**  
Sheet-web Spider | **Linyphiidae** | **Small size (1.75-3.78 mm).**  
Cephalothorax yellow brown. Abdomen grey with folium reduced to two dark stripes. | **Blest (1979) pp 106 – 110.**  
|-----------------|-----------------|-----------------------------|-----------------------------|
| **Haplinis titan**  
Sheet-web Spider | **Linyphiidae** | **Small to medium size (3.5-8 mm).**  
Cephalothorax dark brown. Legs with pattern of very faint annulations. Femur one with prolateral and one dorsal spine. | **Blest (1979) pp 116 – 118.**  
<table>
<thead>
<tr>
<th>Species</th>
<th>Size Range</th>
<th>Description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ostearius melanopygius</em></td>
<td>Small size (2 – 3 mm)</td>
<td>Orange red abdomen is the most distinctive characteristic.</td>
<td>Paquin et al., (2010) pp 55, 56, 106.</td>
</tr>
</tbody>
</table>
| Allotrochosina schauinslandi | Lycosidae | Medium size (2 – 9.7 mm). Eyes in three rows, posterior median and lateral eyes are much larger than anterior eyes. Posterior row is highly recurved. | Vink (2002) pp 18 – 19. 
Paquin et al., (2010) pp 95, 106 |
|-----------------------------|-----------|---------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|
Paquin et al., (2010) pp 95, 106 |
| **Holoplatys apressus**  
| Crevice Jumping Spider | Salticidae | Small to large size (2 – 11 mm). Eyes; AME largest directed forwards. Cephalothorax oblong body remarkably flattened. Colour uniform grey. | No photos available | No photos available | Powell (1873) p 281.  
| | | | | | |
| **Hypobleum scutulatum**  
| House Hopper Spider | Salticidae | Small to large size (2 – 11 mm). Eyes; AME largest directed forwards. Males and females have different colouration and patterns. | | Paquin et al.,(2010) pp 72, 73, 106. |
| Cryptachaea veruculata  
Diamond Comb-footed Spider | Theridiidae | Small size (1.5 – 4 mm).  
| **Steatoda capensis**  
| False Widow Spider |

| Theridiidae |
| Medium size (3-7 mm). Tarsus IV with a row of lightly curved, serrated bristles. Male carapace brown with granulations. Female carapace black to brown. Round black abdomen with some females having white and red markings. |

| **Tenuiphantes tenuis**  
Sheet-web Spider | **Linyphiidae** | **Female** | **Male** |
<table>
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<tbody>
<tr>
<td><img src="image1" alt="Female Spider" /></td>
<td>Small size (2.2 – 2.5 mm). Main feature when looking at the male’s palp is the form of the lamella highlighted in the red circle. All spines are long and strong. Long slender legs.</td>
<td><img src="image2" alt="Female Spider" /></td>
<td></td>
</tr>
<tr>
<td><img src="image3" alt="Male Spider" /></td>
<td><img src="image4" alt="Male Spider" /></td>
<td><img src="image5" alt="Male Spider" /></td>
<td><img src="image6" alt="Male Spider" /></td>
</tr>
</tbody>
</table>

Chapter 4: Discussion

This study is the first New Zealand-based attempt to estimate spider biodiversity in exotic pasture in a standardised way that is comparable with COBRA protocols in other areas around the world.

4.1 Species richness

There are limited publications on spiders in agroecosystems in New Zealand and most are 10 or more years old, which is unexpected as spider diversity in New Zealand is rich and farmland covers about 54.8% of New Zealand (Statistics New Zealand, 2012). There are currently only five research publications that examine spider biodiversity and abundance in agroecosystems in New Zealand (Topping and Lovei, 1997; Hodge and Vink, 2000; McLachlan and Wratten, 2003; Clark et al., 2004; and Vink et al., 2004). There have been other publications on the predatory effects of arthropods and spiders in agroecosystems in New Zealand (Sivasubramaniam et al., 1997; and Bowie et al., 2014).

The species richness found in this study was above the average of 10-45 species found in other pastures in New Zealand. This study found a total of 28 species. Other research papers found approximately 10 - 45 species in pastures (Topping and Lovei, 1997; McLachlan, 2000; McLachlan and Wratten, 2003; Vink et al, 2004; Clark et al., 2004). Toping and Lovei (1997) collected the first quantitative data on the diversity and density of spiders on cultivated land under different farming systems in New Zealand and compared them with similar pastures in England. The main pasture site they used was an AgResearch farm at Flock House in the North Island. There was a total of 23 species found across nine sites at Flock House. The results showed that as disturbance decreased (management/grazing) the number of individuals and species increased, a general trend that is to be expected. However, the pitfall traps were put into the paddock from the edge running to the centre, which ensured that pitfall traps that were close to the edge captured spiders found near the fenced areas, and hence not from a pasture habitat.
McLachlan and Wratten (2003) studied the spider richness and abundance in pasture and field margins across Canterbury, New Zealand. Spider abundance decreased the further that the pitfalls were from a shelterbelt. The pitfall traps near shelterbelts had greater species richness, with 25 species found, compared to only 13 found near the centre of the pasture. Their study was conducted from 1994 to 1997. Thirteen species found in the centre of a pasture is lower than the 28 found in this study but they only sampled using a 21cc McCulloch Super Airstream IV Gas blower/Vac motor (Suction sampler). Topping and Lovei (1997) limited the sampling methods to pitfall traps and a suction sampler. The number of species recorded might have increased in these studies if other sampling methods, such as ground sampling, had been used. This study showed that suction sampling was the least efficient in capturing the maximum number of species. Suction sampling did not capture any new species other than those found in the pitfall traps.

Vink et al., (2004) assessed spider population density and species richness in arable crops and grasses across Canterbury, New Zealand. Across the different samples of grasses and arable crops a total of 20 species of spiders were found. Spider species were found to be higher in pastures compared to arable crops (Vink et al., 2004). Topping and Lovei (1997) found in that spider density increased in pasture compared to that found in wheat crops. One study found cereal crops have a lack of habitat diversity (Vink et al., 2004). This study is different in that it found more species in the cropping pasture (wheat and brassica crops) than the dairy pasture (ryegrass and white clover). This is discussed in detail in section 4.5 (Dairy vs Cropping). Vink et al., (2004) used suction sampling as their only method. This study also found that species richness and abundance was much greater in pastures that had been ungrazed. This is likely caused by less disturbance in the pasture habitat (Vink et al., 2004).

Clark et al., (2004) assessed the effect that cultivation had on spider biodiversity and density in pastures in Waikato, New Zealand. Suction sampling was the only method used and the pasture was sampled five times before cultivation and then monthly intervals after cultivation. Their study also assessed the pasture compositions of grass, clover, weed, bare ground and litter, as it is likely that plant architecture can influence spider distribution rather than influencing a particular species. They found a total of 22 species across 11
families in total. The results showed there was a decrease after the first cultivation sampling, but there was an increase in spider density in the control sites at the same time. The intensity of the grazing did have an impact on spider abundance and diversity as expected. As seen in international studies low intensity farms have been found to create low disturbances and this results in a higher biodiversity compared to high/intermediate intensity farming that has higher disturbance levels resulting in a significant decrease in biodiversity levels (Hoogenveen et al., 2001; Doxa et al., 2010; Gabriel et al., 2013). Hoogenveen et al., (2001) suggest that this scenario is a unimodal relationship where biodiversity increases and drops when intensity increases.

4.1.1 Spider diversity

This study found a total of 28 species from 12 families (Table 7). The most abundant species collected was *Tenuiphantes tenuis*. A total of 484 individuals were caught. This was closely followed by *Anoteropsis hilaris* with a total of 478 individuals collected and 722 individual spiderlings of *Lycosidae*. *Anoteropsis hilaris* is the most abundant predator in these agroecosystems (Vink, 2002). *Linyphiidae* was the most abundant family with a total of 11 species collected. Three species from the genus *Haplinis* were collected, *H. fucatina*, *H. mundenia* (Urquhart, 1894), and *H. titan* (Blest, 1979). *Haplinis fucatina* and *H. titan* had been previously collected in Canterbury pastures (McLachlan and Wratten, 2003; Curtis et al., 2017). In this study there were 20 exotic species and 8 native species found (Table 7). New Zealand pastures often have a lack of habitat diversity partly because they have been established relatively recently with exotic grasses where, previously, forest existed (Topping and Lovei, 1997). Endemic species may not have been able to easily invade the new habitat. Likewise, suitable prey may not have been able to easily invade the new habitats.

4.2 Sampling methods

The results showed the methods were one of the most important factors to consider in detecting species (Appendix A). Pitfall traps and ground sampling captured a larger number of species than sweeping and suction sampling.
4.2.1 Suction sampling

All five studies that sampled spiders in agroecosystems in New Zealand used suction sampling as the only method to capture spiders apart from Topping and Lovei (1997) who also used pitfall traps. In their study, suction sampling collected a total of six species. Five of the six species were captured in cropping pasture while one species was caught in dairy pasture. It is difficult to compare studies as most are spread across different New Zealand pastures, but Vink et al (2004) did sample sites around Lincoln that did vary between crops of ryegrass, fescue (*Festuca arundinacea* S), cocksfoot (*Dactylis golmerata* L.), prairie grass (*Bromus willdenowii* Kunth), wheat and barley, as well as samples in ungrazed pastures. Across all sites the spider populations and species richness were predominately higher in the grasses compared to the cereals (Vink et al, 2004). The results of the present study showed cropping pasture had the highest species richness compared to dairy, which is opposite to Vink et al.,(2004) findings. This may be due to the intensity of grazing, the management practises occurring on this farm, or a more complex habitat structure than other cropping pastures. The demonstration dairy farm was an active farm. Previous to my sampling, two of the sites were grazed by cows and irrigation was consistently on from the morning until night. The sites in the Iversen fields were relatively low intensity farming. There was no irrigation on while sampling, and the hoggets had not been on the pasture for a month.

The limited number of studies make it hard to compare spider richness in pastures. Suction sampling can not be used when it is wet. In dairy pastures, that can be very difficult as irrigation is used frequently and in this study it was very difficult to sample using this method.

Chao 1 and Chao 2 estimators suggest that suction sampling had reached asymptote, but Jackknife 1 and Jackknife 2 estimators suggest that, with one or two more samples, the maximum number of species would have reached asymptote. The species collected from suction sampling were also collected using pitfall traps or using sweeping. There were no unique species collected with the suction sampling method. An aim of this protocol is to make sampling quick and cost efficient. Suction sampling is not cost efficient for most
people as many do not have access to this equipment. However, this method is quick compared to sweeping.

### 4.2.2 Sweeping

Sweeping had the lowest sampling completeness. Less than one quarter of the number of species at the sites were collected by sweeping. This may be due to environmental factors, including pasture dampness and short grasses in the cropping pastures. Sweeping was designed to be used in long grasses and occasionally used in short vegetation (Sutherland, 2006). The advantages of using a sweep net are that nets are easily available and simple to use. The issues that arise from sweeping in pastures, that would cause some problems, include the fact that sheep pastures are normally quite short, whereas dairy pasture have longer grass, useful for sweeping. However, dairy uses irrigation which makes it difficult to sweep. Sweeping, as with suction sampling, cannot be used in wet conditions, which makes it difficult sampling pastures with frequent irrigation. This study was carried out during the summer and it was difficult to sample these pastures using sweeping. It’s important in the COBRA protocol that the methods chosen can be used widely in exotic pastures but the difference in the grass heights and irrigation levels may cause issues with this method.

Sweeping carried out in the dairy pastures collected a large number of slugs compared to the cropping pasture where the majority of the sweep were dipterans (Figure 20). Over six sites on dairy pastures, there was a total of three spider individuals from two species; *Cyclosa fuliginata* (L. Koch, 1872) and *Novakiella trituberculosa*. In the cropping pasture a total of 86 spider individuals were caught from one species: *Cyclosa fuliginata*. The remaining individuals were all juveniles from the family Araneidae. Even though the individual’s numbers were low compared to those from pitfall traps and ground sampling, this is the only method that caught any species from Araneidae (apart from suction sampling that caught one individual from Araneidae). Sweeping is the best way to catch species from this family. One advantage of sweeping is that it is capable of collecting unique species that other methods do not collect. In this study sweeping was they only method that collected *Novakiella trituberculosa*. 

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*60*
Figure 20: Sweeping in the dairy pasture (left) showing a large number of slugs and sweeping in the cropping pasture (right) showing a large number of Diptera.

4.2.3 Pitfall traps

Pitfall traps were the best method for catching spider species with 26 out of the total 28 caught. Topping and Lovei (1997) appears to be the only study in New Zealand that looks at spider diversity and abundance and uses pitfall traps as one of the collection methods. Their pitfall traps collected a total of 10 species. Six out of the ten species collected belonged to the family Linyphiidae. There was one species from Lycosidae, one species from Pisauridae, and two species from Theridiidae. The Topping and Lovei (1997) diversity is low compared to this study, however, their pastures were in the North Island and, because there have been few studies on the diversity of spiders in pastures, it may be that the diversity is lower. Their study showed a similar pattern in that Linyphiidae was the dominant family and this present study found 10 species. There were also another 8 families collected as well. Pitfalls are designed to collected ground dwelling arthropods (Moeed & Meads, 1985; Norbury et al., 2009). There are certain species, like *Tenuiphantes tenuis*, which build webs just above the ground’s surface. This species is unlikely to be caught in pitfall traps and often escapes (Topping, 1993). The data collected showed there were three individuals caught in the pitfall traps compared to 340 individuals found with ground sampling. Only 1.5% of the individuals were *Tenuiphantes tenuis* compared to 56% when ground sampling was used. This suggests
that ground sampling supplements pitfall traps. Only one of the non-parametric estimators suggests that the pitfall traps reached asymptote (Chao 2 = 26). Jackknife 2 suggests there should be an extra four species to reach asymptote. More effort was probably needed when using pitfall traps. An extra four pitfalls traps should be used to increase the chance of capturing extra species.

4.2.4 Ground sampling

Ground sampling was the second-best method for collecting a high number of species. This method collected 21 of the 28 species found in this study. There have been no studies in New Zealand that sample spiders by ground sampling in pastures. There were five species that ground sampling caught that were not found by any other method. These species were: *Taphiassa punctata, Holoplatys appressus, Hypoblemum albovittatum, Eriophora pustulosa*, and *Badumna longinqua*.

This method was the closest to reaching asymptote. Three of the non-parametric estimators; Jackknife 2, Chao 1 and Chao 2 suggested that ground sampling had collected the maximum number of species. Jackknife 1 showed that ground sampling did not reach asymptote and suggested that one more species would reach it. This is shown in the accumulation curves where Chao 1 curve from ground sampling approached the asymptote, whereas Jackknife 1 shows signs of under sampling (Figure 16).

4.3 Time of day

Time of day affected the number of individuals and species caught. Night collecting collected more species than day collecting. Overall, 12 species were caught in total using sweeping, suction sampling and ground sampling. During the day a total of seven species was found compared to 12 species at night. One species *Novakiella trituberculosa* was the only species that was found during the day that wasn’t collected at night. Night sampling collected 91.6% of the species found while day sampling collected 53.8%. If sampling during the daytime 46.1% of diversity is expected to be lost. Time of day could be considered as a different method itself. In all of the New Zealand studies that look into spider diversity and abundance in pastures, it has not been clear whether samples were collected during the day.
or the night. I have assumed that they were collected during the day as, if it was collected during the night, it would be expected to be noted in the methods. Spiders are nocturnal and very active during the night (Nørgaard et al., 2012; Yuen and Bonebrake, 2017). For this reason, sampling should be carried out mainly at night but supplemented with day samplings. The results of this study show that sampling at night collects a lot more species than sampling during the day.

### 4.4 Collection method and time of day interaction

In this study an experienced arachnologist carried out all the sampling methods of the protocol. The methods that are quantitatively defined depend on person-hours. Sweeping, suction sampling and ground sampling are dependent on time spent searching. Because this study only had one collector, the collector effectiveness was not tested. Given the small number of methods used, the species list attained from this protocol was not as exhaustive as the other COBRA protocols around the world (Cardoso et al., 2008a; Cardoso et al., 2008b; Cardoso, 2009). This is due to the simple structure that limits your choice of methods. There was a significant difference between sampling methods. Ground sampling and pitfall traps had no significant difference between them, but there was a significant difference between suction sampling and sweeping. Almost all methods were more effective at collecting species at night. Sweeping was the method that was only marginally better during the day. During April to May, at night the pasture begins to form dew on the surface of the grass, which made it difficult to sweep. Suction sampling and ground sampling collected higher numbers of species during night sampling compared to day sampling. I am unable to say whether time of day and methods were significant as time of day had too many absences and that could not be analysed with a statistical test. COBRA protocols overseas show there is a significant difference between methods and time of day and most conclude night sampling is significantly better than day sampling (Coddington et al., 1991; Coddington et al., 1996; Cardoso et al., 2008a; Cardoso et al., 2008b; Cardoso et al., 2009b; Azevedo et al., 2014). This supports the idea of mainly sampling at night and supplementing with sampling during the day. The length of the time spent day sampling could be reduced compared to the night sampling due to the nocturnal habits of many species of spiders, and the difficulty finding many species during the day.
4.5 Rare species

Rare species are difficult to detect as some are either seemingly rare or are genuinely rare (Cardoso et al., 2008b). Under sampling and edge effect (fences/shelterbelts) are often the reasons why large number of species are found in small numbers (Scharff et al., 2003; Cardoso et al., 2008b). The edge effect is that if sampling is too close to the edge of the pasture then the spiders collected will include species that are found in shelterbelts and fences and not normally in pastures. Cardoso et al (2008b) expands on ideas of the edge effects that include: phenological edge effects (spiders that mature in the winter, rather than in the summer during the breeding season), methodological effects (spiders that live in microhabitats that are challenging to sample with the methods that are used), and spatial edge effects (spiders that live in habitats that are not often found in the present habitat that is being sampled). These three effects are the main reasons why rare species are difficult to find. In New Zealand pastures the limited number of studies means there is not a true estimate on spider diversity. Without knowing the majority of the species found and by not collecting in other nearby habitats or in other seasons, it is hard to conclude what the rare species are. In this study, in a few of the sites in the Lincoln University Demonstration Dairy Farm, there were a number of testing areas that were fenced with wooden poles. The size of the pasture made it hard to keep a reasonable distance from these testing areas. *Badumna longinqua* was collected during ground sampling and is typically found around fences and outside houses (Paquin et al., 2010). The few spiders that were collected from this pasture could be labelled as rare when they are not rare because of the edge effect that occurred. There should be careful consideration when calling a species rare in conclusions. The Department of Conservation’s threatened species classification list for spiders serve as a useful guide to ascertaining the genuine rarity of a species.

4.6 Dairy vs cropping

The results showed there was a significant difference between dairy pasture and cropping pasture. From the cropping sites a total of 26 species were collected and from the dairy sites a total of 15 species were collected. The cropping pasture consisted of Arrow ryegrass and white clover. There was also Californian thistle (*Cirsium arvense*) spread through parts of the pasture. The sites that were used were pasture in rest blocks. The rest blocks are used after
it has been used for intensive cropping. Hoggets also graze the rest blocks on occasions. Irrigation did not seem to be on while sampling was taking place. There were two different types of dairy farms; Lincoln University Demonstration Dairy Farm and the Lincoln University Research Farm. The demonstration dairy farm is an active dairy farm. The pasture consisted of Ronsyn/Impact ryegrass, white clover, and Timothy grass. The cows were on site a week before the sites were sampled and the irrigation was consistently on throughout the sampling process. The research dairy farm is used for research by Lincoln University students as well as for commercial use. The pastures consisted of perennial ryegrass and white clover. There were two sites sampled that consisted of chicory, plantain, and lucerne. The cows had been absent from the pastures for two to four weeks and the irrigation was not on while the sampling process was happening. While the pasture of all three farms were relatively similar, the management practices and how the pasture was previously used differ (Figure 19). It is not conclusive that cropping pasture has a better diversity of spiders than dairy farms, to be more generally applicable it needs to be repeated on other farms throughout NZ. There would need to be future research on this looking at the different farming systems and how they affect biodiversity.
Chapter 5: The COBRA Protocol

5.1 Recommendation of methods to use for sampling

The most important influence that has been found across all previous COBRA protocol studies has been the methods used in the design. When and how a COBRA protocol has been used has shown to be statistically different and therefore has to be treated independently. What has been shown in past research is the lack of influence that the collector has on the data, however, it has been recommended that at least one of the collectors has experience in quantitative sampling. Consideration should also be given to what is the best method for the habitat that samples are being taken from to reduce ineffectual sampling methods. (Cardoso, 2009).

It is important to recognise that although certain methods may not yield high numbers of spiders, this does not mean that a particular sampling method is ineffective. It is the number of species found that is important. Previous research (Cardoso et al., 2008b; Cardoso, 2009) has shown that balanced designs are misrepresentative as they do not accurately represent the population in proportion to their abundance. Conversely, unbalanced designs have been shown to be more representative of the population in proportion to their abundance, such as where sampling of habitats is conducted proportional to their occurrence in the landscape (e.g. common habitats are sampled more intensely than rare habitats). This is due to the fact that many methods overlap and, therefore, it is more productive to design a considered unbalanced design, which will often result in better sample accuracy of the focused population (Cardoso, 2009).

5.2 Recommendations of when to sample

As stated earlier, the time of day that the sampling occurs is important. However, just as important is the time of year the sampling occurs. In all areas of New Zealand, the best time of year for the optimal number of species is summer to autumn. In summer, spiders reproduce, which will yield high numbers. Waiting until early autumn means that the spiders have matured into adulthood, which makes this the optimum time to embark on
sampling. Previous researchers have recommended that autumn is best if a researcher is wanting to get a comprehensive representation of the whole population, compared to sampling in summer, which is likely to result in obtaining fewer species (Cardoso, 2009). In mesic habitats (areas that have no shortage of water) heat is the limiting factor, so spiders will be more abundant when it is the warmest (Jagoba Malumbres-Olarte, pers. comm. 2019). In New Zealand the warmest season is summer.

5.3 Recommendations of where to sample

Essential to the protocol is where to sample. Many studies predetermined areas to be sampled. Conversely when researchers have wanted to study large areas for conservation objectives, they have not selected distinct zones. Pasture area will determine where sampling takes place. For large pasture sites sampling needs to occur 100 m from the fence line because of the edge effect that results in spiders being found that do not normally occur in pastures but can be found in fences, driveways, and shelterbelts. Ideally smaller pasture sizes also need to occur 100 m from the fence line but might not always be possible due to the size of the pasture in this case 5 – 15 m away from the fence line is preferable.

5.4 Recommendation of what to sample

Research using the COBRA protocols have shown that using plotless sampling has been just as effective as using plot-based sampling methods (Cardoso, 2009). Jagoba Malumbres-Olarte (pers. comm. 2019) believes that it is important to delimit the space so the same areas can be compared. Using either method is likely to obtain a high number of species, and the composition does not vary (Cardoso, 2009). Sorensen et al.,(2002) found that this is not always the case and there are no differences between plotless and plot-based sampling. The exception to this is ants (James, 2004) and spiders (Dobyns, 1997), due to the rarity of some species. Due to their low numbers, when sampling specific areas they can be easily missed. It has been suggested by previous researchers that it would be beneficial to compare similar zones and to standardise this to one-hectare blocks. Although this protocol is used for ants and butterflies in overseas studies (Agosti and Alonso, 2000; Pollard and Yates, 1995) to measure biodiversity rapidly, in New Zealand it is less effective due to low species numbers. This study chose spiders to measure biodiversity in New Zealand pastures.
due to the low species numbers of butterflies and ants in New Zealand pastures, compared to species of spiders.

5.5 The COBRA protocol for pastures

These are the steps follow the design this COBRA protocol for exotic pastures in New Zealand. There can be multiple collectors if necessary, but it is highly recommended that at least one collector should be experienced with these types of methods. Sampling should be carried out in summer to autumn. If the pasture is consistently wet from irrigation only pitfall traps and ground sampling will be used. A stopwatch, vials, ethanol, a large white sheet, knife, a ruler, head torch, an A4 piece of paper, and labels are needed.

1. Twenty pitfall traps are to be used 100 metres away from fences or shelterbelts. The pitfall traps need to be in a transect line that is divided into four groups. In the four groups, each pitfall trap will be one metre apart and each group will be a minimum of three metres apart (Figure 7). They should be left out for one week. It is recommended that stock are not in the pasture while the pitfall traps are out, as they contain antifreeze, which can poison stock. However, if this is to be compared with other COBRA protocols, such as COBRA-TF (Malumbres-Olarte, 2017) and COBRA Mediterranean cork forests (Cardoso et al., 2008a), there should be 12 samples with 4 pitfall traps at each sample, totalling 48 pitfall traps. The COBRA setup is the same as the one I used in this study with the sites placed in a square around the pasture.

2. Ground sampling is to be carried out only during the night. Samples should be taken at least 100 metres from any fences or shelterbelts. Samples are be taken from soil to pasture level only. Samples should be taken at random around the pasture. Sampling should be carried out for one hour with each individual sampling consisting of searching for spiders at pasture level for 10 minutes, a total of six samples or one sample of one hectare. If there is thick pasture it can be dug up in a 10 cm square and 2 cm deep section and placed on a white sheet to enable collecting of spiders. Labelling, moving between sites, and sorting specimens into vials are not counted in the hour of sampling. To make it easier pre-made labels will make this process more
efficient. Specimens should be stored in ethanol. Stock should not be in the field while ground sampling is taking place for health and safety reasons.

3. **Sweeping needs to be carried out during the night and needs to be 100 metres away from any fences or shelterbelts.** It should be carried out from 11pm -12am. If ground sampling is carried out first, sweeping can be carried out after 12am-1am or vice versa. If the pasture is damp do not carry out this method. Try to sample the pasture when it is dry. If this cannot be done leave this method out altogether. Sweeping will consist of twelve five-minute of continuous sweeping. Sweeping can be done randomly or up and down a transect line. After five-minutes of sweeping empty the contents onto the large white sheet and collect the spiders. The collection period and moving between sites is not part of the sampling hour.

4. **Suction sampling should only be carried out if a suction device is easily accessible.** This method is an alternative to sweeping. This option is available as it is quicker than sweeping for collecting samples, but it is not cost efficient if this item is not available. If the pasture is wet do not use this method. Suction sampling must be carried out during the early morning and again at night. If there is access to a suction sampler, use it in the middle of the pasture and at least 100 metres away from fences or shelterbelts. To avoid bias when selecting a site, throw a piece of crumpled paper into the pasture and sample the spot it lands on. Suction sampling should be carried out in a one metre square plot. The sampling pipe should be placed firmly on the ground and held for 30 seconds. A pitfall trap cup is placed onto the other end of the pipe to collect the spiders. The suction sampler should be lifted and placed onto another patch of ground in the transect square and held for another 30 seconds and this process should be continued for three minutes. This should be carried out four times in different plots around the pasture.

There are elements of other approaches which could have been woven into this COBRA protocol to improve it. Cardoso et al (2009a) used a number of collectors, while this study only used one collector. A number of collectors that had a range of experience might have been useful in this study, as there may be a range of different people that may use this
protocol e.g. farm managers/biodiversity managers (less experience with spiders) or research students (medium experience). The collector in this study had an advance knowledge on spiders, whether that has had an impact, it has not been researched in this study. Other COBRA protocols (Cardoso et al., 2008a; Cardoso et al., 2008b) also didn’t have sites that were small in size. A few sites form the Lincoln University Research Dairy Farm were combined as the areas alone were too small to conduct extensive sampling. Pastures that have been divided up into small rectangles for research does cause an issue for this protocol. This is due to the fencing. It is hard to sample the true pasture species, as there is interference with the fence line species that are present, which would not normally be captured if sampling a larger pasture field. This studied noted the present of Badumna longinqua which is commonly found on fence lines near pastures, but not normally within the pasture itself. This species was picked up during the sampling of the smaller sites on the Lincoln University Research Dairy Farm that had been lumped together to avoid the spatial effects. In future it would be best to sample pastures that can exceed 100 m from the edge of the pasture and still be extensively sampled within that area. Small pastures can still use this protocol, but it should be noted that edge effect species will be collected.
5.6 Conclusion

Agriculture and biodiversity conservation are often seen as incompatible and there is often conflict between management practices and the way the land is used (Tschanrntke et al., 2005). Agriculture focuses on production, while ecologists and conservationists focus on restoring habitats and biodiversity in the remnants of natural habitats that are left. Biodiversity is beneficial for society but as long as costs are prohibitive in biodiversity up to an optimal level it is more likely biodiversity will be left at sub-optimal levels (Peerlings and Polman, 2004; Wossink and Swinton, 2007; Gullstrand et al., 2014). Agriculture will always be a large part of New Zealand’s and the world’s future (Lanz et al., 2018). With human population rising quickly, food production is continuing to rise accompanied by a loss of forests, oceans, and natural habitats (Mora and Sales; 2011; Crist et al., 2017; Lanz et al., 2018). The push for a sustainable environment has intensified over the past few years. Agriculture remains a potential threat to the environment and agriculture needs to become more sustainable. There are several ways that practices in agriculture cause damage to the environment including; nitrogen leaching into rivers (Cameron et al.2013), gases from animal manure increasing global greenhouse emissions, mainly from methane (McMichael et al., 2007), pesticides that run off into rivers (Triegel and Guo, 2018) and the loss of predatory insects (Bengtsson et al., 2005). When assessing the effects of farming practices on productivity, farmers should also consider the effect on biodiversity. Accepting a slightly lower level of productivity may have beneficial effects on biodiversity.

One of the main purposes of this research is to look at a way to systemise and improve biodiversity assessment protocols for pastures in New Zealand. There are many taxa, including spiders, that are often difficult to sample. This COBRA protocol offers a method to achieve this. Furthermore, this COBRA protocol allows the collecting of the highest diversity with the least amount of effort. Moreover, it appears to be more accurate than previous estimators, especially for species that occur in smaller numbers or are challenging to collect. Multiplying the surveyed richness is more robust and lends itself to less bias than estimating known approximate number of species in individual areas. Using a standardised protocol in pasture allows biodiversity to be monitored over time to accurately assess whether it has increased or decreased as farming strategies change. Different pasture compositions, different applications of fertiliser or insecticides, and different stocking levels are some of
the farming strategies that can affect biodiversity. This study found 28 different species. Cor Vink indicated up to 40 species could be found in Canterbury pasture depending on the season (pers. comm.). As more studies are carried out it will become easier to assess what number of species found represent a high or low level of biodiversity.
References


Blackwall, J. (1841). The difference in the number of eyes with which spiders are provided proposed as the basis of their distribution into tribes; with descriptions of newly discovered species and the characters of a new family and three new genera of spiders. *Transactions of the Linnean Society of London* 18. 601-670.


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Appendix A

General Linear Model: ln versus vegetation type and method; A (ground sampling), P (pitfall trap), Su (Suction sampling), and Sw (sweeping)

Method

Factor coding (-1, 0, +1)

Factor Information

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<th>Factor</th>
<th>Type</th>
<th>Levels</th>
<th>Values</th>
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<td>C, D</td>
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<tr>
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<td>A, P, Su, Sw</td>
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Analysis of Variance

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<th>Adj MS</th>
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<th>P-Value</th>
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<td>method</td>
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<td>6.1247</td>
<td>13.22</td>
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Sidak Pairwise Comparisons: veg type

Grouping Information Using the Sidak Method and 95% Confidence

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<th>Grouping</th>
</tr>
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<tr>
<td>D</td>
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</table>

Means that do not share a letter are significantly different.

Sidak Pairwise Comparisons: meth

Grouping Information Using the Sidak Method and 95% Confidence

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<td>A</td>
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<tr>
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Means that do not share a letter are significantly different.
## Appendix B

### Species collected

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<td><em>Eriophora pustulosa</em> (Waickenaer, 1841)</td>
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<tr>
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<td><em>Cyclosa fuliginata</em> (L. Koch, 1872)</td>
</tr>
<tr>
<td></td>
<td><em>Novakiella trituberculosa</em> (Roewer, 1942)</td>
</tr>
<tr>
<td>Corinnidae</td>
<td><em>Nyssus coloripes</em> (Waickenaer, 1805)</td>
</tr>
<tr>
<td>Desidae</td>
<td><em>Badumna longinqua</em> (L. Koch, 1867)</td>
</tr>
<tr>
<td>Dyseridae</td>
<td><em>Dysdera crocata</em> (C. L. Koch, 1838)</td>
</tr>
<tr>
<td>Gnaphosidae</td>
<td><em>Anzacia gemmea</em> (Dalmas, 1917)</td>
</tr>
<tr>
<td></td>
<td><em>Hemicloea rogenhoferi</em> (L. Koch, 1875)</td>
</tr>
<tr>
<td>Idiopidae</td>
<td><em>Canturia dendyi</em> (Hogg, 1901)</td>
</tr>
<tr>
<td>Lamponidae</td>
<td><em>Lampona cylindrata</em> (L. Koch, 1866)</td>
</tr>
<tr>
<td>Linyphiidae</td>
<td><em>Araeoncus humilis</em> (Blackwall, 1841)</td>
</tr>
<tr>
<td></td>
<td><em>Diploplecta communis</em> (Millidge, 1988)</td>
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<tr>
<td></td>
<td><em>Erigone prominens</em> (Bosenberg and Strand, 1906)</td>
</tr>
<tr>
<td></td>
<td><em>Erigone wiltoni</em> (Locket, 1973)</td>
</tr>
<tr>
<td></td>
<td><em>Haplinis fucatina</em> (Urquhart, 1894)</td>
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<td><em>Haplinis titan</em> (Blest, 1979)</td>
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<tr>
<td>Lycosidae</td>
<td><em>Laetesia germana</em> (Millidge, 1988)</td>
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<tr>
<td></td>
<td><em>Microctenonyx subitaneu</em> (O. Pickard-Cambridge, 1875)</td>
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<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td><em>Tenuiphantes tenuis</em> (Blackwall, 1852)</td>
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<td><em>Anoteropsis hilaris</em> (L. Koch, 1877)</td>
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<td><em>Holoplats apressus</em> (Powell, 1873)</td>
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<td>Theridiidae</td>
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