MOLECULAR DETECTION OF PREDATION: THE EFFECTS OF DETRITIVORE DEVERSITY AND ABUNDANCE ON PEST CONTROL BY GENERALIST PREDATORS

By

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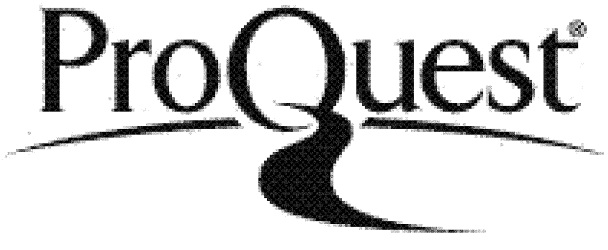

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ABSTRACT

Generalist predators constitute a large proportion of the arthropod fauna in agroecosystems. As generalist predators, Linyphiidae spiders are known to predate on a wide range of prey, including pests, and there is little doubt that they have the potential to make a significant contribution to the natural regulation of pests. Within-field habitat diversification practices, such as undersowing, partial weediness and the addition of mulches or manures to the soil surface, have been known to have negative effects on pest populations. These procedures theoretically increase the abundance of generalist predators by providing suitable habitat, thereby reducing mortality and emigration, and increasing reproduction by enhancing the diversity and abundance of high quality alternative prey species. One potential problem that arises from the enhancement of alternative prey is that some alternative prey species may be more desirable to the predators than the pests. For Linyphiidae spiders in winter wheat, therefore, increased diversity and abundance of Collembola detritivores may detract from, or enhance, predation on cereal aphid pests. The central aim of this project was to investigate the complex predator-prey interactions to reveal possible mechanisms influencing Linyphiidae spider predation on pest and non-pest prey in the field. PCR-based gut contents analysis was conducted to assess the diversity of spider prey consumed and species-specific primers were successfully designed and tested for various species of Diptera, aphid and Collembolla prey. This allowed the quantification of generalist predator diets. Field studies showed a clear aggregation of spiders in the field to areas of high Collembolla and Diptera density. Using a combination of prey sampling and molecular gut content analyses, this study showed that Linyphiidae spiders exhibited preferences for high quality non-pest prey, particularly the Collembolan *Isotoma anglicana,* under normal crop conditions. However, by altering the relative abundance of important non-pest prey, crop enhancements changed spider dietary preferences. In fields supplemented with compost, the diet of spiders was significantly altered to include enhanced predation upon the Collembola species *Entomobrya multifasciata* and the aphid *Sitobion avenae.*

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PUBLICATIONS

The results reported in this thesis have been published:

Agusti N, **Shayler SP**, Harwood JD, Vaughan IP, Sunderland KD, Symondson WOC (2003) Collembola as alternative prey sustaining spiders in arable ecosystems: prey detection within predators using molecular markers. Molecular Ecology 12 (12): 3467-3475.

Results in the above publications were also presented at:

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CONTENTS

Abstract I

Acknowledgements II

Declaration III

Publications IV

Contents V

Chapter 1: Introduction 1

1.1 General introduction 2

1.2 Conventional means of agriculture crop protection 2

1.3 The significance of aphids as pests in agricultural crops 5

1.4 Biology of spiders as biological control agents 6

1.5 Investigating generalist predator-prey interactions 9

1.6 Aims and hypothesis of PhD 15

1.7 References 16

Chapter 2: Investigating web location by Linyphiidae spiders to determine the relative importance of potential prey taxa 28

2.1 Introduction 29

2.1.1 Potential of generalist predators as biocontrol agents 29

2.1.2 Determining the importance of alternative prey to Linyphiidae spiders 30

2.2 Methods 32

2.2.1 Study Site 32

2.2.2 2001 field work 32

2.2.3 2002 field work 35

2.2.4 Weather data 36

2.2.5 Analysis of results 36

2.3 Results 37

2.3.1 Analysis of 2001 web data 37

2.3.2 Analysis of 2002 web data 49

2.3.2.1 Ground and aerial sticky trap analysis 60

2.3.3 2001 vs 2002 comparisons 68

2.4 Discussion 70

2.5 References 73

Chapter 3: Potential prey molecular marker development and validation 77

3.1 Introduction 78

3.1.1 Identification of potential non-pest prey of Linyphiidae Spiders 78

3.1.2 Aims of this study 80

3.2 Methods 83

3.2.1 Potential prey primer design 83

3.2.1.1 DNA extraction 83

3.2.1.2 PCR of DNA extractions using general primers 83

3.2.1.3 Sequencing of PCR products 86

3.2.1.4 Species specific primer design and optimization 86

3.2.2 DNA decay rate experiments 88

3.2.2.1 Arthropod cultures 88

3.2.2.1.1 Spiders 88

3.2.2.1.2 Diptera 88

3.2.2.1.3 Collembola 88

3.2.2.1.4 Aphids 89

3.2.2.2 Feeding experiments 90

3.3 Results 92

3.3.1 Primer cross-reactivity 92

3.3.2 Feeding experiment results 94

3.4 Discussion 97

3.5 References 99

Chapter 4: Predation by Linyphiidae spiders on non-pest and pest prey in winter wheat: spiders preferences in the field 102

4.1 Introduction 103

4.1.1 General background of biological control 103

4.1.2 Biological control potential of spiders 104

4.1.3 Investigating predator-prey interactions 105

4.1.4 Predation by Linyphiidae spiders in winter wheat 107

4.2 Methods 109

4.2.1 Field site 109

4.2.2 Spider collection and potential prey density monitoring 109

4.2.3 Screening of spiders for target prey 109

4.2.4 Analysis of results 111

4.3 Results 113

4.3.1 Spider preferences from sticky traps and quadrats 113

4.3.1.1 Predation on *Isotoma anglicana* 116

4.3.1.2 Predation on *Entomobrya multifasciata* 118

4.3.1.3 Predation on *Lepidocyrtus cyaneus* 118

4.3.1.4 Predation on *lycoriella castanescens* 118

4.3.1.5 Predation on *Sitobion avenae* 119

4.3.2 Spider preferences for ground and aerial webs 124

4.3.2.1 Predation on *Isotoma anglicana* 128

4.3.2.2 Predation on *Entomobrya multifasciata* 128

4.3.2.3 Predation on *Lepidocyrtus cyaneus* 131

4.3.2.4 Predation on *lycoriella castanescens* 131

4.3.2.5 Predation on *Sitobion avenae* 131

4.4 Discussion 136

4.5 References 142

Chapter 5: The effect of compost augmentation in winter wheat on predation By Linyphiidae spiders 150

5.1 Introduction 151

5.1.1 Pest control background 151

5.1.2 Predation by Linyphiidae spiders 153

5.1.3 The aims of this study 155

5.2 Methods 156

5.2.1 Field site 156

5.2.2 Spider collection 156

5.2.3 Sampling of spider web sites 156

5.2.4 Screening of spiders for target prey 157

5.2.5 Analysis of results 159

5.3 Results 160

5.3.1 Analysis of arthropods captured in non-compost and compost plots 160

5.3.1.1 Collembola analysis in compost and

non-compost plots 162

5.3.1.2 Diptera analysis in compost and non-compost plots 164

5.3.1.3 Hemiptera analysis in compost and non-compost plots 164

5.3.1.4 Hymenoptera analysis in compost and non-compost plots 164

5.3.1.5 Coleoptera analysis in compost and non-compost plots 165

5.3.2 Results from Linyphiidae spider preferences analysis 170

5.3.2.1 Predation on *Isotoma anglicana* 175

5.3.2.1 Predation on *Entomobrya multifasciata* 175

5.3.2.1 Predation on *Lepidocyrtus cyaneus* 175

5.3.2.1 Predation on *Lycoriella castanescens* 180

5.3.2.1 Predation on *Sitobion avenae* 180

5.4 Discussion 183

5.5 References 192

Chapter 6.1: Conclusions 202

Appendices 204

Chapter 1:

Introduction

1 Introduction

1.1 General introduction

A wide variety of methods exist for the control of pests in agriculture. Although many methods have been used for decades, concerns over specificity, efficiency, cost effectiveness and pollution have driven the need for constant research into new methods and into improving existing methods. This project is an investigation into a possible alternative method of pest control which uses a naturally occurring predator. This study aims to investigate the complex interactions that occur between the predator, pest and alternative non-pest prey in order to determine the potential of the generalist predator to control populations of a pest within cereal crops.

1.2 Conventional means of agricultural crop protection

Cereal crops are a valuable resource and the products of cereal crops form an important part of the human diet (Stanford 1934). However, they are vulnerable to attack from insect pests such as aphids and so active measures have to be employed to protect them. Chemical pesticides can be sprayed onto the crop in response to a pest infestation. These chemicals generally work by disrupting metabolic pathways and nervous systems or use juvenile hormone mimics to prevent life cycle completion (Brown 1978). This can be extremely efficient in significantly reducing numbers of a pest population (Neil *et al.* 1997) and can kill invading pest populations within 24 hrs (Matthews 1979; Venkatarajappa 2001). However, despite recent developments in the efficiency and specificity of chemical pesticides (Perrin 1997) there are still some disadvantages to using them. Many pesticides are readily washed out of the crop environment by precipitation (McDonald *et al.* 1999) making their way into other,

mainly aquatic, ecosystems where the potential for bioaccumulation exists resulting in adverse effects on other organisms (Feldman *et al.* 2000). Conversely, the development of technology to analyse foodstuff has shown that pesticide residues can remain on them after harvesting (Stan 2000). The pests themselves can develop resistance to pesticides by the overproduction of enzymes that sequester, degrade or alter the toxic chemicals (Foster *et al.* 2000) especially where repeated applications results in unintentional artificial selection of those mutants within the pest population that happen to be resistant to the poison used (Ripper 1944). Pests can also develop behavioural responses to the application of chemical pesticides and are known to actively avoid applied chemicals (Edwards *et al.* 1994) which are often applied ineffectively, creating refuges within the crop (Matthews & Thomas 2000). Studies into pest population dynamics showed that populations also fluctuate causing economic loss irregularly (George & Gair 1979), making it difficult to predict when to apply pesticides even with complex modelling systems (Trumper & Holt 1998). These drawbacks have driven the need for research into improving pesticides and investigating alternative methods of pest control.

One such alternative is to use natural enemies of the pest. These can either be specialists or generalists. A range of specialist predators have been used to control pests. Laboratory studies using stenophagous Syrphidae showed that there were significant attack and kill rates on aphids in culture (Adams *et al.* 1987; Michaud & Belliure 2001) and this, combined with information on Syriphidae life history (Ankersmit *et al.* 1986) strengthens their potential as a biocontrol agent against cereal aphids in the field. Similar studies have also been undertaken with other stenophagous aphid predators such as lacewings and ladybirds. Cage experiments in the field

containing known densities of these organisms have shown significant decreases in numbers of aphids on crop plants (Messina & Sorenson 2001). Other specialists can also be used. Parasitoids are commercially available and have been effectively used as an aphid control agent (Giller *et al.* 1995). However, there are disadvantages to using such selective predators. The predator population is intimately linked with the pest population so they are often most active at the peak or beginning of pest population decline (Pankanin-Franczyk & Ceryngier 1995) allowing for the potential of the pest to damage the crop before it is under control. Also, once a pest population has been destroyed there is little incentive for predators to remain and so they often vacate the area in search of further prey (Ripper 1944). The potential also exists to use generalist predators to control a pest. Feeding studies show that carabid beetles included large numbers of aphids in their diet (Bilde & Toft 1997; Kielty *et al.* 1999) and even climb plants in search of them (Mundy *et al.* 2000) whereas staphylinid larvae have also been shown to consume large numbers of aphids (Petersen 1998). Feeding studies have also shown that spiders will also include aphids in their diet with numerous studies on the feeding habits of linyphiid spiders (Sunderland *et al.* 1986b; Beck & Toft 2000; Bilde & Toft 2000) and lycosid spiders (Kielty *et al.* 1999; Mayntz & Toft 2000). The effects of generalist predators on pest populations has been shown in field studies. When linyphiid spiders and carabid beetles are prevented from entering a crop, aphid populations are shown to increase (Chiverton 1986) and an inverse relationship can be seen between the numbers of generalist predators and aphids. A similar effect has also been shown in caging experiments where staphylinid beetles have been shown to significantly reduce aphid numbers (Dennis & Wratten 1991).

Unlike specialist predators, generalist predators can subsist on alternative prey so a population of generalist predators is uncoupled from a pest population. This means that they can be present within a crop system before the arrival of a pest (Chang & Kareiva 1999). By allowing non-pest prey populations to naturally build up early in the growing season, generalist predator populations also increase, allowing a greater impact on later developing pest populations before the pest can establish itself (Settle *et al.* 1996). As generalist predators, linyphiid spiders in winter wheat may have the potential to suppress aphid pest populations through interactions with non-pest prey populations.

1.3 The significance of aphids as pests in agricultural crops

Aphids are considered important pests of cereal crops. Severe outbreaks of aphids frequently occur across Europe (Fletcher & Bardner 1969; Carter *et al.* 1989) highlighting the importance of protecting crops from this pest. Damage as a result of aphid infestations can be attributed to direct or indirect damage. Aphids are known to act as vectors for plant viruses such as the Barley Yellow Dwarf Virus which causes yellowing and stunted growth in cereal crops, including winter wheat, often leading to failure of the plant to seed (Oswald & Houston 1951; Oswald & Houston 1953). Direct damage is caused by the action of phloem feeding by aphids which weakens the plant via the removal of nutrients and also causes ultrastructural and tissue level damage (Telang *et al.* 1999). Once they have invaded a crop, an aphid population can rapidly increase due to their parthenogenetic nature to quickly reach damaging levels (Blackman 1973; Vickerman & Wratten 1979; Dolling 1991). In a typical infestation, the number of aphids has been recorded at 16 – 22 aphids per wheat ear (Fletcher & Bardner 1969). In crop loss assessments, the damage caused by aphid infestations can

result in yield losses ranging from 25 to 37 % (Montandon *et al.* 1993; Butts *et al.* 1997).

1.4 Biology of spiders as biological control agents

Spiders are naturally present in agricultural crops and can contribute to the control of pests such as aphids. In the UK, spiders can be found in densities of up to 200 m^{-2} (Nyffeler & Sunderland 2003) forming a large proportion of the epigeal predators. There is also a large diversity of spiders found in agricultural crops. In the US, spiders from the families Tetragnathidae, Areneidae, Theridiidae, Linyphiidae, Thomisidae, Salticidae and Lycosidae are commonly found (Nyffeler 1999; Nyffeler & Sunderland 2003) showing that the spider guild structure is very complex whereas in Europe Linyphiidae are the most numerically dominant family in a wide range of crops (Nyffeler & Sunderland 2003).

Spiders in crop systems have different strategies to capture their prey. Active hunting spiders, such as lycosids and salticids, will forage for their prey on the ground and on the available vegetation. This mode of prey capture primarily relies on sight to locate their prey. The vision of these spiders can be highly evolved with different eyes specialized for different functions such that eyes can be specialized to detect movement or to identify prey (Schmid 1998). Their eyes can also detect polarized light which allows for more accurate navigation at dawn and dusk (Dacke *et al.* 2001) and this can be essential for some species that require visual input in order to navigate (Ortega-Escobar 2002). The visual acuity of these spiders can be sufficient to allow high levels of prey discrimination. These spiders can identify prey that can only be

captured using specific hunting strategies and can alter their hunting strategies accordingly (Harland & Jackson 2000; Harland & Jackson 2001) and they can even identify stationary prey (Jackson & Pollard 1996). Although these spiders will actively hunt prey, they can also exhibit 'sit-and-move' strategies whereby a suitable site is selected that the spider will remain at until a prey item is identified which triggers an attack response (Samu *et al.* 2003).

Alternatively, spiders such as linyphiids and tetragnathids use webs as a mode of prey capture. Here, spiders rely on detecting the vibrations of prey that are caught in their webs. This requires a well developed mechanosensory system to verify the location of prey within the web and may also enable the spider to identify the prey type (Venner *et al.* 2000). Spiders will also adjust their webs so that a particular size of prey can be more efficiently detected (Watanabe 2000) and modify their webs to optimize prey capture efficiency (Hauber 2002). The importance of webs can be shown with the linyphiid spider *Tenuiphantes tenuis*. Areas considered as favourable potential web sites are rapidly colonized (Alderweireldt 1994a) and there are frequent territorial contests between web owners and invading spiders (Samu *et al.* 1996). The webs of linyphiid spiders are constructed as a horizontal sheet with web size and location varying depending on the species. Web dependent Linyphiinae construct their webs several centimetres above the ground whereas Erigoninae, which can hunt away from the web, construct smaller webs on the ground (Sunderland *et al.* 1986a; Alderweireldt 1994b).

Although they prey primarily on insects, spiders rarely show specificity towards their prey, generally attacking prey relative to the rate of encounter (Riechert & Lockley

1984). As a result, spider behaviour includes wasteful killing (also termed 'superfluous killing') and partial consumption of prey. Spiders may kill a pest but subsequently ingest little which is advantageous for pest control because it will result in more pests being killed per unit of spider food demand (Sunderland 1999). The process of external ingestion creates a delay between prey capture and ingestion. Spiders will continue to attack and secure prey until the first prey can be ingested. This may explain why spiders capture more prey than they eventually consume (Riechert & Lockley 1984) which has been observed in linyphiid spiders against aphids (Mansour & Heimbach 1993).

Being uncoupled from the target pest population enables spider populations to increase independently and earlier than the pest by preying on alternative, non-pest prey present in large numbers earlier than the pest. This closely fits the 'ideal' definition for a biocontrol agent whereby 'any predator can reduce high densities of a pest but only good ones can prevent high densities from developing in the first place' (Huffaker & Kennett 1969). Generalist feeding ensures survival and this should be considered when examining spiders as potential biological control agents. If a pest reaches high numbers, then spiders will feed preferentially on it. However, once the numbers decrease they may then run to alternative types of food. This could be advantageous provided the preference remains until the pest is below economic threshold. Thus spiders may act as buffers to limit initial exponential growth of pest populations (Riechert & Lockley 1984). However, this may also enable them to survive periods of low pest density.

The annual recolonization of many agroecosystems by spiders is accomplished more by aerial deposition of ballooning spiders than by cursorial invasion from refugia such as forests and fence lines (Suter 1999). Ballooning involves emitting a long strand of silk into air currents until there is enough drag on the silk to overcome the pull of gravity on the spider. Ballooning requires specific micrometeorological conditions where the mean wind speed is less than 3.0 ms and has a strong vertical gradient in horizontal wind speed. The importance of ballooning as a source of influx of spiders was demonstrated by Bishop & Riechert (1990) using a combination of pitfall traps and aerial sticky traps positioned across a range of habitats, including small-scale crop fields, flower beds and straw augmented cabbage plots. Comparison of the pitfall traps and aerial traps showed that there was a peak of activity early in spring in the aerial trap samples, a majority of which were juveniles (98 %) of the families Linyphiidae, Clubionidae and Thomsidae, with little activity in the pitfall traps. Samples taken later in the year showed that there was little aerial activity with a small rise in ground activity, mostly in Lycosidae, with overall aerial deposition of spiders of 41-50 %. The characteristics of spiders show that they have the potential to be used to control pests in agroecosystems. As such, spiders and other generalist predators have been the subject of a wide variety of studies into predator-prey interactions.

1.5 Investigating generalist predator-prey interactions

The complexity of the interactions between generalist predators and their prey are inherently difficult to investigate and a wide range of techniques have been developed to enable studies to be carried out (Sunderland 1988; Greenstone 1999; Symondson 2002).

Laboratory based studies reduce the number of variables and allow investigations to be carried out in controlled conditions. Feeding trials can be used in this way to determine basic interactions between spiders and their prey. It is possible to determine the quality of a prey item by monitoring the growth and fecundity of a spider on single (Bilde & Toft 2000; Toft & Wise 1999b) or mixed (Bilde & Toft 2000; Sigsgaard *et al.* 2001; Oelbermann & Scheu 2002) diets. They can also show the limits for intake of poor quality prey (Toft & Wise 1999a) as well as showing which prey are toxic for the spider (Fisker & Toft 2004). Such studies are easily expanded to include mesocosm work where a limited amount of potential prey and the predators are kept in a small enclosure containing the basic constituents found in the field (Dinter & 2002; Madsen *et al.* 2004) and introducing natural structural refuges, such as plants, providing a level of realism. Investigating predator-prey interactions under more realistic but still controlled conditions can be done using field cages and exclusion techniques to manipulate the predator or prey populations. This type of manipulation places the subjects in natural conditions whilst enabling the direct interactions to be investigated (Dennis & Wratten 1991). However, the exclusion of other organisms and the confinement of the subjects could produce false results as in laboratory experiments. This is also true, though to a lesser extent, of field manipulation experiments. Here, predator-prey populations are manipulated using pitfall trap exclusion, barriers or using pesticide application (Giller *et al.* 1995; Snyder & Wise 1999) and the effects are compared with control plots. This can give an indication of the effects of generalist predators but it does not allow direct investigation of the interactions involved.

A direct method of investigating these interactions is to examine the gut contents of field caught predators. By dissecting the gut out of a predator it is possible to determine what the prey was by identifying any solid remains. This method can be used to identify the diet of particle feeding predators such as beetles (Sunderland *et al.* 1987; Chiverton & Sotherton 1991; Triltsch 1997). However, alternative methods of gut content identification have to be employed for fluid feeding predators such as spiders.

One possible method is the use of protein electrophoresis. Here, prey proteins can be separated out using an electric field in a polyacrylamide gel and the gel can then be stained to reveal enzyme bands that are characterized for each species (Murray & Solomon 1978; Fitzgerald *et al.* 1986; Solomon *et al.* 1996). This method was first described by Murray & Solomon (1978) and it was shown to be an effective tool for investigating predation in the field. However, although this method has been successfully used to identify the prey of Hemiptera (Corey *et al.* 1998) and Coleoptera (Camara *et al.* 2003; Traugott 2003) from the field this method has never been used to identify the prey of spiders.

The use of antibodies to detect prey proteins has also been employed to investigate predation by generalist predators. Polyclonal antibodies can be obtained by immunizing a mammal with an extract from the target prey and the antibodies are then harvested from blood sera. The polyclonal antibodies can then be exposed to extracts from a predator gut and any reaction with them can then be observed using a variety of techniques. Polyclonal antibodies have often been used to investigate predation both in terrestrial systems (Sunderland 1988; Symondson 2002) and in

marine systems (Hoyt *et al.* 2000). However, acquiring a high specificity for a target prey item is difficult and polyclonal antibodies will often cross react with non-target organisms (Mayfield *et al.* 2000). Despite methods to limit cross reactivity (Symondson & Liddell 1993), the additional difficulty in reproducing polyclonal antibodies has lead to investigating alternative methods. Monoclonal antibodies are more specific and are produced by fusing myeloma cells and antibody-producing spleen cells from an immunized mammal to produce hybridoma cells. These are then selected and cloned until a culture of cells that produce the required antibody are produced. Monoclonal antibodies have been used to identify prey in the guts of several predators including carabid beetles (Symondson *et al.* 1999; Symondson *et al.* 2000) and linyphiid spiders (Harwood *et al.* 2004). Monoclonal antibodies even can be made to be specific enough to determine the life cycle stage the prey item was in when it was eaten (Greenstone & Hunt 1993). Although they can be difficult and expensive to produce, once developed the cell lines producing these monoclonal antibodies can be kept for long periods of time providing a supply of antibodies whenever needed.

Alternatively, it is possible to identify prey DNA from invertebrate predator guts. This is done using primers in a polymerase chain reaction (PCR) to produce copies of the targeted DNA. These amplified fragments of DNA are then separated using electrophoresis to determine their size and to visualize them. Using DNA to investigate terrestrial arthropod predation was first carried out by Agusti *et al.* (1999, 2000) and Zaidi *et al.* (1999). Agusti *et al.* (1999) investigated predation by a heteropteran predator on a Lepidopteran pest was investigated. Random primers were used to randomly amplify the DNA of both predator and prey. When the DNA was

examined on a gel, bands that were present in the prey but not the predator were identified and sequenced to produce prey specific primers that would amplify DNA of varying lengths. In feeding trials it was found that shorter fragments of prey DNA could be amplified for longer periods of time after ingestion by the predator which was also found in Agusti *et al.* (2000) where the prey was an Aleyrodidae pest. In both cases this was attributed to larger fragments of DNA exhibiting a higher susceptibility to digestion than shorter fragments. This was also shown by Zaidi *et al.* (1999) where carabid beetles were fed an organophosphate resistant strain of mosquito. By using primers that amplified the multiple copy esterase genes from the mosquitoes, this system showed that multiple copy DNA could be amplified from the gut of a predator and that this would increase the chance of successfully amplifying DNA by increasing the number of potential primer sites. The use of the nuclear ribosomal RNA internal transcribed spacer region (ITS-1) was investigated by Hoogendoorn & Heimpel (2001) as a source of multiple copies of a target for detecting the presence of prey. In laboratory trials, this method successfully detected the presence of DNA from the eggs of a lepidoperan pest within the gut of a coccinellid beetle predator. Also, the possibility of using a range of different sized fragments of DNA to determine the time since ingestion was demonstrated. However, copies of the ITS-1 region can show variation in length within individuals (Hillis *et al.* 1996; Tang *et al.* 1996) which could make the interpretation of results difficult. In Chen *et al.* (2000), DNA from the mitochondrial genome was targeted as a source of multiple copy DNA. Fragments of the Cytochrome Oxidase II gene from cereal aphids were successfully amplified from the gut of coccellinid beetles and chrysoid lacewings during laboratory feeding trials showing that this could be a reliable method of investigating predation in the field. An earlier study by Asahida *et al.*

(1997) also used the mitochondrial genome in an investigation of predation by a sand shrimp on fish juveniles. This shows that the mitochondrial genome is a reliable target that can be used in a variety of predation studies. Subsequent predation studies have used the Cytochrome Oxidase I (COI) gene. In Agusti *et al.* (2003a), COI primers were designed to amplify a Psyllidae pest. These were tested against a range of potential predators and alternative prey and the primers were shown to be species specific showing their potential as a tool to determine which predators could contribute to the control of the pest. The first study using DNA to investigate predation in the field was carried out by Agusti *et al.* (2003b). Species specific COI primers were designed to amplify DNA from three species of Collembola. In winter wheat, Collembola are thought to be important non-pest prey of linyphiid spiders and may be an influencing factor of the ability of linyphiid spiders to suppress aphid pest populations (Harwood *et al.* 2004). By testing linyphiid spiders from the field, Agusti *et al.* (2003a) showed that these spiders do predate Collembola and that linyphiid spiders show preferences towards certain prey species. Recent developments in the use of PCR to investigate predation have shown that it is possible to screen generalist predators for large number of different potential prey using multiplex PCR techniques (Harper *et al.* 2005). This method has been effectively applied to predators caught in the field showing that the use of PCR to investigate predation can be a highly effective and versatile tool for investigating predation. Such studies have shown that using DNA is a viable option for investigating predator-prey interactions in the field and the ready availability of DNA sequences and primers mean that this method can be easily applied to investigate new predator-prey systems.

1.6 Aims and hypothesis of PhD

This Ph.D. is an investigation into predation by linyphiid spiders on non-pest (Collembola and Diptera) and pest (Aphid) prey in winter wheat. Using a combination of prey population monitoring and identification of prey DNA from the gut of the spiders, this project aims to ascertain how the diversity and abundance of non-pest prey affect predation on pest prey by linyphiid spiders. This project attempts to develop primers to potential Diptera non-pest prey and investigate predation by linyphiid spiders within winter wheat and linyphiid spiders occupying different spatial niches within the crop. This project also attempts to investigate the differences between Linyphiid spider predation under normal crop conditions and under crop conditions thought to increase non-pest prey populations. The primary hypothesis of this Ph.D. was that temporal shifts in the availability of non-pest resources (especially Collembola and Diptera) affect rates of predation by Linyphiidae on a pest (aphid) population.

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Chapter 2:

Investigating web location by linyphiid spiders to determine the relative importance of potential prey taxa

2.1 Introduction

2.1.1 Potential of generalist predators as biocontrol agents

The use of chemical pesticides as an exclusive measure of controlling pests in agriculture is not thought to be a suitable long term solution. Despite advances in chemical pesticides, such as increased specificity of active ingredients (Perrin 1997), the possible environmental impacts of pollution (McDonald *et al.* 1999) and the development of pesticide resistance by pests (Ripper 1944) have provided an incentive to investigate alternative methods of control. Biological control is thought to be a viable alternative to the use of conventional chemical methods of pest control. Specialist predators can be reared for release in response to an increased pest population. Specialist predators of aphids such as coccellinid beetles (Kehrli & Wyss 2001) and parasitoids (Giller *et al.* 1995) have been shown to be effective at reducing pest populations. Alternatively, density manipulations have shown that naturally occurring generalist predators spiders, carabid and staphylinid beetles can be used to control a pest (Chiverton 1986; Duffield *et al.* 1996). As generalist predators can subsist on non-pest prey, populations of a generalist predator can be present early in the growing season of the crop and increase by predating non-pest prey. When the pest invades the crop, the increased predator population would impact on the pest preventing the pest from becoming established (Settle *et al.* 1996). This shows that there is the potential to use generalist predators to suppress pests through the manipulation of alternative prey.

2.1.2 Determining the importance of alternative prey to linyphiid spiders

As a generalist predator, linyphiid spiders have the potential to control cereal aphids in winter wheat. Laboratory feeding studies have shown that linyphiid spiders will consume aphids (Beck & Toft 2000; Bilde & Toft 2000) and spiders collected from the field have been found to contain the remains of aphids (Sunderland *et al.* 1987; Harwood *et al.* 2004). However, feeding studies including non-pest prey, such as Collembola, have shown that the value of prey items for reproduction and development can differ (Marcussen *et al.* 1999; Toft & Wise 1999; Sigsgaard *et al.* 2001; Oelbermann & Scheu 2002; Fisker & Toft 2004) and a predator may preferentially feed on higher quality alternative prey instead of the pest. In the field, the presence of alternative prey may also affect predation on the pest (Ostman 2004). However, alternative prey may be temporally separated from the pest. Harwood *et al.* (2004) showed that early in the season, Collembola were abundant and that linyphiid spiders located their webs in areas of highest Collembola density whereas later in the season, when Collembola density was low, the spiders were found to be predating aphids. Monitoring the web sites of spiders, (Harwood *et al.* 2001; Harwood *et al.* 2003) indicated which alternative prey were important for sustaining the spiders until the arrival of the pest. The webs of linyphiid spiders are constructed horizontally and can be situated either on the ground or just above it (Sunderland *et al.* 1986a; Alderweireldt 1994). These webs are frequently abandoned intact when the spider moves to another web site and this can lead to large areas of a crop being covered (Sunderland *et al.* 1986b) which can trap large numbers of aphids and so contribute to their control (Sunderland *et al.* 1986a; Alderweireldt 1994). Different species of linyphiid spiders have different degrees of dependency on their webs to obtain food and this is reflected in the size of the web. The sub-family Erigoninae have small

ground based webs and will hunt away from their web whereas Linyphiinae have larger aerial webs and are highly dependent on them for food (Sunderland *et al.* 1986a; Alderweireldt 1994). The different web strategies may be an indication of different dietary requirements. Using sticky traps to represent small linyphiid webs, Harwood *et al.* (2003) showed that Erigoninae located their webs in areas of high Collembola density and Linyphiinae were locating their webs in areas of high aphid density. This showed that the two sub-families were exploiting different resources potentially to avoid direct competition. Although the sticky traps used were representative of Erigoninae webs, the different size and vertical location of Linyphiinae webs could mean that prey taxa density and diversity were misrepresented for this sub-family. This study aims to address this by using sticky traps modelled on the two web types to investigate the density and diversity of potential prey captured by the two sub-families. This would allow for a more accurate determination of which potential prey could be more important as alternative non-pest prey for the linyphiid spiders. It also allows comparisons between the two prey capture strategies to be carried out and aims to accurately determine the mechanisms underlying the prey capture strategies and which prey taxa are important non-pest prey that may influence predation on pest prey in the field.

2.2 Methods

2.2.1 Study site

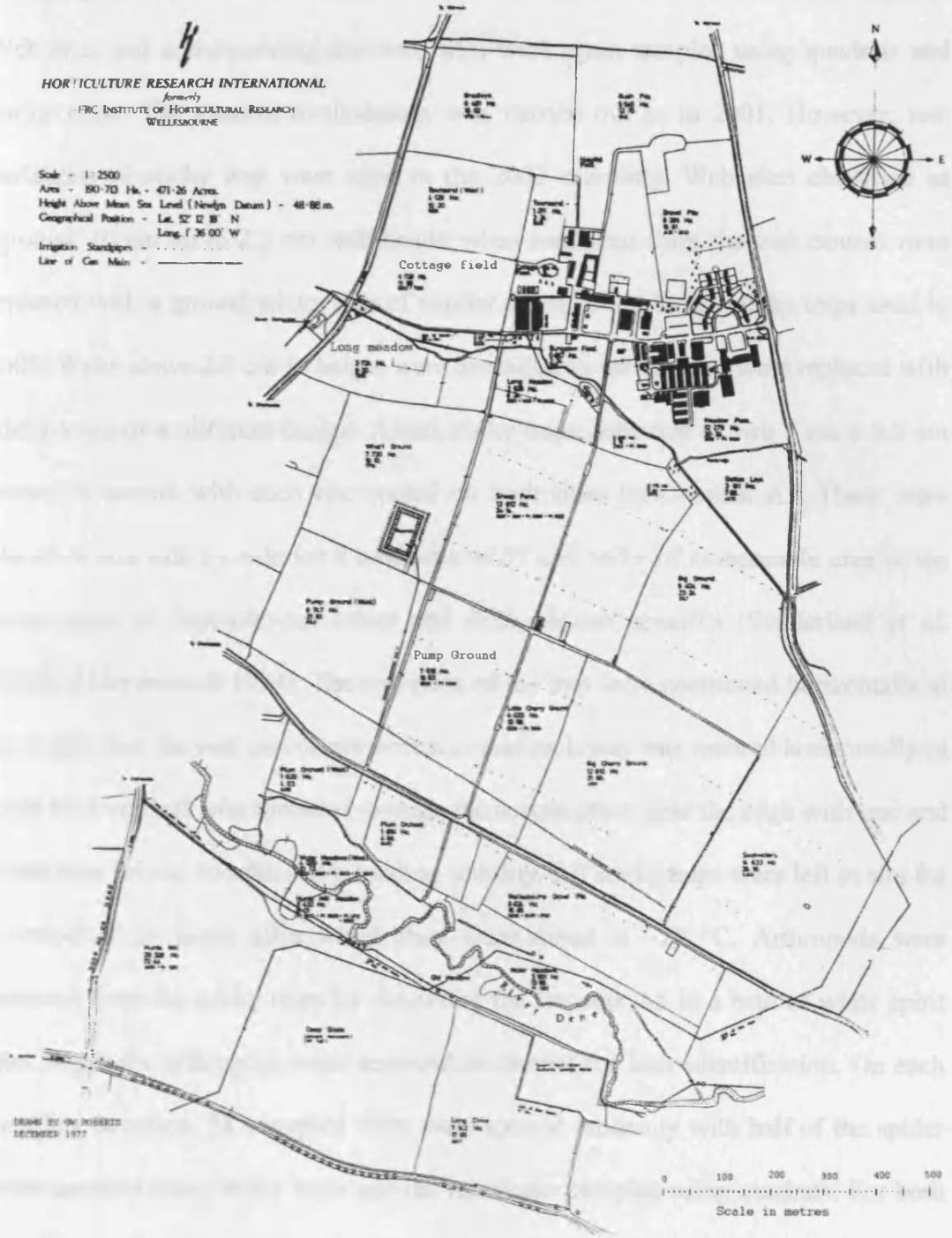
The sampling sites were fields of winter wheat (cv. 'Hereward') planted in predominantly sandy loamy soil at Horticulture Research International (HRI) in Wellsbourne, Warwickshire, UK (52°12.18'N, 1°36.00'W). The fields used during field work in 2001 were Long Close and Pump Ground whereas the fields used in 2002 were Cottage Field and Long Meadow. Figure 2.1 is a map showing the locations of all the fields at the HRI site. All fields were managed according to standard farming practices minus the use of pesticides.

2.2.2 2001 field work

Sampling was carried out every two weeks from late May until harvest (in late July to early August). Two ground-based sampling methods were used as described by (Harwood *et al.* 2001; Harwood *et al.* 2003) to form sampling strategies that would accurately sample web sites for potential arthropod prey of linyphiid spiders. The use of sticky traps is a passive sampling strategy relying on the activity of arthropods to bring them into contact with the trap for capture whereas using quadrats forms an active strategy, sampling inactive arthropods potentially concealed within vegetation, small cracks in the ground or under small stones. Sticky traps placed at the sampling site can sample arthropods that fall or descend from higher strata over a given time period whereas the use of quadrats samples arthropods that are present during a limited period of time and therefore give an instantaneous measurement of arthropod density. When sampling the web site, all traces of the web were removed to eliminate any possible interference with sampling and also to prevent the attraction of other

spiders to the web site (Hodge & StorferIsser 1997). The sticky traps consisted of a 1.5 cm x 5 cm x 2 mm base covered in black acrylic paint for camouflage. A 1.5 cm x 5 cm piece of acetate was attached to the upper surface using a thin layer of Oecotak A5 (Oecos, Kimpton, UK), an ecologically neutral adhesive, with another thin layer of Oecotak A5 applied to the upper surface of the acetate to provide a horizontal sampling area of 7.5 cm^2. This sampling area is comparable to the area of ground webs constructed by linyphiid spiders such as *Erigone atra* and E. *dentipalpis* (Sunderland *et al.* 1986a; Alderweireldt 1994). For each web site sampled using a sticky trap, a corresponding non-web site was also sampled with a sticky trap situated at a distance of 30 cm from the web site. All sticky traps were placed horizontally in situ for a period of 24 hours after which they were stored at –20 °C for later identification of arthropods captured. Removal of arthropods for identification was carried out by soaking the sticky trap in a bath of white spirit to dissolve the Oecotak A5. The quadrats used were circular with an area of 78.5 cm^2. The quadrat was placed at the sampling site and all arthropods were removed from the area using a pooter taking care to search amongst any vegetation present. All arthropods captured were placed directly in alcohol for later identification. For each web site sampled using a quadrat a corresponding non-web site was also was sampled as described with a pooter. On each sampling occasion, 30 occupied linyphiid spider webs were located randomly in each field and half the web sites were sampled using sticky traps and the remainder were sampled using quadrats.

Figure 2.1: Map of the field site at Horticulture Research International (HRI), Wellsbourne, Warwickshire



2.2.3 2002 field work

Sampling was carried out from mid May until harvest (in late July / early August). Web sites and corresponding non-web sites were again sampled using quadrats and sticky traps. The quadrat methodology was carried out as in 2001. However, two variations of sticky trap were used in the 2002 sampling. Web sites classified as 'ground' (0 cm up to 2.5 cm web height when measured from the web centre) were replaced with a ground sticky trap of similar construction to the sticky traps used in 2001. Webs above 2.5 cm in height were classified as 'aerial' and were replaced with sticky traps of a different design. Aerial sticky traps consisted of two 7 cm x 5.5 cm pieces of acetate with each one coated on both sides by Oecotak A5. These were placed *in situ* side by side for a total area of 77 cm^2 to be of comparable area to the aerial webs of *Tenuiphantes tenuis* and *Bathyphantes gracillis* (Sunderland *et al.* 1986a; Alderweireldt 1994). The two parts of the trap were positioned horizontally at the height that the web previously occupied and each part was secured horizontally in place by a vertical wire threaded through the acetate sheet near the edge with one end of the wire driven into the soil providing stability. All sticky traps were left in situ for a period of 24 hours after which they were stored at –20 °C. Arthropods were removed from the sticky traps by dissolving the Oecotak A5 in a bath of white spirit after which the arthropods were removed to alcohol for later identification. On each sampling occasion, 28 occupied webs were located randomly with half of the spider webs sampled using sticky traps and the remainder sampled using quadrats. For both sampling methods, a 50 : 50 ratio of ground to aerial webs was sampled.

2.2.4 Weather data

Meteorological data for each year was obtained from the HRI weather station at Wellsbourne which was within 1200 m of all fields.

2.2.5 Analysis of results

Prior to analysis all population data was log10(x+1) transformed to normalize data and ensure homogeneity of variances. Where different sampling methods were used in an analysis, data was converted to number per cm^2 as a standard unit to allow for direct comparisons. Multifactoral Analysis of Variance (ANOVA) was used to investigate the effect of different factors and their interactions on the total prey sampled. Individual factors of interest were further analysed for each individual potential prey population. One-Way ANOVA was used to investigate the effect of sampling date on potential prey populations and Paired t-tests or Two-sample t-tests were used to compare web versus non-web sites, sticky traps versus quadrats and ground versus aerial sticky traps on potential prey population pooled over time. Where the distribution of a prey population could not be normalized, mean population per cm^2 at each sampling date was used.

Diversity indices were calculated using the Shannon diversity index (H):

$H = - \sum pi \ln pi$

where *pi* is the proportion of total individuals found in the *i*th species. The use of proportions of individuals instead of number of individuals allows for an accurate measure of diversity (May 1975) that can be analysed using parametric analysis by converting the indices to mean Shannon values per sampling occasion.

2.3 Results

2.3.1 Analysis of 2001 web data

A complete list of all arthropods collected during 2001 is shown in Appendix 1. The numbers of each species of spider collected from webs prior to analysis with sticky traps or quadrats is shown in Table 2.1.

Table 2.1: Numbers of each species of spider collected from webs in 2001 prior to sampling of their web sites using either sticky traps or quadrats.

Spider species	Sticky trap sampled web site	Quadrat sampled web site	Total
Tenuiphantes tenuis	39	41	80
Erigone atra	25	28	53
E. dentipalpis	4	3	7
Bathyphantes gracillis	21	22	43
Oedothorax sp.	20	21	41
Meioneta rurestris	7	4	11
Pachygnatha degeeri	8	2	10
Microlinyphia pusilla	0	2	2
Erigoninae juveniles	11	10	21
Linyphiinae juveniles	15	18	33
Total	150	150	300

A 4-way balanced ANOVA was carried out to investigate the effect of field, sampling method (sticky traps or quadrats), sampling location (web site or non web site) and sampling date on the number of total prey sampled per cm^2. The ANOVA output is shown in Table 2.2. Each factor was shown to have a significant effect on the total prey sampled. In addition, a number of interactions between the factors were shown to have a significant effect on the total prey sampled. Temporal variation of total prey per cm^2 was shown to be different in each field and when using different sampling methods. Field also had an effect on prey captured at web and non-web sites.

Table 2.2: ANOVA showing the effects of sampling date, sampling method, web site or corresponding non-web site location and field on numbers of potential prey of linyphiid spider caught in 2001. All data was Log_{10}([number captured per cm^2] + 1) transformed prior to analysis.

Source	d.f.	SS	MS	F	P
Field	1	0.138438	0.138438	44.74	0.000
Date	4	0.206524	0.051631	16.68	0.000
Method	1	0.846341	0.846341	273.50	0.000
Location	1	0.021232	0.021232	6.86	0.009
Field x Date	4	0.146029	0.036507	11.80	0.000
Field x Method	1	0.001425	0.001425	0.46	0.498
Field x Location	1	0.017964	0.017964	5.81	0.016
Date x Method	4	0.259924	0.064981	21.00	0.000
Date x Location	4	0.013013	0.003253	1.05	0.380
Method x Location	1	0.000103	0.000103	0.03	0.855
Field x Date x Method	4	0.173695	0.043424	14.03	0.000
Field x Date x Location	4	0.011272	0.002818	0.91	0.457
Field x Method x Location	1	0.001465	0.001465	0.47	0.492
Date x Method x Location	4	0.011953	0.002988	0.97	0.426
Field x Date x Method x Location	4	0.016870	0.004218	1.36	0.246
Error	560	1.732936	0.003095		
Total	599	3.599183			

The differences between web and non-web sites of each taxa are shown in Table 2.3. Though there were slightly higher mean numbers per cm^2 at web sites of Aphididae and Coleoptera when sampled with sticky traps and Diptera, Aphdidae, Hymenoptera and Coleoptera when sampled using quadrats, none of these differences were found to be significantly different. Where Hymenoptera were sampled using sticky traps, there was also significant difference between web and non-web sites, however, the mean population per cm^2 was slightly higher in the non-web samples. The mean number per cm^2 of Collembola was found to be significantly higher at web sites when sampled using both sticky traps and quadrats. There were also highly significantly more Diptera caught at web sites when using stickys traps.

Table 2.3: Paired t-test results for the difference between five potential prey taxa at web sites and non-web sites of linyphiid spiders when sampled using either sticky traps or quadrats in 2001. All data was $Log_{10}([\text{potential prey per } cm^2] + 1)$ transformed prior to analysis.

Potential prey item	t	n	Mean per web site ± SE	Mean per non web site ± SE	P
(a) Sticky traps					
Collembola	2.06	150	0.0151 (±0.0026)	0.0093 (±0.0019)	0.048
Diptera	3.90	150	0.0461 (±0.0046)	0.0260 (±0.0033)	0.000
Aphididae	1.45	10	0.0266 (±0.0145)	0.0233 (±0.0140)	0.181
Hymenoptera	0.97	10	0.0244 (±0.0048)	0.0289 (±0.0057)	0.358
Coleoptera	0.34	10	0.0061 (±0.0031)	0.0050 (±0.0011)	0.738
(b) Quadrats					
Collembola	2.59	150	0.0105 (±0.0015)	0.0070 (±0.0010)	0.011
Diptera	1.51	10	0.0012 (±0.0006)	0.0006 (±0.0004)	0.166
Aphididae	1.48	10	0.0020 (±0.0009)	0.0013 (±0.0005)	0.172
Hymenoptera	0.36	10	0.0004 (±0.0002)	0.0003 (±0.0002)	0.727
Coleoptera	1.98	10	0.0019 (±0.0008)	0.0007 (±0.0006)	0.080

Figure 2.2 shows the mean number of each taxa at web sites when sampled using each method. Two sample t-tests caried out on the mean number per cm^2 of each potential prey population showed that sticky traps collected significantly more of each taxa ($P < 0.05$). T-tests using the Shannon Diversity index calculated each week showed that the diversity of potential prey captured using sticky traps throughout the sampling period was significantly higher ($t = 2.72$, $P = 0.030$) than in the quadrat samples.

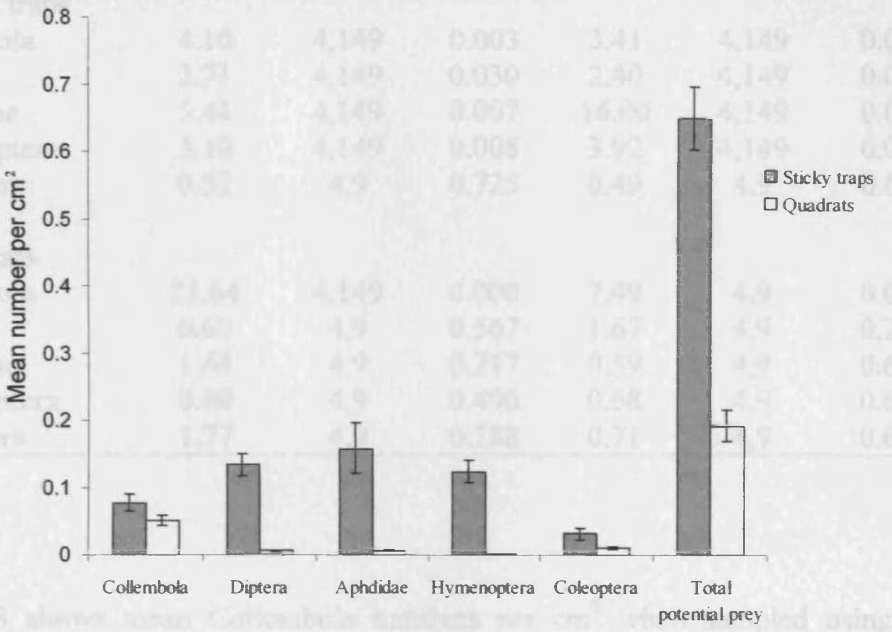


Figure 2.2: Bar chart of showing the Mean number of each potential prey taxa (±SE) at linyphiid spider web sites when sampled using sticky traps or quadrats 2001.

Using Two sample t-test, no significant difference ($P < 0.050$) was found between the two fields for each of the taxa allowing the fields to be pooled for analysis. Table 2.4 shows the significance in the variation of each taxa at web and non-web sites.

Table 2.4: ANOVA results testing for the variation over time in the Mean number per cm^2 of each potential prey taxa sampled using a) Sticky traps or b) Quadrats at either web sites or non-web sites.

Variable	Web sites			Non-web sites		
	F	d.f.	P	F	d.f.	P
a) Sticky traps						
Collembola	4.10	4,149	0.003	3.41	4,149	0.021
Diptera	2.71	4,149	0.030	2.40	4,149	0.036
Aphididae	5.41	4,149	0.007	16.00	4,149	0.000
Hymenoptera	5.10	4,149	0.008	3.92	4,149	0.005
Coleoptera	0.52	4,9	0.725	0.49	4,9	0.640
b) Quadrats						
Collembola	21.64	4,149	0.000	7.49	4,9	0.010
Diptera	0.69	4,9	0.567	1.67	4,9	0.292
Aphididae	1.64	4,9	0.217	0.59	4,9	0.685
Hymenoptera	0.90	4,9	0.490	0.68	4,9	0.636
Coleoptera	1.77	4,9	0.188	0.71	4,9	0.617

Figure 2.3 shows mean Collembola numbers per cm^2 when sampled using each method over time. ANOVA results in Table 2.4 showed that there was significant variation over time in both sticky traps and quadrats. The graphs show that the population of Collembola had two peaks, the highest population density occurring early to mid June with a smaller peak later in the season. This was consistent between sampling techniques though the effect was most prominent at web sites sampled using sticky traps. The graphs for Diptera (Figure 2.4) also show significant variation over time for sticky traps but not when sampled using quadrats. The graphs show that

Diptera were most abundant during early and mid July. The variation over time for Aphididae was also significant when sampled using sticky traps (Figure 2.5) which shows an exponential growth curve until a population crash at the end of July. This pattern was not replicated using quadrats where the variation over time was shown to be not significant (Figure 2.5). Figure 2.6 shows the population over time for Hymenoptera. When sampled with sticky traps the variation was significant and showed a similar increase to Aphididae during mid July though this increase was not as pronounced in Hymenoptera. No significant variation was found in the quadrat samples over time. No significant variation over time was found for Coleoptera when sampled with sticky traps or quadrats as shown in Figure 2.7.

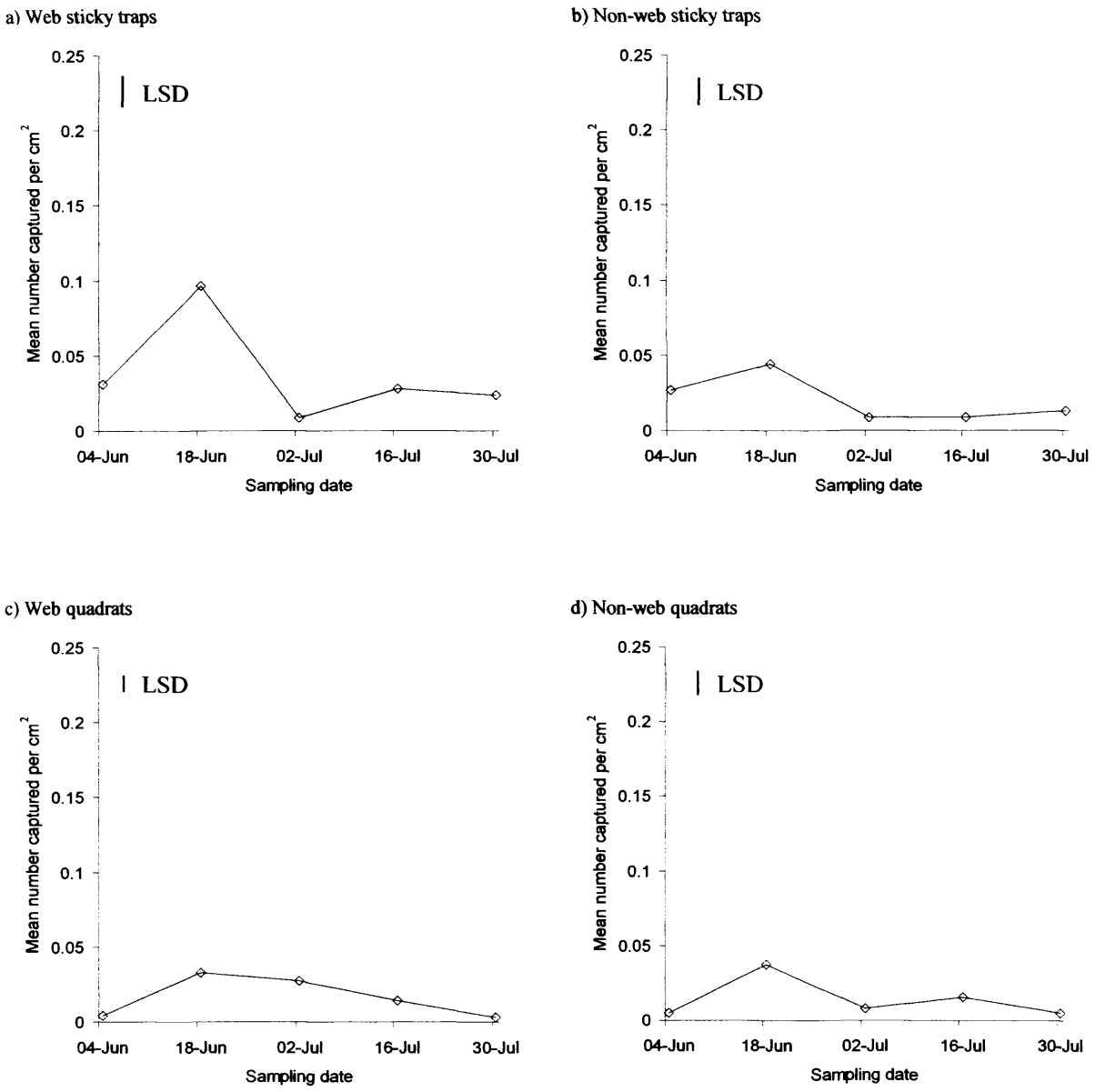


Figure 2.3: Mean number of Collembola per cm^2 sampled during 2001 at a) web site sticky traps, b) non-web site sticky traps, c) web site quadrats and d) non-web site quadrats. Least significant difference (LSD) shown by solid bars for comparison of variation in density between sampling dates.

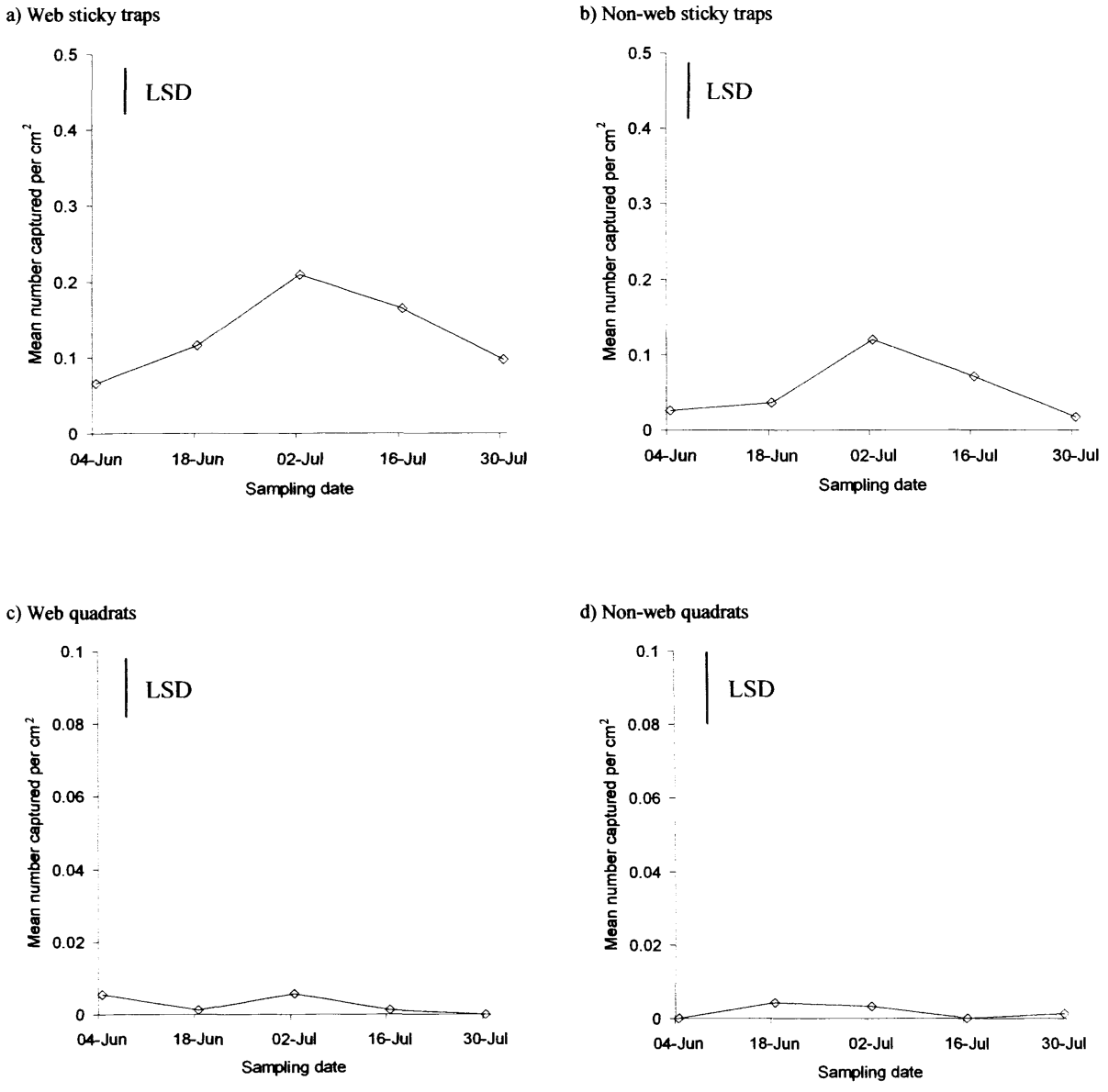


Figure 2.4: Mean number of Diptera per cm^2 sampled during 2001 at a) web site sticky traps, b) non-web site sticky traps, c) web site quadrats and d) non-web site quadrats. LSD shown by solid bars for comparison of variation in density between sampling dates.

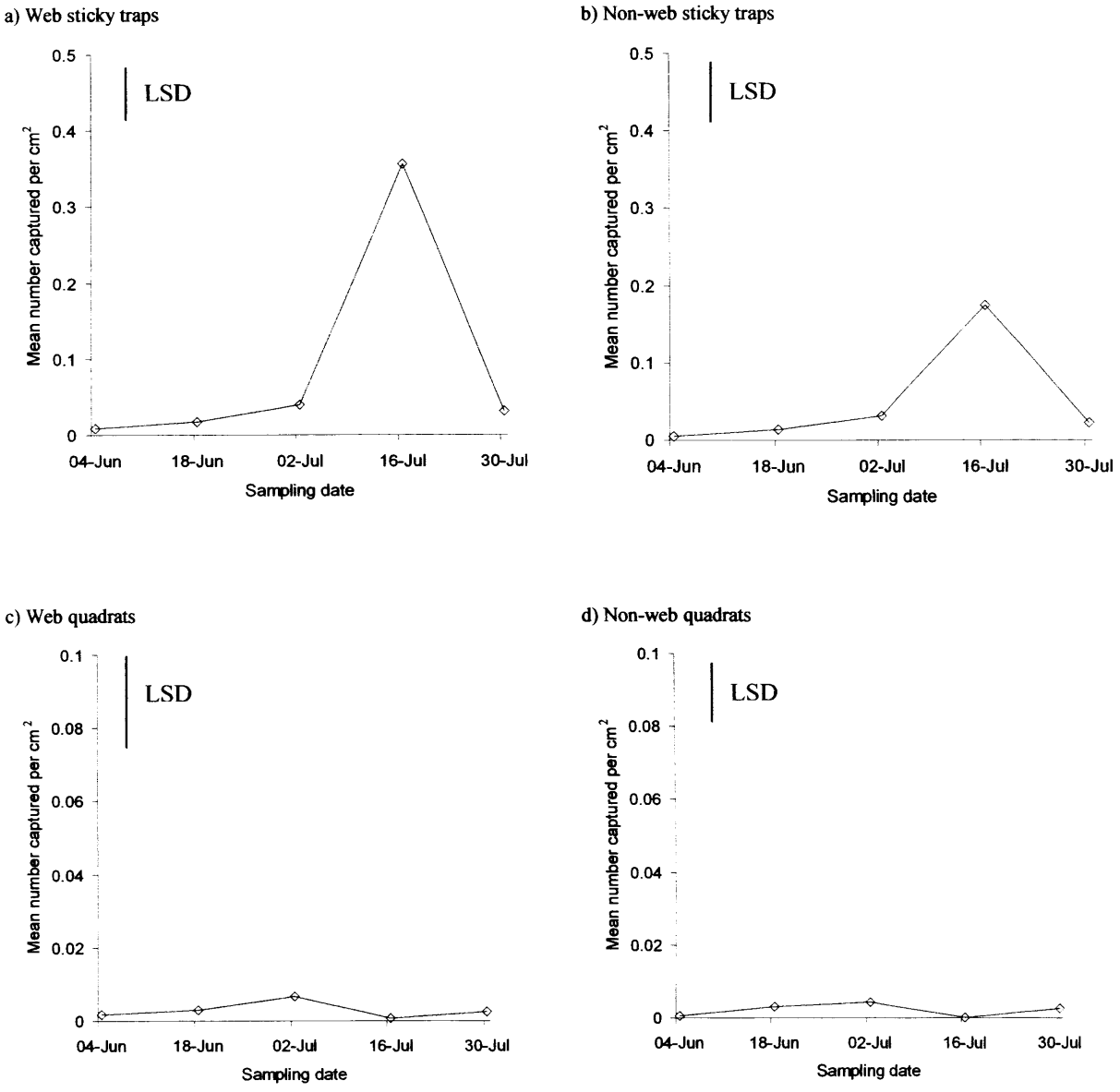


Figure 2.5: Mean number of Aphididae per cm^2 sampled during 2001 at a) web site sticky traps, b) non-web site sticky traps, c) web site quadrats and d) non-web site quadrats. LSD shown by solid bars for comparison of variation in density between sampling dates.

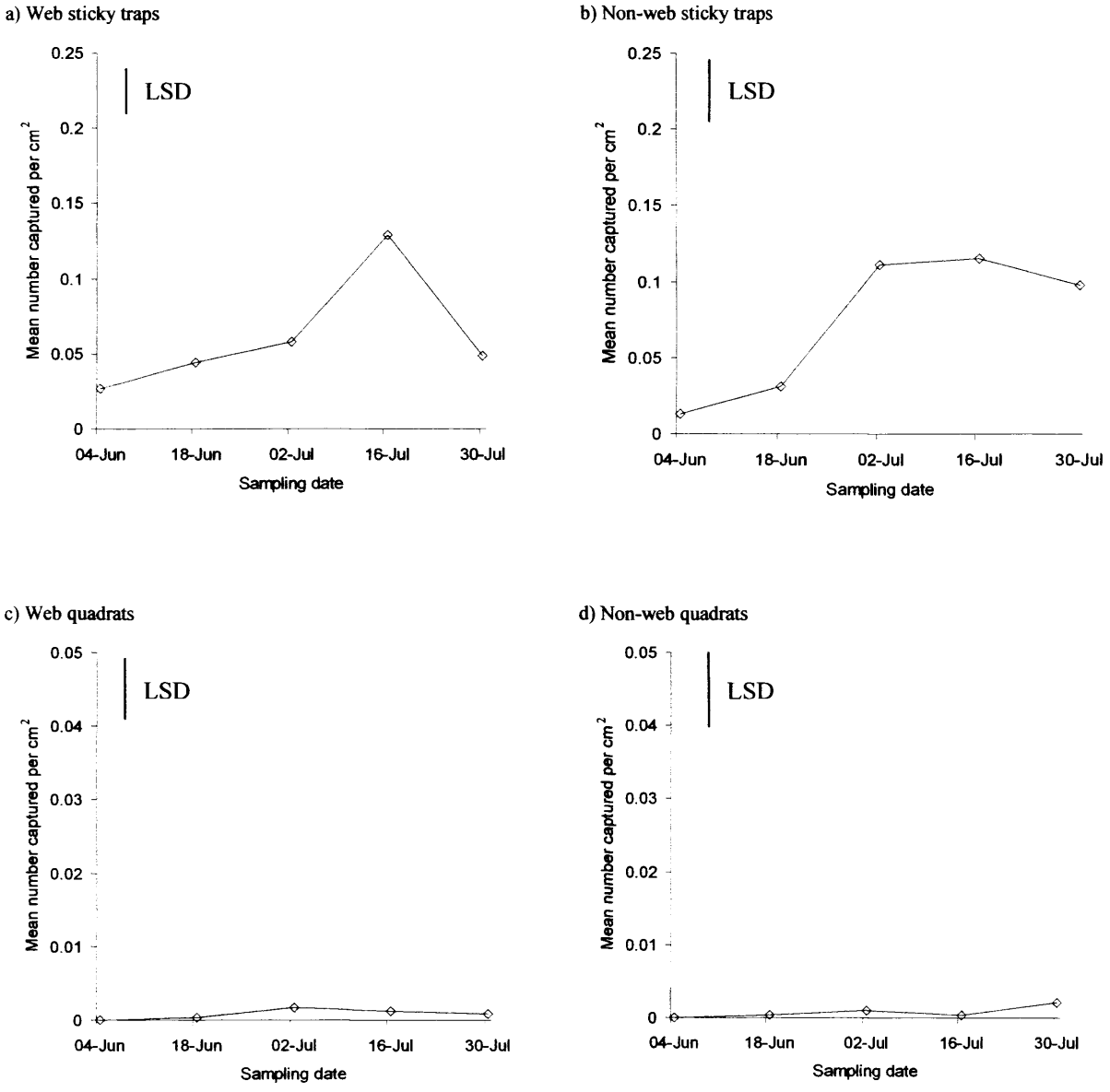


Figure 2.6: Mean number of Hymenoptera per cm^2 sampled during 2001 at a) web site sticky traps, b) non-web site sticky traps, c) web site quadrats and d) non-web site quadrats. LSD shown by solid bars for comparison of variation in density between sampling dates.

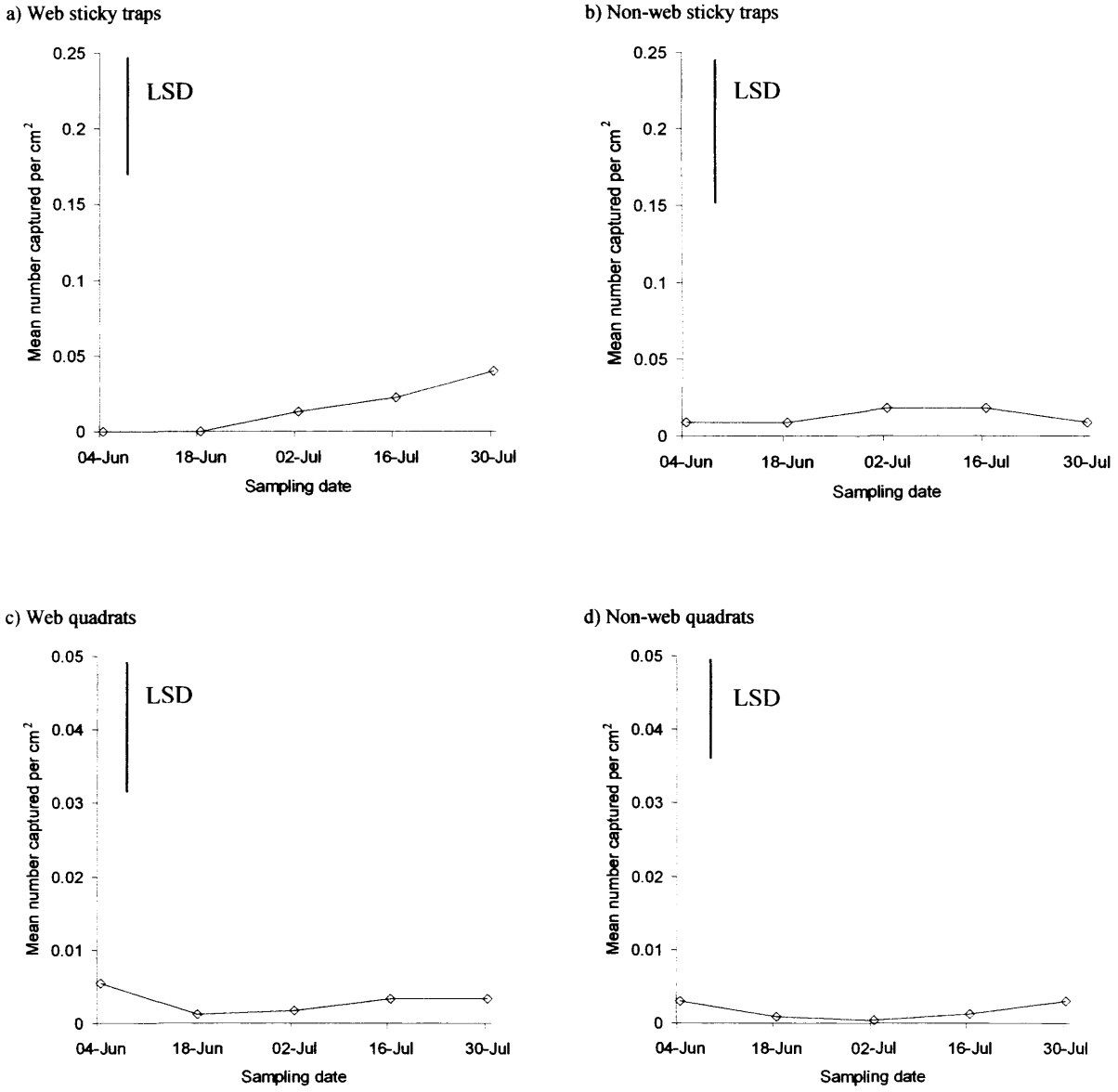


Figure 2.7: Mean number of Coleoptera per cm^2 sampled during 2001 at a) web site sticky traps, b) non-web site sticky traps, c) web site quadrats and d) non-web site quadrats. LSD shown by solid bars for comparison of variation in density between sampling dates.

2.3.2 Analysis of 2002 web data

A complete list of all arthropods collected in 2002 is shown in Appendix 2. The numbers of each species of linyphiid spider collected from each web prior to analysis are shown in Table 2.5.

Table 2.5: Numbers of each species of spider collected from webs in 2002 prior to sampling of their web sites with using either sticky traps or quadrats.

Spider species	Sticky trap sampled web site	Quadrat sampled web site	Total
Tenuiphantes tenuis	62	81	143
Erigone atra	27	19	46
E. dentipalpis	11	5	16
Bathyphantes gracillis	39	29	68
Oedothorax sp.	8	6	14
Meioneta rurestris	7	3	10
Pachygnatha degeeri	0	3	3
Savignya frontata	10	17	27
Erigoninae jeuveniles	4	3	7
Linyphiinae jeuveniles	0	2	2
Total	168	168	336

An analysis of the effects of field, sampling date, sampling method and web or non-web site location on the total potential prey captured was carried out using a multifactor ANOVA (Table 3.6). Each of the factors was shown to have a highly significant effect apart from field where the effect was significant but not as prominent at $P = 0.041$. Significant interactions were found between field and sampling date, sampling date and method as well as field and sampling date and method.

Table 2.6: ANOVA showing the effects of sampling date, sampling method, web site or corresponding non-web site location and field on numbers of potential linyphiid spider prey caught in 2002. All data was $Log_{10}([number\ captured\ per\ cm^2] + 1)$ transformed prior to analysis.

Source	d.f.	SS	MS	F	P
Field	1	0.022454	0.022454	4.18	0.041
Date	5	0.178709	0.035742	6.66	0.000
Method	1	1.608174	1.608174	299.72	0.000
Location	1	0.058838	0.058838	10.97	0.001
Field x Date	5	0.226503	0.045301	8.44	0.000
Field x Method	1	0.016155	0.016155	3.01	0.083
Field x Location	1	0.015005	0.015005	2.80	0.095
Date x Method	5	0.144602	0.028920	5.39	0.000
Date x Location	5	0.049150	0.009830	1.83	0.105
Method x Location	1	0.007378	0.007378	1.37	0.241
Field x Date x Method	5	0.164293	0.032859	6.12	0.000
Field x Date x Location	5	0.029665	0.005933	1.11	0.356
Field x Method x Location	1	0.015821	0.015821	2.95	0.086
Date x Method x Location	5	0.024629	0.004926	0.92	0.469
Field x Date x Method x Location	5	0.027056	0.005411	1.01	0.412
Error	622	3.337429	0.005366		
Total	669	5.928769			

In Table 2.7, the differences between web and non-web sites for each of the major taxa identified is shown. For Collembola, the difference between web and non-web sites was significant with more potential prey caught at web sites when sampled with sticky traps and quadrats. Coleoptera were also caught in greater densities when sampled at web sites with quadrats, however, no difference was found between web and non-web sites when sampled using sticky traps. More Diptera and Aphididae were also found at web sites with both sampling methods though these differences were found to be not significant. Hymenoptera were captured at higher densities in non-web sites though this was also found to be not significant.

Table 2.7: Paired t-test results for the difference between five potential prey taxa at web sites and non-web sites of linyphiid spiders when sampled using either sticky traps or quadrats in 2002. All data was $Log_{10}([\text{potential prey per cm}^2] + 1)$ transformed prior to analysis.

Potential prey item	t	n	Mean per web site ± SE	Mean per non web site ± SE	P
(a) Sticky traps					
Collembola	2.56	167	0.0629 (±0.0075)	0.0448 (±0.0055)	0.012
Diptera	0.50	167	0.0124 (±0.0013)	0.0112 (±0.0018)	0.617
Aphididae	0.92	167	0.0268 (±0.0046)	0.0212 (±0.0046)	0.359
Hymenoptera	0.61	167	0.0221 (±0.0024)	0.0245 (±0.0030)	0.542
Coleoptera	1.58	24	0.0062 (±0.0019)	0.0029 (±0.0012)	0.127
(b) Quadrats					
Collembola	4.16	168	0.0296 (±0.0031)	0.0194 (±0.0020)	0.000
Diptera	0.21	12	0.0012 (±0.0001)	0.0010 (±0.0001)	0.732
Aphididae	0.73	12	0.0050 (±0.0003)	0.0032 (±0.0002)	0.481
Hymenoptera	0.52	12	0.0029 (±0.0007)	0.0036 (±0.0008)	0.615
Coleoptera	2.82	12	0.0013 (±0.0008)	0.0006 (±0.0003)	0.017

Figure 2.8 shows the mean number of each taxa at web sites when sampled using sticky traps and quadrats Two sample t-tests caried out on the mean number per cm^2 of each potential prey population showed that sticky traps collected significantly more of each taxa ($P < 0.05$). The diversity of the potential prey was also shown to be significantly higher in sticky traps than in quadrats ($t = 2.57$, $P = 0.043$).

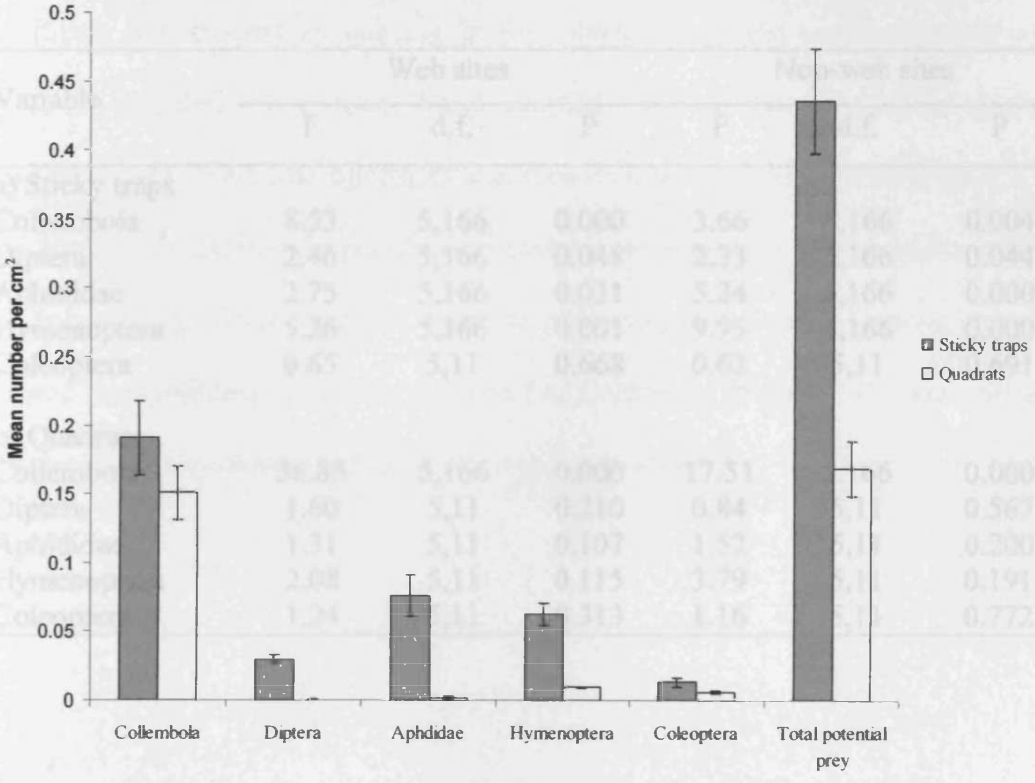


Figure 2.8: Bar chart of showing the mean number of each potential prey taxa (+SE) at linyphiid spider web sites when sampled using sticky traps or quadrats in 2002.

Two sample t-tests were used to show that there was no significant difference ($P < 0.050$) between the mean population per cm^2 of each taxa between the two fields sampled allowing the data from both fields to be pooled for analysis. Table 2.8 shows the significance in the variation of each taxa at web and non-web sites.

Table 2.8: ANOVA results testing for the variation over time in the Mean number per cm^2 of each potential prey taxa sampled using a) Sticky traps or b) Quadrats at either web sites or non-web sites.

Variable	Web sites			Non-web sites		
	F	d.f.	P	F	d.f.	P
a) Sticky traps						
Collembola	8.53	5,166	0.000	3.66	5,166	0.004
Diptera	2.46	5,166	0.048	2.33	5,166	0.044
Aphididae	2.75	5,166	0.031	5.24	5,166	0.000
Hymenoptera	5.26	5,166	0.001	9.95	5,166	0.000
Coleoptera	0.65	5,11	0.668	0.62	5,11	0.691
b) Quadrats						
Collembola	36.85	5,166	0.000	17.51	5,166	0.000
Diptera	1.60	5,11	0.210	0.84	5,11	0.567
Aphididae	1.31	5,11	0.107	1.52	5,11	0.200
Hymenoptera	2.08	5,11	0.115	3.79	5,11	0.191
Coleoptera	1.24	5,11	0.313	1.16	5,11	0.772

Collembola variation over time for each of the sampling methods is shown in Figure 2.9. For both sticky traps and quadrats the highest density of Collembola was late in May with a smaller peak in early July. This variation throughout sampling was significant for both sticky traps and quadrats. Figure 2.10 shows the variation over time for Diptera when sampled using each sampling method. Sticky traps showed significant variation over time with a peak in mid June. A comparatively low number of individuals were collected using quadrats and no significant variation was found. Where Aphididae were sampled using sticky traps (Figure 2.11) the variation was significant and showed an increase in the numbers captured until early July after which the population decreased. When sampled using quadrats, Aphididae numbers were lower and showed no significant variation over time. Hymenoptera (Figure 2.12) also showed an increase in population density over time with a subsequent crash in late July and significant variation when sampled using sticky traps. Quadrat sampling showed no significant variation over time. For Coleoptera (Figure 2.13) no significant variation over time was found for sticky traps or quadrats.

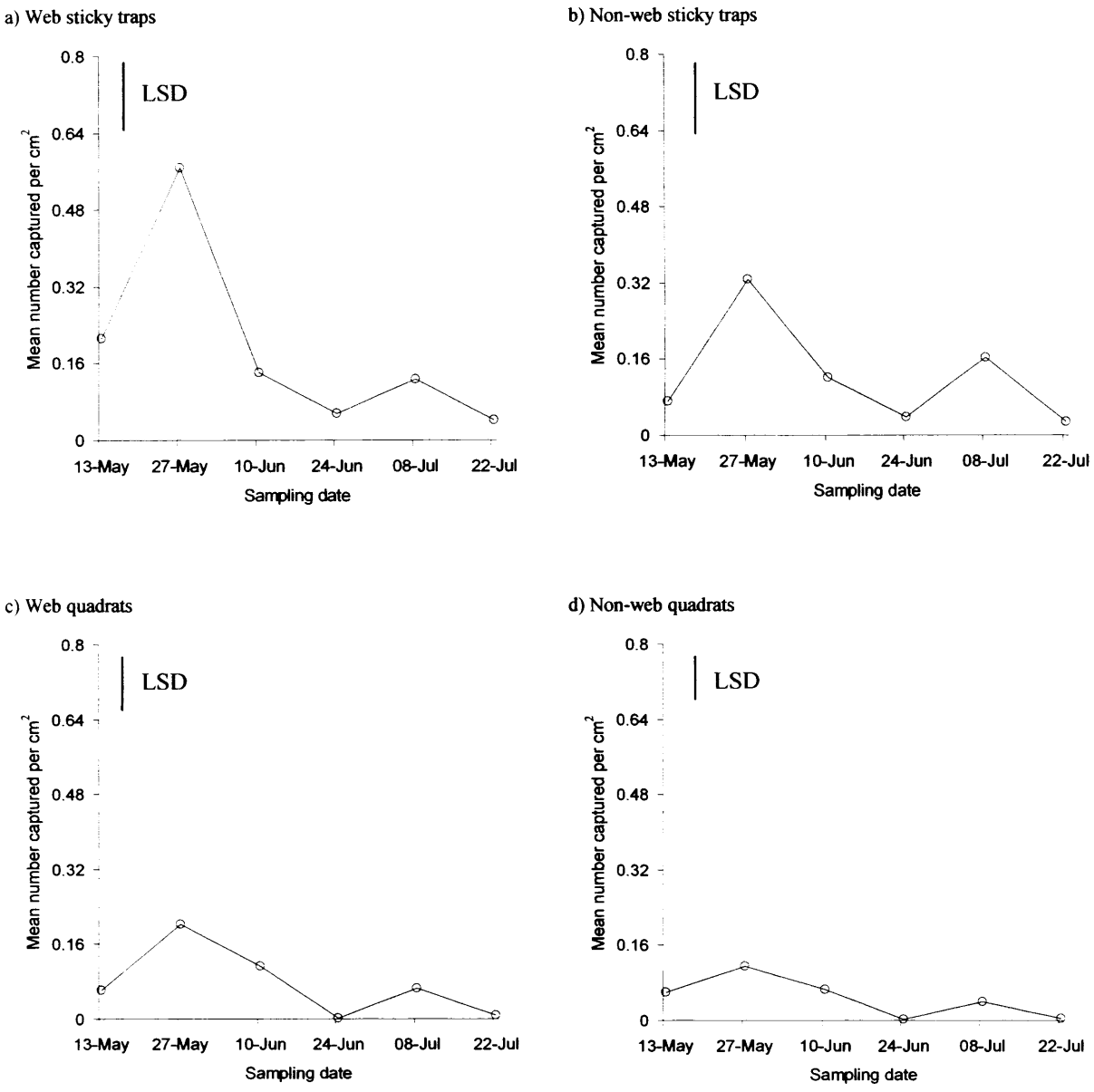


Figure 2.9: Mean number of Collembola per cm^2 sampled during 2002 at a) web site sticky traps, b) non-web site sticky traps, c) web site quadrats and d) non-web site quadrats. LSD shown by solid bars for comparison of variation in density between sampling dates.

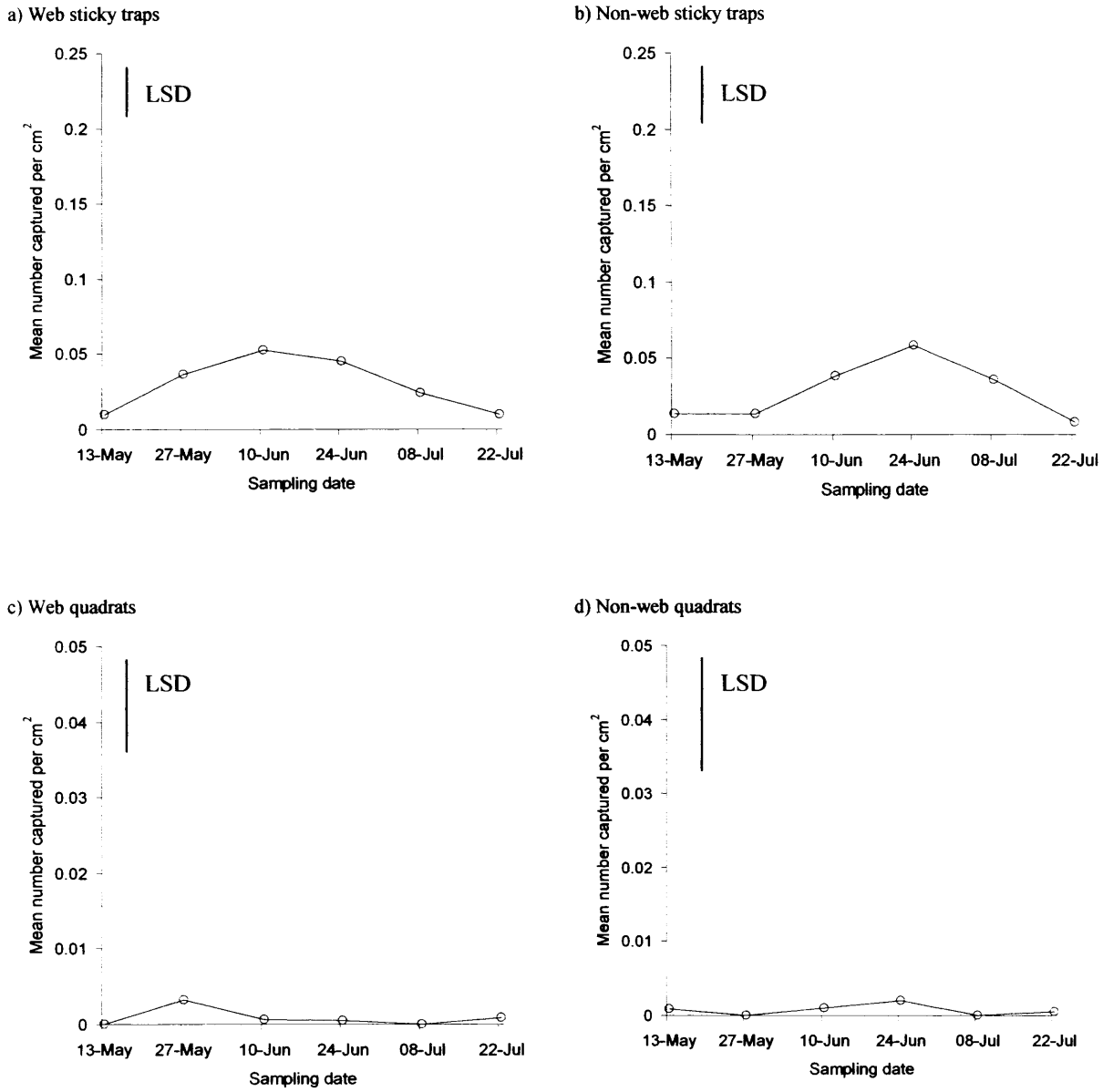
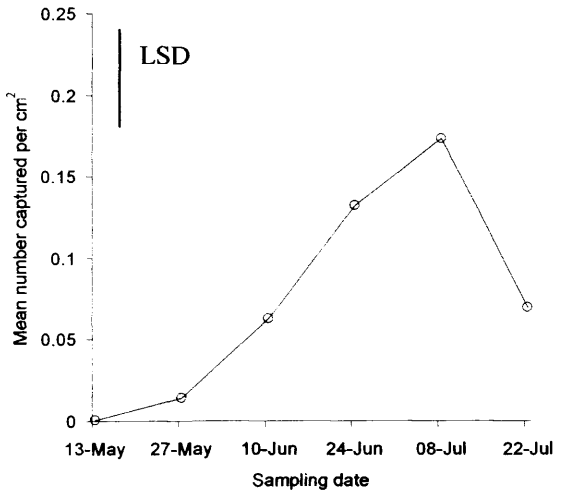
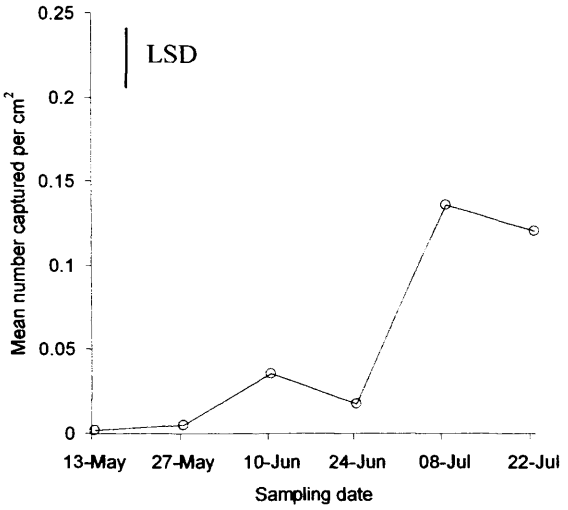


Figure 2.10: Mean number of Diptera per cm^2 sampled during 2002 at a) web site sticky traps, b) non-web site sticky traps, c) web site quadrats and d) non-web site quadrats. LSD shown by solid bars for comparison of variation in density between sampling dates.

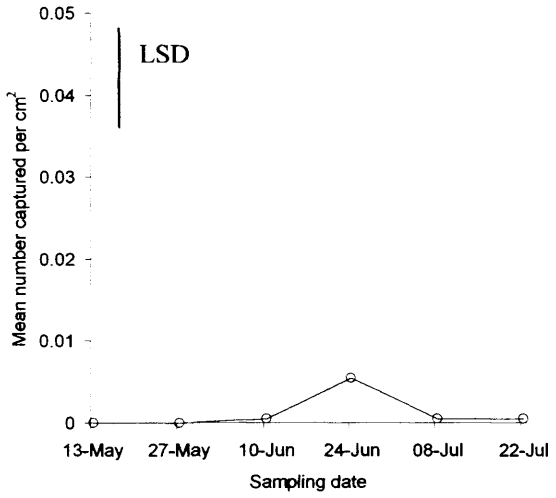
a) Web sticky traps



b) Non-web sticky traps



c) Web quadrats



d) Non-web quadrats

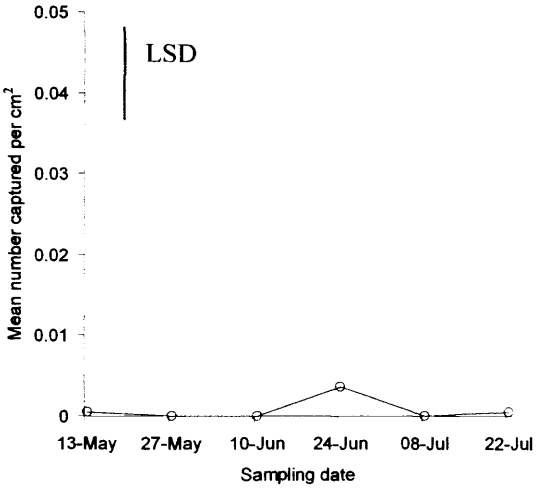


Figure 2.11: Mean number of Aphididae per cm^2 sampled during 2002 at a) web site sticky traps, b) non-web site sticky traps, c) web site quadrats and d) non-web site quadrats. LSD shown by solid bars for comparison of variation in density between sampling dates.

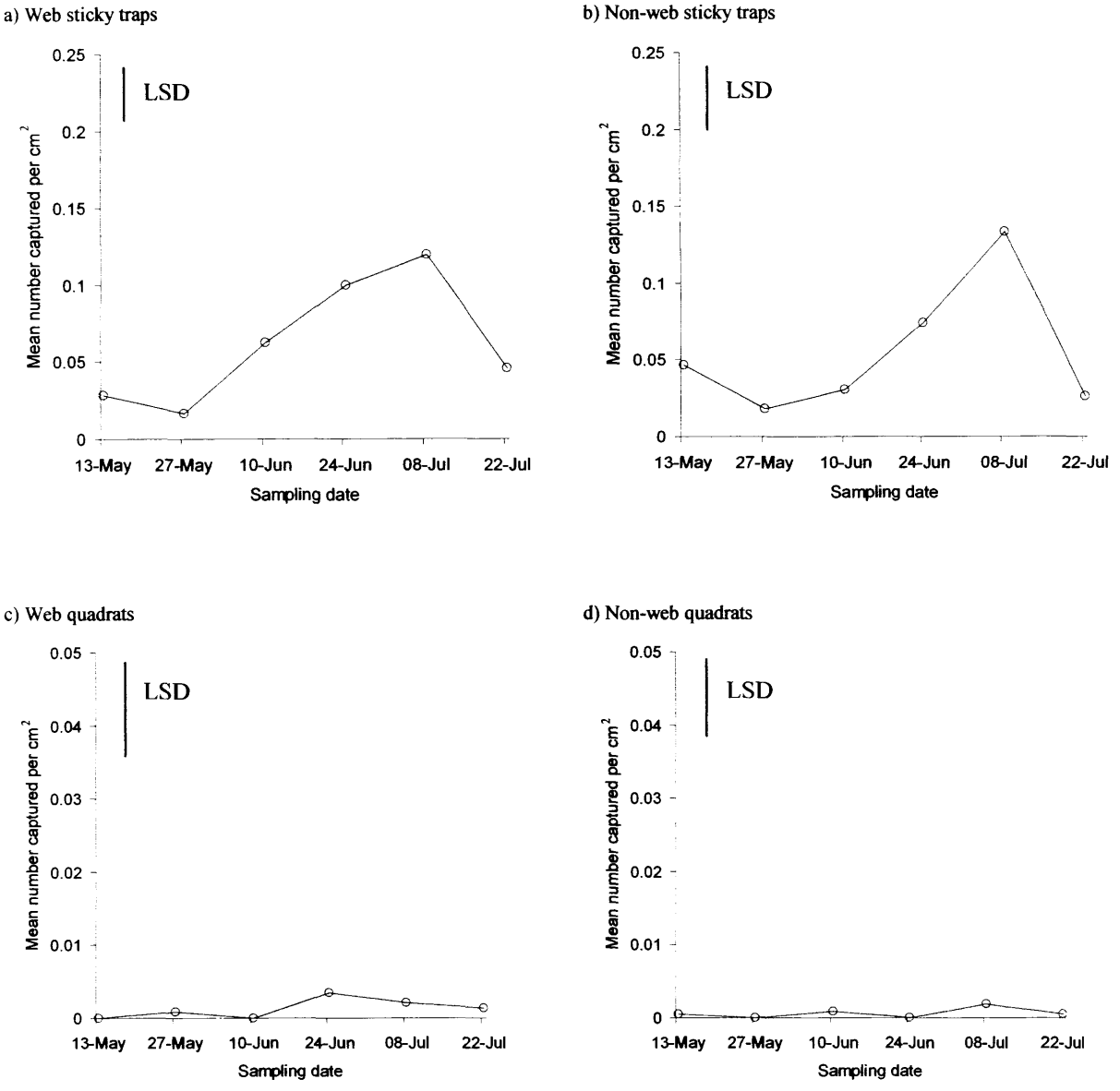


Figure 2.12: Mean number of Hymenoptera per cm^2 sampled during 2002 at a) web site sticky traps, b) non-web site sticky traps, c) web site quadrats and d) non-web site quadrats. LSD shown by solid bars for comparison of variation in density between sampling dates.

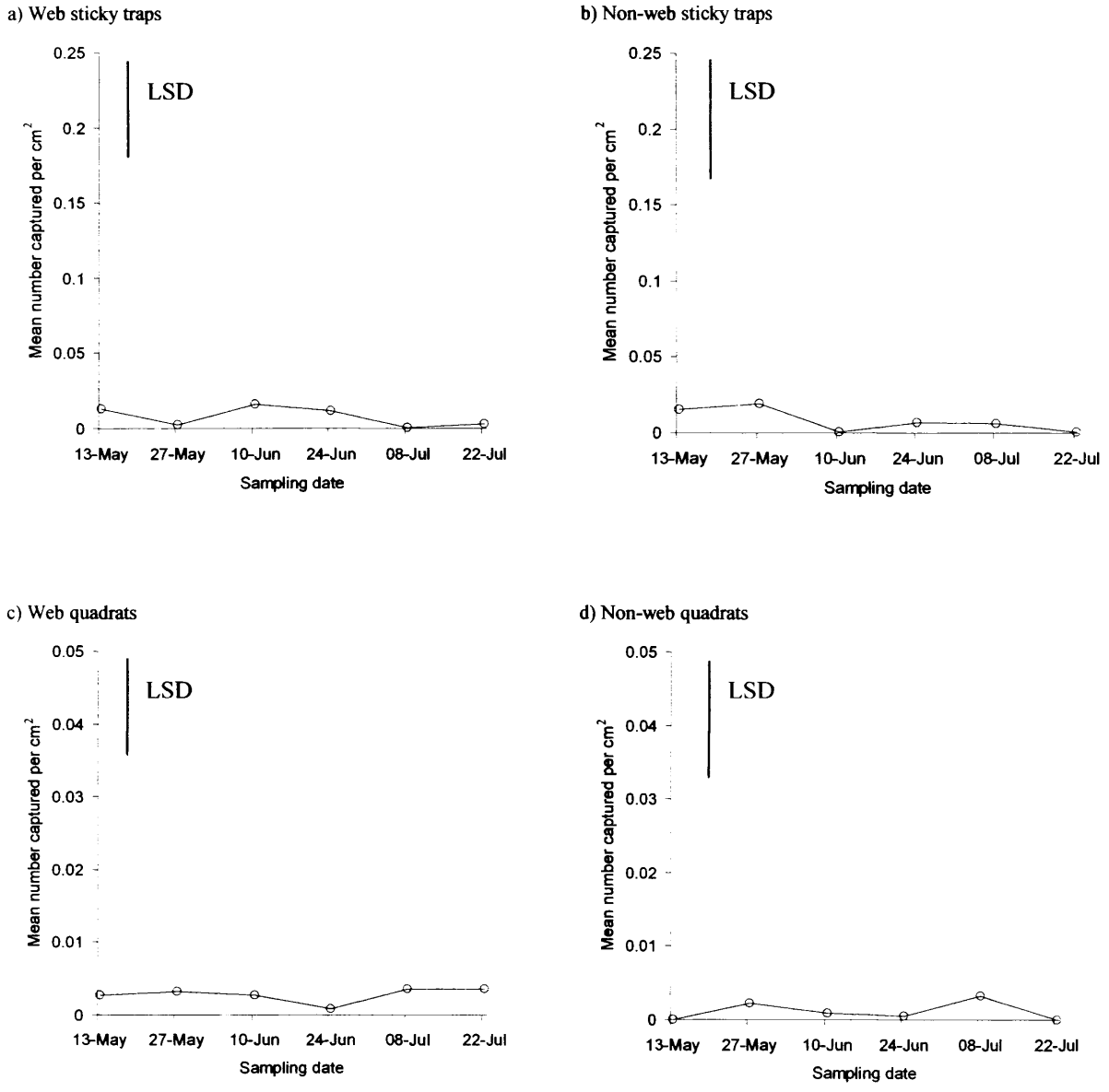


Figure 2.13: Mean number of Coleoptera per cm^2 sampled during 2002 at a) web site sticky traps, b) non-web site sticky traps, c) web site quadrats and d) non-web site quadrats. LSD shown by solid bars for comparison of variation in density between sampling dates.

2.3.2.1 Ground and aerial sticky trap analysis

Table 2.9 shows the numbers of each species of spider when collected from either ground webs or aerial webs.

Table 2.9: Numbers of each species of spider collected from either ground or aerial webs in 2002 prior to sampling using the corresponding sticky traps.

Spider species	Ground web site	Aerial web site	Total
Tenuiphantes tenuis	6	56	62
Erigone atra	27	0	27
E. dentipalpis	11	0	11
Bathyphantes gracillis	7	32	39
Oedothorax sp.	6	2	8
Meioneta rurestris	4	3	7
Pachygnatha degeeri	0	0	0
Savignya frontata	9	1	10
Erigoninae jeuveniles	1	3	4
Linyphiinae jeuveniles	0	0	0
Total	71	97	168

Figure 2.14 shows the difference in mean numbers per cm^2 between ground sticky traps and aerial sticky traps. The total number of potential prey per cm^2 was significantly higher for the ground sticky traps than for the aerial sticky traps ($t = 10.04$, $P = 0.000$). This trend was also found for Collembola ($t = 7.47$, $P = 0.000$), Aphididae ($t = 3.18$, $P = 0.002$), Hymenoptera ($t = 3.17$, $P = 0.002$) and Coleoptera ($t = 2.36$, $P = 0.035$). However, no significant difference was found between ground and aerial sticky traps for Diptera ($t = 0.35$, $P = 0.729$). The total diversity of potential prey throughout sampling was found to be significantly higher in aerial web sites than in ground web sites ($t = 3.56$, $P = 0.016$).

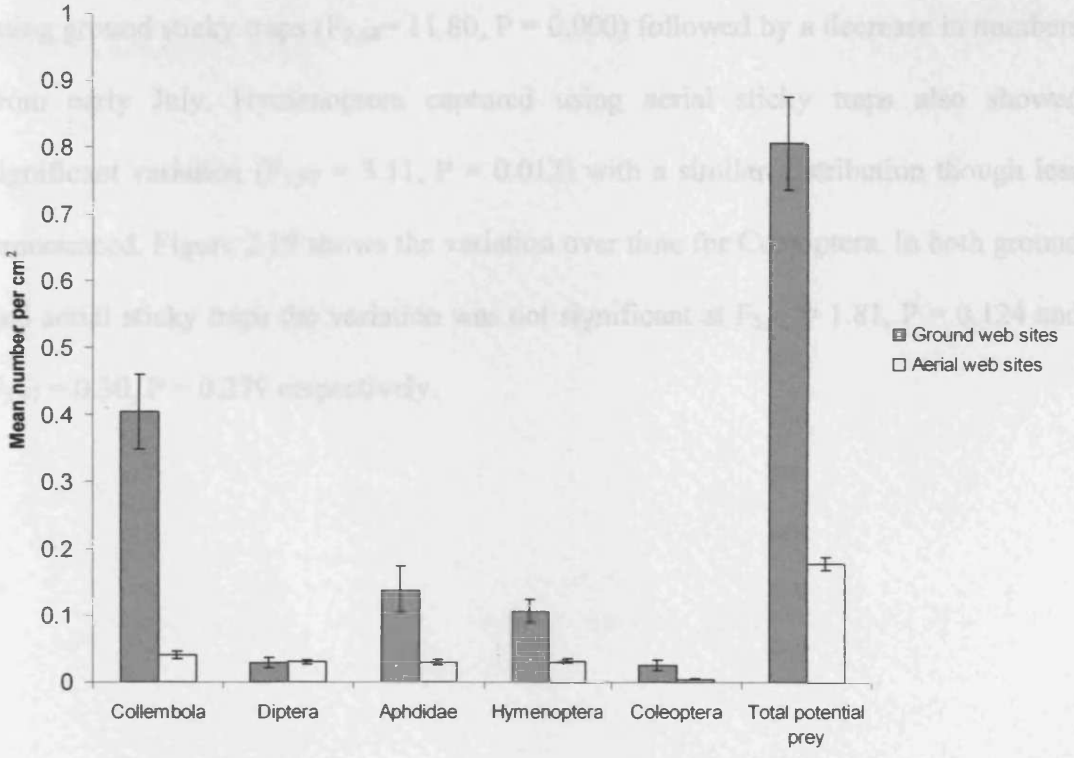
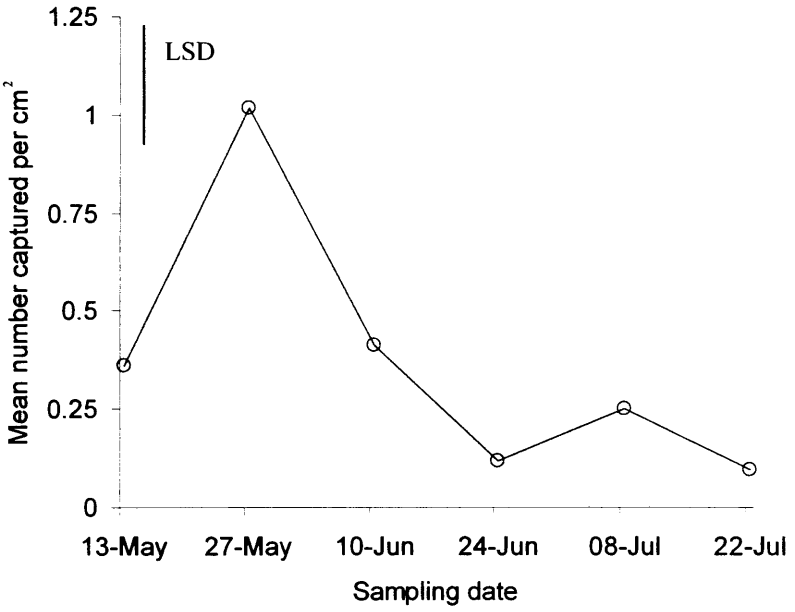


Figure 2.14: Bar chart of showing the mean number of each potential prey taxa (±SE) at ground or aerial linyphiid spider web sites when sampled using the corresponding ground or aerial sticky traps.

Figure 2.15 shows the variation of Collembola over time when sampled using ground sticky traps and aerial sticky traps. The variation was significant for both ground ($F_{5,68}$ = 5.58, P = 0.000) and aerial ($F_{5,97}$ = 5.40, P = 0.000) sticky traps with a peak in number per cm^2 during late May that was greater at ground sites. In Figure 2.16, the variation of Diptera over time was shown to have a normal distribution and to be significant for both ground ($F_{5,68}$ = 4.37, p = 0.002) and aerial ($F_{5,97}$ =6.02, P = 0.000) sticky traps. For Aphdidae (Figure 2.17), there is a significant increase in numbers until early July for ground sticky traps ($F_{5,68}$ = 3.04, P = 0.016) before numbers decrease. For aerial sticky traps there is also a significant increase ($F_{5,97}$ = 2.92, P = 0.021) though this increase is less pronounced. Hymeoptera (Figure 2.18) show a similar significant increase in number per cm^2 throughout the sampling season caught using ground sticky traps ($F_{5,68}$= 11.80, P = 0.000) followed by a decrease in numbers from early July. Hymenoptera captured using aerial sticky traps also showed significant variation ($F_{5,97}$ = 3.11, P = 0.012) with a similar distribution though less pronounced. Figure 2.19 shows the variation over time for Coleoptera. In both ground and aerial sticky traps the variation was not significant at $F_{5,68}$ = 1.81, P = 0.124 and $F_{5,97}$ = 0.30, P = 0.279 respectively.

a) Ground web sticky traps



b) Aerial web sticky traps

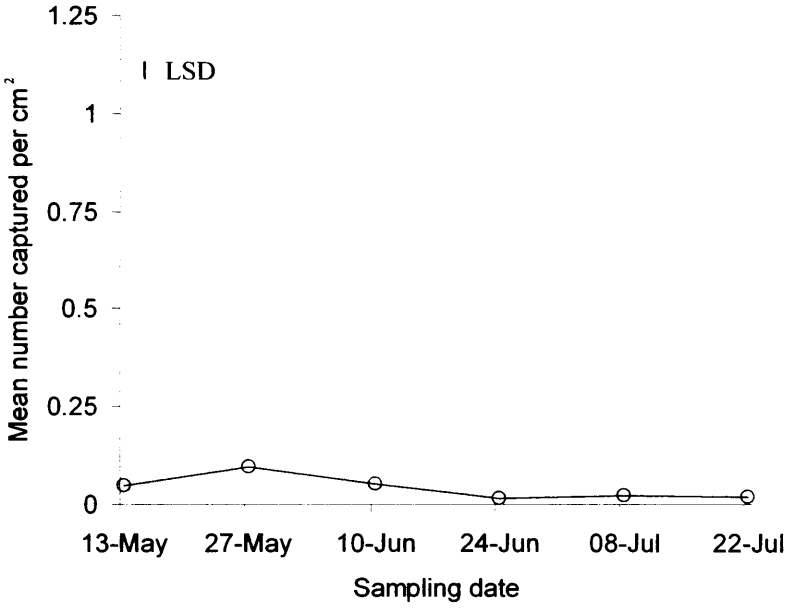
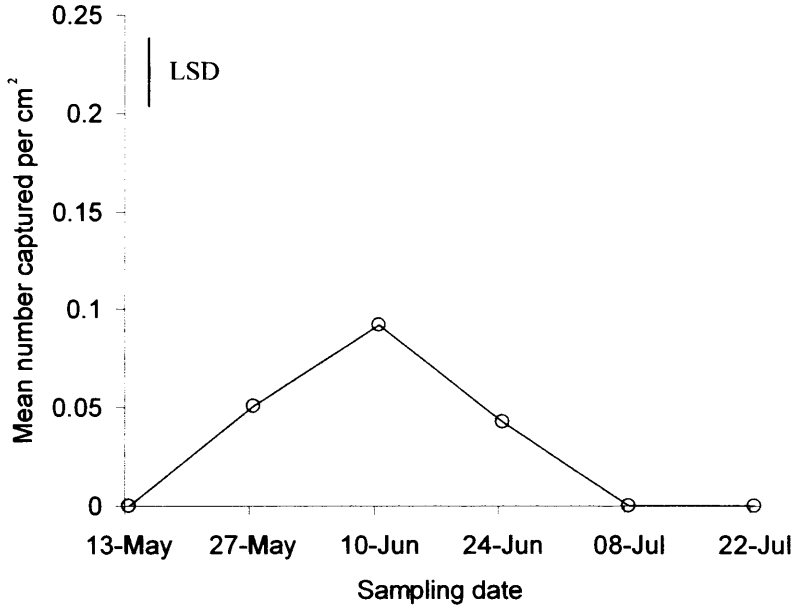


Figure 2.15: Mean number of Collembola per cm^2 sampled during 2002 at a) ground web site sticky traps and b) aerial web site sticky traps. LSD shown by solid bars for comparison of variation in density between sampling dates.

a) Ground web sticky traps



b) Aerial web sticky traps

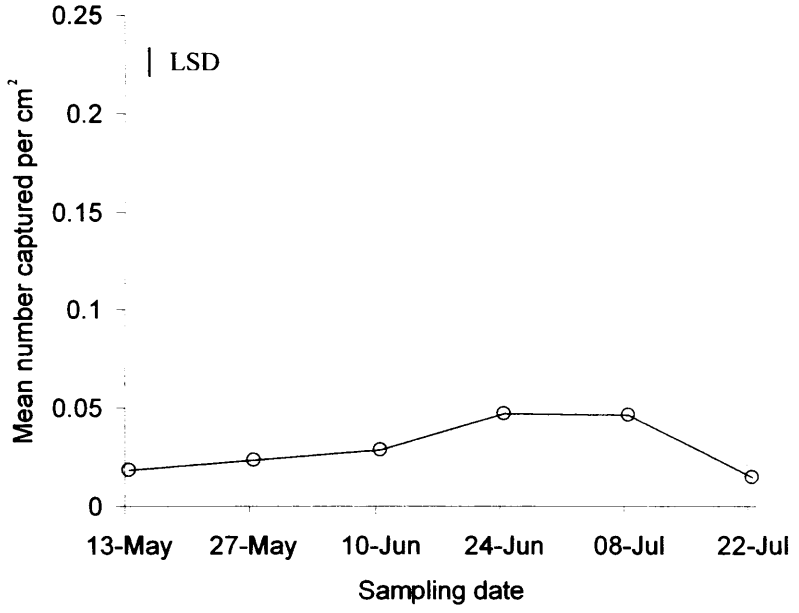
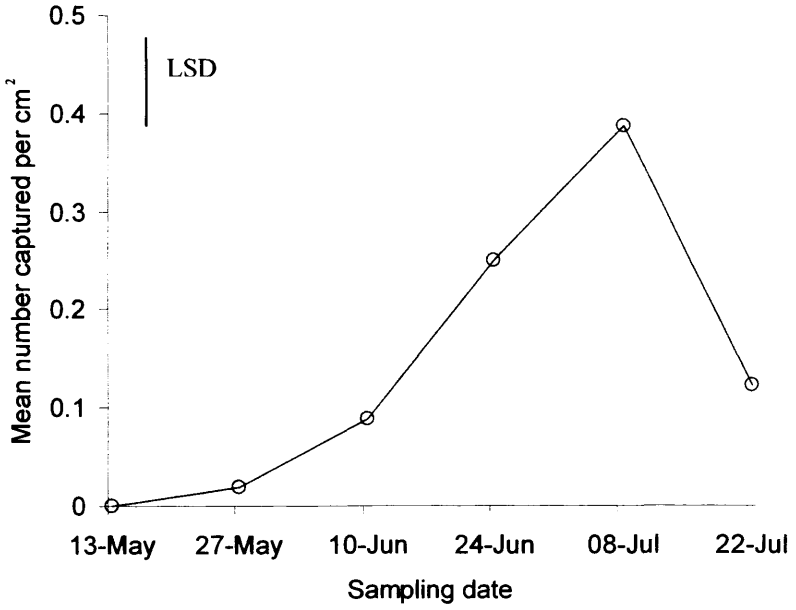


Figure 2.16: Mean number of Diptera per cm^2 sampled during 2002 at a) ground web site sticky traps and b) aerial web site sticky traps. LSD shown by solid bars for comparison of variation in density between sampling dates.

a) Ground web sticky traps



b) Aerial web sticky traps

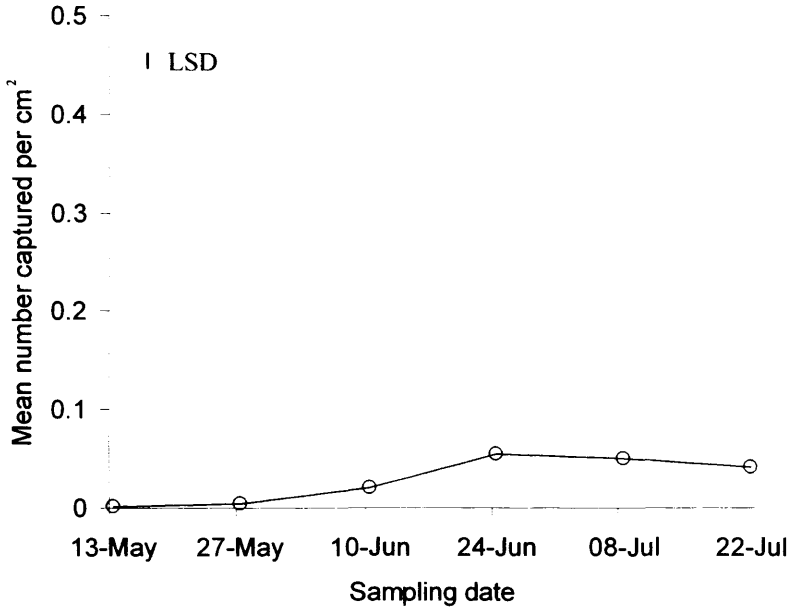
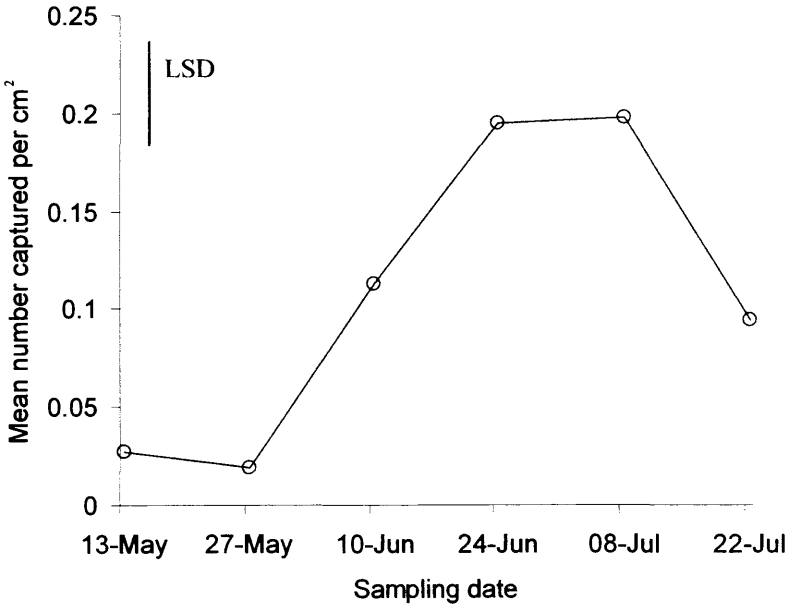


Figure 2.17: Mean number of Aphididae per cm^2 sampled during 2002 at a) ground web site sticky traps and b) aerial web site sticky traps. LSD shown by solid bars for comparison of variation in density between sampling dates.

a) Ground web sticky traps



b) Aerial web sticky traps

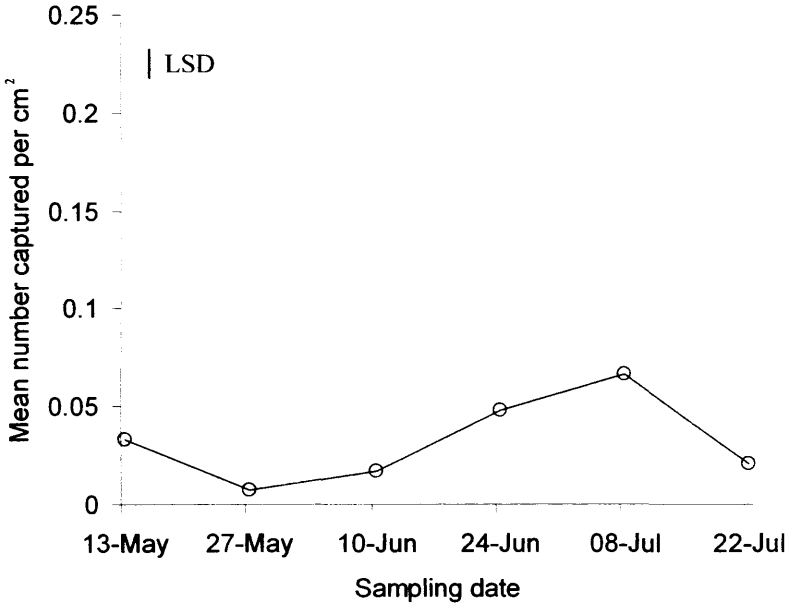
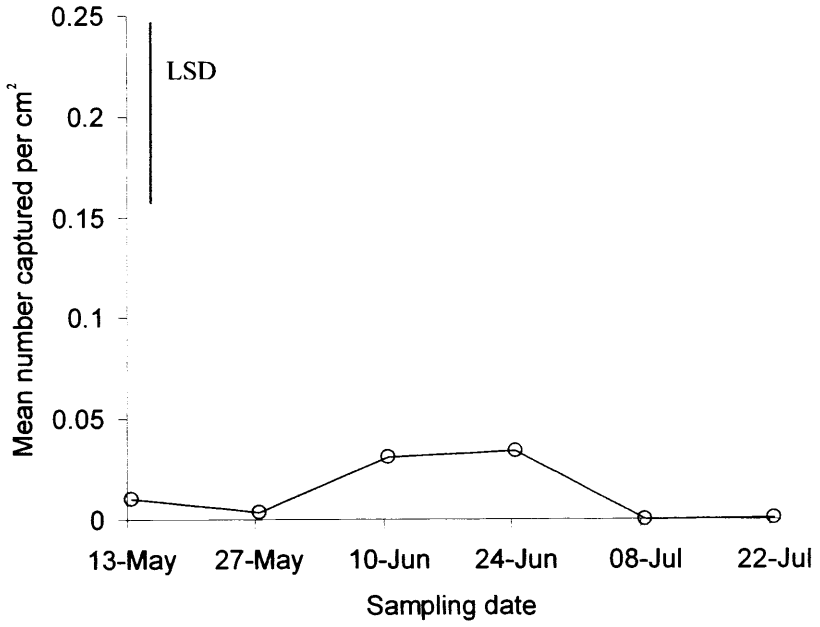


Figure 2.18: Mean number of Hymenoptera per cm^2 sampled during 2002 at a) ground web site sticky traps and b) aerial web site sticky traps. LSD shown by solid bars for comparison of variation in density between sampling dates.

a) Ground web sticky traps



b) Aerial web sticky traps

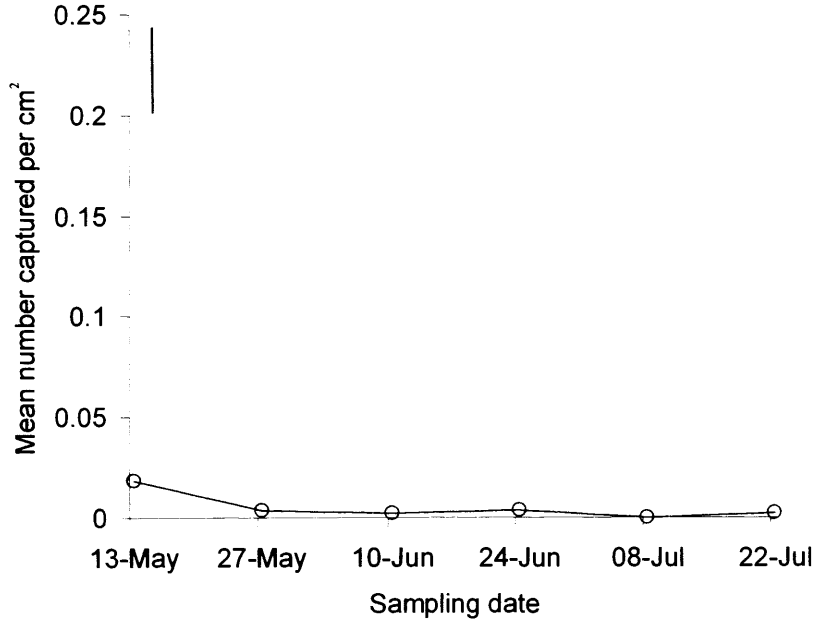


Figure 2.19: Mean number of Coleoptera per cm^2 sampled during 2002 at a) ground web site sticky traps and b) aerial web site sticky traps. LSD shown by solid bars for comparison of variation in density between sampling dates.

2.3.3 2001 vs 2002 comparisons

The differences between weather variables in 2001 and 2002 are shown in Table 2.10. Of the variables tested, only temperature was found to be significantly different with both the mean maximum and mean minimum being higher in 2001. When the diversity of total potential prey was compared between 2001 and 2002 no significant difference was found for either sticky traps ($t = 0.42$, $P = 0.690$) or quadrats ($t = 0.10$, $P = 0.925$). Differences between the two years in the mean number per cm^2 of each taxa captured at web sites was also investigated and the results are shown in Table 2.11. No significant difference was found except for Collembola where significantly more were caught at web sites in 2002. Non-web sites were not analysed.

Table 2.10: Differences between weather conditions during the sampling periods in 2001 and 2002.

Weather variable	Mean per day ± SE		F	P
	2001	2002		
Maximum air temperature (° C)	21.867 (±0.523)	19.989 (±0.375)	8.91	0.003
Minimum air temperature (° C)	11.549 (±0.406)	9.916 (±0.333)	8.80	0.004
Rainfall (mm)	2.170 (±0.906)	1.415 (±0.299)	0.73	0.393
Sunshine (h)	6.108 (±0.505)	6.516 (±0.481)	0.34	0.562
Wind speed (m.p.h.)	8.492 (±0.573)	9.107 (±0.585)	0.55	0.460
Relative humidity	70.64 (±1.43)	71.59 (±1.23)	0.25	0.615

Table 2.11: Two sample t-test results for the difference between five potential prey taxa at web sites in 2001 and 2002 when sampled using either sticky traps or quadrats. All data was $Log_{10}([potential\ prey\ per\ cm^2] + 1)$ transformed prior to analysis.

Variable	t	d.f.	Mean number per cm^2 in 2001 ± SE	Mean number per cm^2 in 2002 ± SE	p
a) Sticky traps					
Collembola	3.58	11	0.0151 (±0.0026)	0.0629 (±0.0075)	0.004
Diptera	1.94	19	0.0461 (±0.0046)	0.0124 (±0.0013)	0.067
Aphdidae	1.36	19	0.0266 (±0.0145)	0.0268 (±0.0046)	0.190
Hymenoptera	1.92	17	0.0244 (±0.0048)	0.0221 (±0.0024)	0.072
Coleoptera	0.93	19	0.0061 (±0.0031)	0.0062 (±0.0019)	0.364
b) Quadrats					
Collembola	2.10	16	0.0105 (±0.0015)	0.0296 (±0.0031)	0.052
Diptera	1.64	9	0.0012 (±0.0006)	0.0012 (±0.0001)	0.135
Aphdidae	1.32	15	0.0020 (±0.0009)	0.0050 (±0.0003)	0.207
Hymenoptera	0.98	13	0.0004 (±0.0002)	0.0029 (±0.0007)	0.346
Coleoptera	0.83	10	0.0019 (±0.0008)	0.0013 (±0.0008)	0.427

2.4 Discussion

The fine scale sampling methods employed in this study provide accurate methods of investigating the availability of potential prey to linyphiid spiders. In 2001, both sticky trap and quadrat data showed that web sites were located in areas of high Collembola density. This was also shown by Harwood *et al.* (2001, 2003) and supports feeding trial data showing that Collembola can be a high quality prey item (Marcussen *et al.* 1999; Bilde *et al.* 2000; Dinter 2004). The possible importance of Collembola for linyphiid spiders in the field was also shown by Agusti *et al.* (2003) where spiders were shown to be predating three species of Collembola and that there was preferential predation on one of the three species. The temporal variation of Collembola in 2001, showing that there was a high abundance early in the season but low density when aphids are present, also shows that Collembola may be an important alternative prey that allows the linyphiid spider population to increase prior the arrival of the pest. This pattern was also shown by Harwood *et al.* (2004) where once Collembola density was low, there was predation on the increasing population of aphids. Web sites were also found to be located in areas of high Diptera density. This was not found by Harwood *et al.* (2001, 2003) or in the 2002 linyphiid spider data. However, in 2001 the air temperature was significantly higher than in 2002 and there were significantly more Collembola captured using sticky traps at web sites in 2002 than in 2001. Although it is not statistically significant, this was some evidence that quadrats were also capturing more Collembola in 2002. This shows that the difference in Collembola density between the two years was not an artefact of using two types of sticky trap to sample potential prey. This could indicate that the density of Collembola in 2001 was insufficient to support linyphiid spiders so linyphiid spiders were using

Diptera as an additional food source. In 2002, the same pattern of high Collembola density early in the season and a high density of Aphdidae later in the season was shown. Despite the inclusion of using aerial sticky traps to more accurately model aerial webs, there was no difference between web and non-web sites for Diptera indicating that Collembola could be more important as an overall non-pest food source for linyphiid spiders.

The use of two different types of sticky trap allowed for comparisons between the two types of web strategy employed by the two sub-families of linyphiid spiders. Significantly higher densities of Collembola, Aphididae, Hymenoptera, Coleoptera and total potential prey were caught at the web sites of Erigoninae than at the web sites of Linyphiinae. This shows that there is a higher availability of potential prey at ground web sites meaning that the small ground based webs have a higher efficiency per cm^2 at capturing prey than the larger aerial webs. However, there was no significant difference between Diptera captured per cm^2 at ground web sites and aerial web sites. This indicates that aerial webs are positioned to maximize the possibility of encountering Diptera. However, the total diversity of potential prey was higher at aerial web sites than at ground web sites. This indicates that aerial webs of Linyphiinae are positioned to increase the diversity of potential prey. As feeding studies have shown that some Collembola are high quality prey items for linyphiid spiders (Marcussen *et al.* 1999; Bilde *et al.* 2000), the higher density of Collembola at ground web sites would provide Erigoninae spiders with a high quality diet. This may explain why Erigoninae do not invest in large webs and often hunt away from the web. At aerial web sites, the lower density of Collembola may mean that the Linyphiinae spiders have limited access to high quality prey. To increase the overall

quality of the diet, spiders at aerial web sites would have to invest more energy into constructing larger webs to obtain the higher diversity of prey needed for a favourable nutrient balance in their diet. This is supported by Greenstone (1979) who showed that lycosid spiders would consume prey in ratios that would optimize the amino acid intake. If Linyphiinae were unable to directly compete with Erigoninae for high quality prey at ground web sites, then this could explain why the Linyphiinae are so dependent on their web to obtain their dietary requirements. Such a high dependency on a web would mean that any favourable web sites would be highly contested and this is supported by Samu *et al.* (1996) which showed that *Tenuiphantes tenuis* web owners would vigorously defend their web in intraspecific competition with other individuals attempting to take over the web.

This study provides further evidence in support of Harwood *et al.* (2003) that the two linyphiid sub-families are exploiting different ecological resources by resource partitioning. Investigating these interactions by using direct methods, such as using molecular techniques to determine the gut contents of linyphiid spiders, would reveal the mechanisms by which the linyphiid spiders and non-pest prey interact to influence the potential of linyphiid spiders to predate pests.

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Chapter 3:

Potential prey molecular marker development and validation

3.1 Introduction

3.1.1 Identification of potential non-pest prey of linyphiid spiders

The theory of how generalist predators may act to control a pest population is that a population of generalist predators can increase by predating non-pest prey early in the growing season of a crop such that when pest prey invade the crop later in the growing season, there is a large population of predators that would prevent the establishment of the pest (Settle *et al.* 1996). This theory places a high level of importance on non-pest prey populations and predation on those non-pest prey by the generalist predator. Although there is evidence that generalist predators can exert a measure of control on a pest (Chiverton 1986), the interactions between predator, pest prey and non-pest prey are often poorly understood. As a generalist predator in winter wheat, linyphiid spiders have the potential to control populations of cereal aphids and are known to include cereal aphids in their diet (Sunderland *et al.* 1987; Harwood *et al.* 2004). However, the diversity and abundance of non-pest prey may influence linyphiid spider predation on aphids. Early in the season, Collembola are abundant (Sunderland *et al.* 1987; Harwood *et al.* 2001; Harwood *et al.* 2004; Chapter 2, this thesis) and Agusti *et al.* (2003) showed that linyphiid spiders predated several Collembola species in the field indicating that Collembola could be an important part of the linyphiid spider diet. Linyphiid spiders have been shown to locate their webs in areas of high Collembola density (Harwood *et al.* 2001; Harwood *et al.* 2003; Chapter 2, this thesis) and feeding studies have shown that some Collembola species are high quality prey items for linyphiid spiders (Marcussen *et al.* 1999). Higher densities of Diptera have also been found at web sites of spiders (Harwood *et al.* 2001; Harwood

et al. 2003) indicating that some species of Diptera may also form an important part of the linyphiid spider diet in the field.

Determining the extent of predation on non-pest prey and pest prey by linyphiid spiders would give an indication of the relative importance of each prey item to the predator and hence their role in determining linyphiid spider potential to control aphids. Studies using molecular tools to identify the remains of prey within the gut of a predator have been shown to be effective at investigating predation by linyphiid spiders (Sunderland *et al.* 1987; Agusti *et al.* 2003; Harwood *et al.* 2004). Polymerase chain reaction (PCR) primers used to amplify DNA of prey can be easily optimized for use in different predation studies. Identification of potential prey from Chapter 2 (this thesis) showed that three species of Collembola (*Isotoma anglicana*, *Entomobrya multifasciata* and *Lepidocyrtus cyaneus*) (see Appendix 1 and Appendix 2) are common in winter wheat and this is supported by (Harwood *et al.* 2001). Further identification of Diptera from samples taken from winter wheat in 1998 and 1999 at HRI in Wellsbourne during a study by (Harwood *et al.* 2001) showed that the species *Lycoriella castanescens* and the family Cecidomyiidae are also common potential prey for linyphiid spiders (see Table 3.1 and Table 3.2). This was also shown by identifying Diptera sampled in winter wheat in Chapter 2 (this thesis) (see Appendix 1 and Appendix 2).

3.1.2 Aims of this study

In a previous study by (Agusti *et al.* 2003) into linyphiid spider predation on Collembola, primers were designed for *I. anglicana*, *E. multifasciata* and *L. cyaneus* to show that these spiders would predate such non-pest prey in the field. Primers have also previously been designed for the pest cereal aphid, *Sitobion avenae* by Chen *et al.* (2000). This study aims to optimize these pest and non-pest primers to eliminate cross-amplification of non-target potential prey and predators to facilitate their use for investigating the complex predator-prey interactions involving these prey and linyphiid spiders in winter wheat. Non-target potential prey were identified and selected on the basis of high relative abundance and measuring less that 5 mm in length as linyphiid spiders are known to preferentially predate prey in this size range (Nyffeler 1999). Primers to *L. castanescens* and Cecidomyiidae have not been previously designed and this study aims to design primers to these Diptera for use on linyphiid spiders collected from the field. Feeding experiments using linyphiid spiders and these prey items would enable the half life of detection of the prey DNA within the gut of the predator to be determined. This study aims to assemble these tools to enable their use in investigating linyphiid spider predation in the field.

Table 3.1: List of Diptera identified from samples taken in 1998 from winter wheat during a study by Harwood *et al.* (2001) showing the relatively high numbers of *Lycoriella castanescens* that were captured.

Suborder	Family	Species	Number
Nematocera	Sciaridae	*Bradysia confines* m.	4
		B. triseriata m.	4
		Bradysia sp. f.	9
		Lycoriella castanescens m.	9
		L. castanescens f.	12
		Lycorriella sp. f.	2
		Trichosia sp. f.	1
	Cecidomyiidae	Unidentified	27
	Mycetophilidae	*Exechia pseudofestiva*	3
Brachycera (Cyclorrapha)	Spheroceridae	*Pteremis fenestralis*	32
	Muscidae	*Coenosia tigrina*	7
	Phoridae	*Megaselia* sp.	10
		Spiniphora dorsalis	1
		Triphleba luteifemorata	1
	Lonchopteridae	*Lonchoptera lutea*	9
Brachycera (orthorrapha)	Empididae	*Platypalpus* sp.	2
	Dolichopodidae	*Campsicnemus curvipes*	1

Table 3.2: List of Diptera identified from samples taken in 1999 from winter wheat during a study by Harwood *et al.* (2001) showing the relatively high numbers of *Lycoriella castanescens* that were captured.

Suborder	Family	Species	Number
Nematocera	Tipulidae	*Nephrotoma submaculosa*	1
	Psychodidae	*Psychoda phalenoides*	1
	Scatopsidae	Unidentifiable	1
	Chironomidae	*Krenosmitta camptopheps*	1
	Sciaridae	*Bradysia* sp. f.	6
		Bradysia triseriata m.	2
		Epidapus edwardsi m.	3
		Lycoriella sp. f.	9
		Lycoriella castanescens m.	12
		Lycoriella castanescens f.	5
	Cecidomyiidae	*Peromya* sp.	1
		Jenetiella sp.	2
		Putoniella sp.	1
		Mayetiola sp.	2
		Clinodiplosis sp.	2
		Unidentifiable	36
	Mycetophilidae	*Exechia pseudofestiva*	1
Brachycera (Cyclorrapha)	Spheroceridae	*Pteremis fenestralis*	7
		Sphaerocera monilis	1
		Leptocera fontinalis	1
	Opomyzidae	*Geomyza tripunctata*	1
	Agromyzidae	*Liriomyza pedestris*	1
	Sepsidae	*Sepsis orthocnemis*	1
	Chloropidae	*Meromyza* sp.	1
	Anthromyiidae	*Delia platura*	1
		Delia sp.	1
		Fucellia sp.	1
	Muscidae	*Coenosia tigrina*	2
	Phoridae	*Megaselia* sp.	27
	Lonchopteridae	*Lonchoptera lutea*	3
		Lonchoptera furcata	1
	Syrphidae	*Episyrphus balteatus*	1
		Syrphus vitripennis	2
Brachycera (Orthorrapha)	Empididae	*Platypalpus pallidiventris*	2
		Empis nuntia	1
	Dolichopodidae	*Campsicnemus curvipes*	17

3.2 Methods

3.2.1 Potential prey primer design

3.2.1.1 DNA extraction

DNA was extracted from all predators and potential prey in the same way using a DNeasy® Tissue Kit (Qiagen Ltd). Individual whole organisms were placed in 1.5 ml Eppendorf tubes and 180 µl of Buffer ATL was added. The arthropod was then homogenized using a plastic pestle and the DNA was extracted as directed by the manufacturer's DNeasy® Tissue Kit Handbook (2003) using the protocol "Isolation of Total DNA from Animal Tissues" from step 2. A full list of the species from which DNA was extracted is shown in Table 3.3.

3.2.1.2 PCR of DNA extractions using general primers

A 473 bp segment of the cytochrome oxidase I (COI) mitochondrial gene was amplified from each predator and potential prey DNA extracts by polymerase chain reaction (PCR) using the general primers CI-J-1718 5' GGCGGGTTTGGAAATTGATTAGTGCC 3' and CI-N-2191 5' CCCGGTAAAATTAAAATATAAACTTC 3' (Simon *et al.* 1994). The PCR reaction volume was 25 µl containing 4 µl DNA extract, 1 X PCR Buffer (containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl) (Invitrogen™, Life Technologies®), 2 mM $MgCl_2$, 0.05 mM each dNTP (ABgene®), 0.5 µM each primer, 0.625 units of Taq DNA Polymerase (Invitrogen™, Life Technologies®) and made up to final volume with sterile water. PCR was carried out in a Perkin-Elmer 9700 Automated Thermocycler with PCR conditions optimized to initial denaturation for 2 minutes at 94 °C, then 35

cycles of denaturation for 1 minute 10 seconds at 94 °C, annealing for 1 minute 10 seconds at 58 °C and extension for 1 minute 10 seconds at 72 °C, then final extension for 5 minutes at 72 °C. 5 µl of PCR product was separated on 1.2 % agarose gel stained with ethiduim bromide for visual confirmation of successful amplification with a GeneRuler™ 100 bp ladder (Fermentas) used as a size standard.

Table 3.3: List of DNA extractions from arthropods for use in primer development and cross reactivity tests. Arthropods were collected from the field site at HRI, wellsbourne during either 1998 and 1999 (Harwood *et al.* 2001) or 2001 and 2002 (Chapter 2, this thesis).

Order	Family	Species
Araneae	Linyphiidae	*Erigone atra* (Blackwall) *E. dentipalpis* Blackwall) *Tenuiphyphantes tenuis* (Blackwall)
Arthropleona	Entomobryidae	*Entomobrya multifaciata* (Tullberg) *Lepidocyrtus cyanus* (Tullberg)
	Isotomuridae	*Isotoma anglicana* (Lubbock)
	Sminthuridae	*Sminthurus elegans* (Fitch)
Diptera	Cecidomyiidae	*Sitodiplosis mosellana* (Géhia) *Clinodoplosis* sp. (Kieffer) *Mayetola* sp. (Kieffer) *Peromyia* sp. (Kieffer) *Putoniella* sp. (Kieffer) *Resseliella* sp. (Seitner)
	Dolichopodidae	*Campsicnemus curvipes* (Fallén)
	Drosophilidae	*Drosophila melanogaster* (Meigen)
	Phoridae	*Megaselia* sp. (Rondani)
	Sciaridae	*Bradysia confinis* (Winnertz) *B. trieriata* (Winnertz) *Lycoriella castanescens* (Lengersdorf)
	Sphaeroceridae	*Pteremis fenestralis* (Fallén)
Hemiptera	Aphididae	*Metopolosiphum dirhodum* (Walker) *Rhopalosiphum padi* (Fitch) *Sitobion avenae* (Fabricius)

3.2.1.3 Sequencing of PCR products

Prior to sequencing, PCR products were cleaned using Geneclean® Turbo for PCR Kit (Boi101) as per the manufacturer's instructions. Separate forward and reverse sequencing PCRs were carried out on the PCR products using 2 µl cleaned PCR product, 1 µl of 0.8 pmol / µl of either forward or reverse primer, 1 µl of Terminator mix (ABI Prism® BigDye™ Terminator Cycle Sequencing v2 Ready Reaction kit,) and 1µl of sterile water. The reaction conditions were 25 cycles of 10 seconds at 96 °C, 50 seconds at 50 °C and 4 minutes at 60 °C. The resulting products were then sequenced on an ABI Prism 3100 Genetic Analyzer Perkin-Elmer automated DNA sequencer.

3.2.1.4 Species specific primer design and optimization

All sequences were aligned using BioEdit™ Sequence Alignment Editor (Hall 1999). Potential species specific primer sites were identified by eye using guidelines from (Innis & Gelfand 1990), and using Primer3 (Rozen & Skaletsky 2000). Primers were designed and optimized using guidelines from (Innis & Gelfand 1990) and (Saniki 1990). Sequence alignments and primer sites are shown in Appendix 5. All primer pairs were optimized for a PCR reaction volume of 12 µl containing 2 µl DNA extract, 1 X PCR Buffer, 2 mM $MgCl_2$, 0.05 mM each dNTP, 0.5 µM each primer, 0.625 units of *Taq* DNA Polymerase and made up to final volume with sterile water. The reaction conditions were: initial denaturation for 2 minutes at 94 °C, then 35 cycles of denaturation for 1 minute 10 seconds at 94 °C, annealing for 1 minute 10 seconds at a temperature specific for the primer pair (see Table 3.4) and extension for 1 minute 10 seconds at 72 °C, then final extension for 5 minutes at 72 °C. Primers were tested for cross amplification using the DNA extracts shown in Table 3.3.

Table 3.4: Complete list of all primer pairs developed and used in this study with optimal annealing temperature for each pair

Target organism	Primer pair	Primer sequence 5'-3'	Amplicon size (bp)	Annealing temperature	Source
Lycoriella castanescens	L.castF1	CAGATATAGCATTCCCCCGTT	210	66	New primer pair
	L.castR1	CCCAAGATTGAGGAAATACCC			
	L.castF1	As above	218	66	New primer pair
	L.castR2	TTACTGCCCCCAAGATTGAGG			
	L.castF1	As above	337	60	New primer pair
	L.castR3	TAAAACGGGTAGGGATAGAAG			
	L.csatF2	GAACAGTTTATCCTCCCCTATC	229	60	New primer pair
	L.castR3	As above			
Cecidomyiidae	CecidF1	CCCGATATAGCATTTCCACG	415	67	New primer pair
	CecidR2	GGATCTCCTCCTCCTATAGG			
	CecidF1	As above	413	62.5	New primer pair
	CecidR3	ATCTCCTCCTCCTATAGG			
	CecidF4	CATACAGGATCATCAGTAGA	271	57.5	New primer pair
	CecidR2	As above			
	CecidF4	As above	269	52	New primer pair
	CecidR3	As above			
Isotoma anglicana	Ia1F	CTCTTCTATTGGCCGGAGGACTTG	276	68	Agusti *et al.* (2003)
	Ia4R	GCACAGGAAGTGATAGTAAAAGTAA			
Lepidocyrtus cyanus	Lc2F	CCCACTAGCTGCTGGAATCGCCC	216	69	Agusti *et al.* (2003)
	Lc4R	GCACTGGGAGGGATAGTAGTAATA			
Entomobrya multifactiata	Em1F	CCCTCCTTCTTACAGGAGGTTTAG	211	64	Agusti *et al.* (2003)
	Em3R	TGATCTCAAGATATTCCAGGGGT			
Sitobion avenae	EgaCOIIF1	TATTTGAACTACAACTCCTC	231	62	Chen *et al.* (2000)
	EgaCOIIR	AGTTTTATTGTCTACTTCAATTAAA			

3.2.2 DNA decay rate experiments

3.2.2.1 Arthropod cultures

3.2.2.1.1 Spiders

Spiders were collected from winter wheat fields at Horticulture Research International, Wellesbourne, UK. *Tenuiphantes tenuis* (Blackwall) (Araneae: Linyphiidae) and *Erigone atra* (Blackwall) (Araneae: Linyphiidae) were identified and maintained individually in 5 cm Φ Petri dishes containing damp Plaster of Paris and charcoal base on a diet of live *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae). Spiders were maintained in culture in a 16 h light / 8 h dark cycle at 16 °C.

3.2.2.1.2 Diptera

Wingless *D. melanogaster* (Meigen) were cultured in a 16 h light / 8 h dark cycle at 20 °C on Drosophila Ready Mix (Phillip Harris Education). *Lycoriella castanescens* (Lengersdorf) (Diptera: Sciaridae) were collected from winter wheat fields at Horticulture Research International, Wellesbourne, UK. *Lycoriella castanescens* were cultured on a mixture of 5 % soya flour and compost in a 16 h light / 8 h dark cycle at 20 °C.

3.2.2.1.3 Collembola

Isotoma anglicana (Lubbock) (Collembola: Isotomidae) were collected from fields of winter wheat at Horticulture Research International, Wellesbourne, UK. *Isotoma*

anglicana were cultured on damp Plaster of Paris and activated charcoal and maintained on a diet of organic potato in a 16 h light / 8 h dark cycle at 20 °C.

3.2.2.1.4 Aphids

Sitobion avenae (Fabricius) (Homoptera: Aphididae) were cultured within cages on winter wheat (Herewood) grown in peat in a 16 h light / 8 h dark cycle at 16 °C.

3.2.2.2 Feeding experiments

Feeding experiments were carried out to determine the length of time DNA from a prey item could be detected once ingested. All spiders were starved for two weeks prior to the feeding experiment. Individual male and female *L. tenuis* were offered *L. castanescens* during a 2 h feeding period. Each spider was allowed to ingest an individual *L. castanescens* and the spider was then removed to a clean Petri dish. Once the initial feeding period had been completed, spiders were maintained in culture in a 16 h light / 8 h dark cycle at 16 °C and allowed to feed on *S. avenae ad libatum* for the remainder of the feeding experiment. Five females and five males were frozen at –80 °C after 0, 2, 4, 8, 12, 24, 36, 48 and 72 hrs taking t=0 hrs as the mean point of the feeding period due to variation of spider feeding rates on *L. castanescens*. Five starved males and five starved females were also frozen at –80 °C as controls. Due to insufficient numbers of male *L. tenuis*, only 3 individuals were frozen at t=36 hrs and none were frozen at t=48 and 72 hrs. The feeding experiment carried out by (Agusti *et al.*, 2003) using female *E. atra* fed *I. anglicana* attained a DNA detection rate of 100 % at 24 hours. To determine the limit of detection a further feeding experiment was done using female *E. atra* fed on *I. anglicana*. Individual *E. atra* were allowed to feed on single *I. anglicana* during a feeding period of 2 hrs. Spiders were then maintained in clean Petri dishes and allowed to feed on *S. avenae ad libatum* as before. Five spiders were frozen at –80 °C after 0, 12, 24, 36, 48 and 72 hrs where t=0 hrs is the mean of the feeding period. Five starved spiders were also frozen at –80 °C as controls. PCRs were carried out on all feeding experiment spiders to determine the success rate of amplification of target DNA after the various digestion times. The primer pair used to detect *L. castanescens* DNA was L.castF1 / L.castR1 whereas the primers used to detect *I. anglicana* DNA were Ia1F / Ia4R.

Statistical analysis of feeding experiments carried out using Minitab® release 13 (Minitab Inc.). Regression analysis was used to determine decay rate of each experiment and analysis of covariance (ANCOVA) was used to compare the decay rates.

3.3 Results

3.3.1 Primer cross-reactivity

Gel pictures from the cross-reactivity testing are shown in Appendix 4 and the results from the cross-reactivity testing on all primer pairs is summarized in Table 3.5. Three primer pairs designed to amplify *L. castanescens* was successfully optimized to be species specific. Three primer pairs for Cecidomyiidae were shown to cross amplify non-target Diptera and one primer pair was shown to amplify DNA from two different genera of Cecidomyiidae. Primer pairs for the three species of Collembola and the aphid were successfully optimized to be species specific.

Table 3.5: Cross amplification tests using optimised primer pairs. Primer pair shown in bold was used for feeding decay experiments

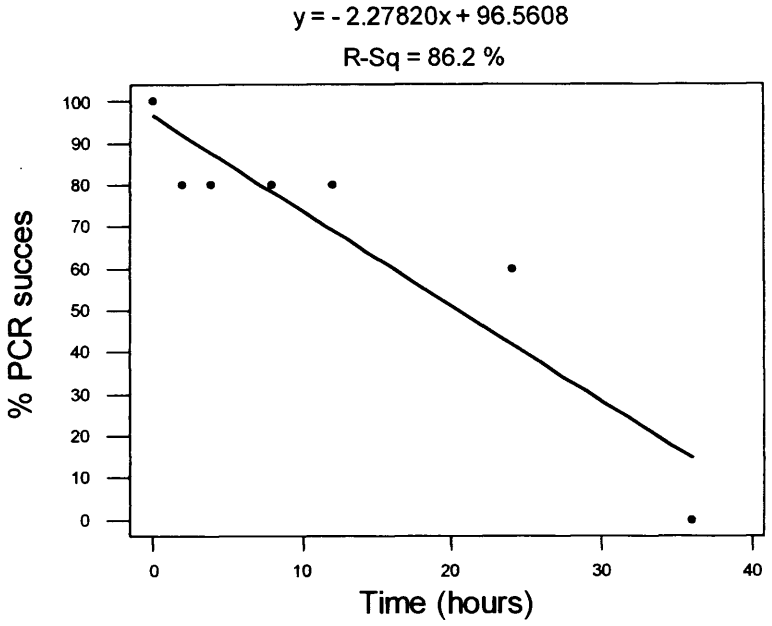
DNA extract	Primer pair: amplification = √ no amplification = -											
	L.castF1 L.castR1	L.castF1 L.castR2	L.castF1 L.castR3	L.castF2 L.castR3	CecidF1 CecidR2	CecidF1 CecidR3	CecidF4 CecidR2	CecidF4 CecidR3	Ia1F Ia4R	Lc2F Lc4R	Em1F Em3R	EgaCOIIF1 EgaCOIIR
Erigone atra	-	-	-	-	-	-	-	-	-	-	-	-
E. dentipalpis	-	-	-	-	-	-	-	-	-	-	-	-
Lephyphantes tenuis	-	-	-	-	-	-	-	-	-	-	-	-
Entomobrya multifaciata	-	-	-	-	-	-	-	-	-	-	√	-
Lepidocyrtus cyanus	-	-	-	-	-	-	-	-	-	√	-	-
Isotoma anglicana	-	-	-	-	-	-	-	-	√	-	-	-
Sminthurus elegans	-	-	-	-	-			-	-	-	-	-
Sitodiplosis mosellana	-	-	-	-	√	√	√	√	-	-	-	-
Clinodoplosis sp.	-	-	-	-	-	-	-	-	-	-	-	-
Mayetola sp.	-	-	-	-	-	-	-	-	-	-	-	-
Peromyia sp.	-	-	-	-	-	-	-	-	-	-	-	-
Putoniella sp.	-	-	-	-	√	-	-	-	-	-	-	-
Resseliella sp.	-	-	-	-	-	-	-	-	-	-	-	-
Campsicnemus curvipes	-	-	-	-	-		-	√	-	-	-	-
Drosophila melanogaster	-	√	-	-	-	√	√	-	-	-	-	-
Megaselia sp.	-	-	-	-	-	-	-	-	-	-	-	-
Bradysia confines	-	-	-	-	-	-	-	-	-	-	-	-
B. trieriata	-	-	-	-	-	-	-	-	-	-	-	-
Lycoriella castanescens	√	√	√	√	-	-	-	-	-	-	-	-
Pteremis fenestralis	-	-	-	-	-	-	-	-	-	-	-	-
Metopolosiphum dirhodum	-	-	-	-	-	-	-	-	-	-	-	-
Rhopalosiphum padi	-	-	-	-	-	-	-	-	-	-	-	-
Sitobion avenae	-	-	-	-	-	-	-	-	-	-	-	√

3.3.2 Feeding experiment results

Gel pictures showing the results of the feeding experiments are shown in Appendix 4 and Figure 3.1 and Figure 3.2 show the regression plots for the decay rates. For both female *T. tenuis* and female *E. atra* there was 100 % detection for the first five and three time periods respectively and regression analysis was carried out using subsequent time periods. In all three decay rate regression plots there were high correlation coefficient (r^2) values indicating a high amount of the Y variable (% PCR success) that is explained by the X (time since ingestion) variable. This also shows that the decay rate was linear. By re-arranging the regression equation $y = a + bx$, where 'b' is the gradient and 'a' is the Y intercept, to $x = (y - a) / b$ is was possible to calculate the DNA decay half life by substituting Y as 50 % PCR success rate. The half life for each experiment is shown in Table 3.6. The results from the ANCOVA tests are shown in Table 3.7. The decay rate regression lines were shown to be significantly different from zero at $P < 0.001$ in the three feeding experiments. The ANCOVA results also show that the decay rate of *L. castanescens* DNA is faster in male *T. tenuis* than in female *T. tenuis* by a significantly different slope ($P = 0.013$).

Figure 3.1: Regression graphs showing the rate prey DNA decay within the gut of a linyphiid spider

a) Male *Tenuiphantes tenuis* fed *Lycoriella castanescens*



b) Female *Tenuiphantes tenuis* fed *Lycoriella castanescens*

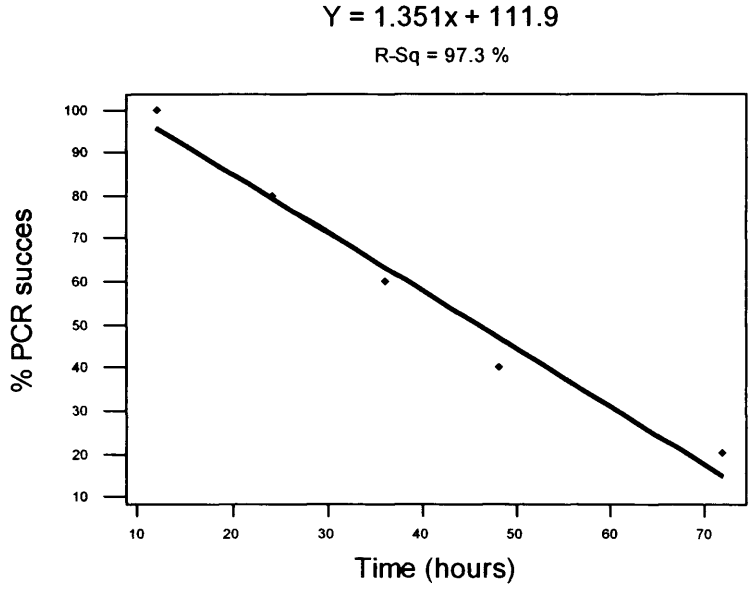


Figure 3.2: Regression graph showing the rate of DNA decay of *Isotoma anglicana* within the gut of female *Erigone atra*

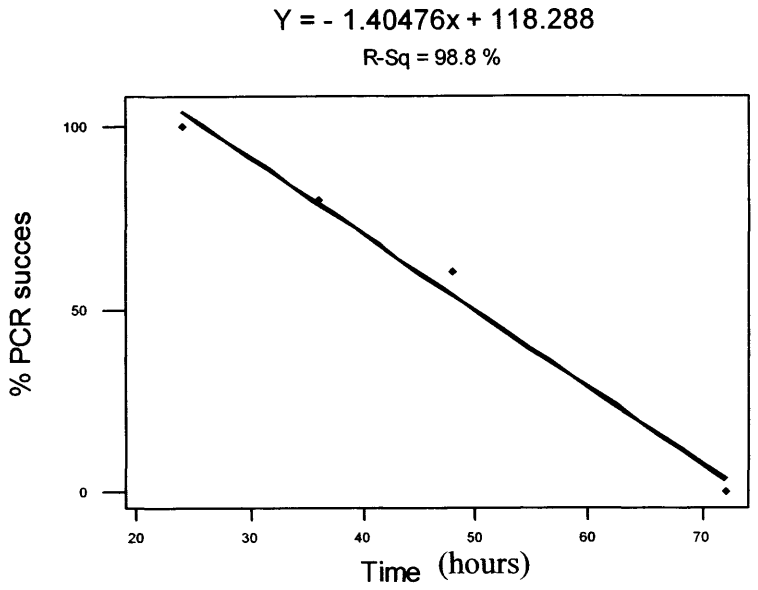


Table 3.6: DNA decay rate half life for each feeding experiment

Spider species	Sex	Experimental prey item	Amplicon size (bp)	r^2	DNA half life (hours)
L. tenuis	Male	*L. castanescens*	210	0.862	20.438
L. tenuis	Female	*L. castanescens*	210	0.973	45.515
E. atra	Female	*I. anglicana*	276	0.98.8	48.612

Table 3.7: Feeding experiments regression analyses and analysis of covariance (ANCOVA) results

Regression comparison	Slope		Y intercept	
	F	P	F	P
Male L. tenuis vs Female L. tenuis	12.21	0.013	0.01	0.974

3.4 Discussion

The feeding experiments showed that DNA from individual prey items could be detected after ingestion by linyphiid spiders for extended periods of time. The detection times for *T. tenuis* females and *E. atra* females were similar at 45.5 and 48.6 hrs respectively and both spider species readily consumed the prey items offered. However, male *T. tenuis* did not readily consume prey items offered with some individuals rejecting prey items after only a short ingestion time. In addition, disproportionately large numbers of male *T. tenuis* died in culture before the feeding experiment indicating that they may not be well suited to culture.

By determining the detection limits of ingested prey DNA, these laboratory experiments show that this is a viable system for use on field caught spiders. The chance of successfully amplifying prey DNA has been increased by using approaches previously shown to be advantageous. All the amplicons are comprised of short sequences (between 200 – 300 bp) as in previous studies (Agusti *et al.* 1999; Zaidi *et al.* 1999) where shorter sequences were shown to resist digestion and so be detectable for longer periods of time. In addition, (Zaidi *et al.* 1999) demonstrated successful amplification of ingested prey DNA present in multiple copies at the cellular level and theorized that this would increase the likelihood of successful amplification. Subsequent studies targeted genes from the mitochondrial genome (Agusti *et al.* 2003; Chen *et al.* 2000) as an obvious source of multiple copy DNA. Also, by allowing the predator to consume additional alternative prey for the duration of the feeding experiment, the possibility of obtaining detection times analogous to those for field caught spiders is increased. Field caught spiders are likely to have partially consumed

multiple prey items regardless of satiation state (Maupin & Riechert 2001) which may affect the detection time of any one prey item consumed.

The successful development of this method provides a powerful tool for investigating predation. This method was used to investigate the predation dynamics of linyphiid spider and their potential prey within fields of winter wheat elsewhere in Chapters 4 and 5.

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Chapter 4:

Predation by Linyphiidae spiders on non-pest and pest prey in winter wheat: spiders preferences in the field

4.1 Introduction

4.1.1 General background of biological control

A large variety of arthropods are known to inhabit agricultural crops. Herbivorous arthropods that attack the crop are regarded as pests and those with a high intrinsic capacity for a rapid increase in population are likely to cause a reduction in crop yields. Infestations of aphids within cereal crops can cause significant damage (George & Gair 1979; Niehoff & Stablein 1998) and even if relatively small numbers initially invade the crop, their parthenogenetic nature can result in a rapid population increase (Vickerman & Wratten 1979). Chemical pesticides can be used to control such pests but the long term effects, including the development of pest resistance (Ripper 1944; McDonald *et al.* 1999; Perez *et al.* 2000) and environmental pollution (McDonald *et al.* 1999) indicate the need to investigate alternatives. The rearing and release of specialist biological control agents such as parasitoids (Giller *et al.* 1995; Pankanin-Franczyk & Ceryngier 1995; Kehrli & Wyss 2001) can be carried out in response to pest outbreaks, however, the possibility also exists to use endemic biological control agents. Naturally present specialist predators such as Syrphidae (Diptera), Coccinellidae (Coleoptera) and parasitoids (Pankanin-Franczyk & Ceryngier 1995; Michaud & Belliure 2001) will attack pests such as aphids but will often only be present once a pest population has reached numbers large enough to attract them (Pankanin-Franczyk & Ceryngier 1995). Generalist predators also include aphids in their diet with carabid beetles, Staphylinidae beetles and spiders all observed to attack and consume aphids (Sunderland *et al.* 1986a; Bilde & Toft 1997; Cardinale *et al.* 2003; Harwood *et al.* 2004). Generalist predators can subsist on alternative prey and so can be present within a crop prior to the arrival of a pest (Chang & Kareiva

1999). As they are not dependent on specific prey, a population of generalist predators can increase early in the season. This leads to greater predation pressure on an invading pest before it can establish itself (Settle *et al.* 1996). The effective control of a pest by generalist predators is likely to occur due to an assemblage of polyphagous predators with variable feeding habits (Provencher & Riechert 1994; Riechert & Lawrence 1997). Field studies where populations of generalist predators have been manipulated have shown that they can suppress pest populations (Chiverton 1986; Duffield *et al.* 1996) showing a potential for generalist predators as biological control agents.

4.1.2 Biological control potential of spiders

Spiders have a number of attributes that are favourable as biological control agents. Spiders are highly mobile and large numbers can be found within agricultural crops. They can quickly colonize a crop early in the season primarily by aerial ballooning (Suter 1999). Spiders within a crop will rapidly produce webs and large areas of a crop can be covered by spider webs, especially those of linyphiid spiders (Sunderland *et al.* 1986a). These webs are located in areas of high prey density (Harwood *et al.*, 2001; Harwood *et al.* 2003a) trapping large numbers of potential prey (primarily insects) a high proportion of which can be pests such as aphids (Sunderland *et al.* 1986b). Although many of these potential prey items are uneaten (Alderweireldt 1994b), few potential prey items escape the webs (Nentwig 1982). Linyphiid spiders will frequently abandon their webs for new web sites increasing the total web cover in the crop and adding further biological control potential (Sunderland 1999). Spiders rarely show specificity towards prey generally attacking prey relative to the rate of encounter (Riechert & Lockley 1984) and therefore if there is a high pest population

the spider will preferentially feed on it. Laboratory studies have shown that spiders will readily consume aphids. Aphids are considered a low quality food item for spiders and spiders have shown an aversion to consuming them if they were the sole food item (Toft 1997 & 2000). However, such aversions are not complete and spiders can tolerate consuming toxic prey (Mayntz & Toft 2000) with the natural genetic variation of a population enabling some spiders to tolerate an aphid exclusive diet for several instars (Beck & Toft 2000). Alternative non-aphid prey can dilute the effect of aphid toxins providing a more favourable amino acid balance (Greenstone 1979) and it has been shown that spiders with a good nutrient balance will readily consume more of a toxic prey item such as aphids (Mayntz & Toft 2000). Spiders will also kill prey without consuming them or with only partial consumption which occurs even when the spider is fully satiated (Maupin & Riechert 2001) increasing the potential for spiders to control a pest. Overall, spiders show a high potential to control pests and this study is an investigation into the complex interactions between linyphiid spiders and their prey within cereal crops in an attempt to determine the potential of linyphiid spiders to control aphids.

4.1.3 Investigating predator-prey interactions

An understanding of the complex predator-prey interactions involving generalist predators is essential to assess their potential as biological control agents. Although it can be shown that spiders play a role in influencing the population of a pest using microcosms to limit the possible interactions making their effects easier to observe (Snyder & Ives 2003) or by the use of manipulation experiments in the field (Chiverton 1986), it is necessary to investigate these interactions directly to gain an accurate insight into the mechanisms involved. Examining the gut contents of spiders

collected from the field would provide a direct account of their predatory activity. As spiders are fluid feeding, the identification of particulate prey remains is not possible (Sunderland 1975) and other methods must be employed. In previous predation studies, antibodies have been used to identify the gut contents of generalist predators such as Nabidae bugs (Bacher *et al.* 1999) and Carabidae beetles (Symondson *et al.* 1999; Symondson *et al.* 2000). Antibodies have also successfully been used to investigate spider predation. Sunderland *et al.* (1987) showed that spiders in cereal crops predated on aphids in cereal crops and that this could be reliably detected using antibodies. Harwood *et al.* (2004), revealed that linyphiid spiders predated aphids but that predation could vary according to the availability of Collembola, an alternative, non-pest potential prey. They showed that Collembola are important for maintaining linyphiid spiders early in the season prior to the arrival of aphids. Despite these successes, the production of antibody cell lines is difficult and expensive (Chen *et al.* 2000; Symondson *et al.* 2002) limiting further studies. As an alternative, the identification of prey DNA has been used recently in several studies.

DNA sequences are widely available and designing DNA primers necessary for amplifying prey DNA can be carried out with relative ease. In the absence of sequence data, universal primers can be used to amplify DNA from a wide range of organisms for sequencing (Simon *et al.* 1994). Predation studies using terrestrial arthropods showed that the length of a DNA sequence affected the length of time it could be amplified for after ingestion (Agusti *et al.* 1999; Zaidi *et al.* 1999; Agusti *et al.* 2000; Hoogendoorn & Heimpel 2001; Agusti *et al.* 2003b). Shorter DNA sequences could be detected for longer periods of time indicating that longer DNA sequences were at higher risk of digestion (Agusti *et al.* 1999). In Zaidi *et al.* (1999),

the target DNA was multiple copy esterase genes. Multiple copies of a target DNA increase the chances of successfully amplifying prey DNA and this in combination with short target sequences make DNA a viable alternative to antibody work. Possible multiple copy target prey DNA sequences include the nuclear internal transcribed spacers (ITS) regions as used by Hoogendoorn & Heimpel (2001). Using this region, several different lengths of Lepidoptera pest DNA were amplified from coccinellid beetles, however, the ITS regions exhibit intraspecific variation in length potentially impairing the ability to interpret results. An alternative source is the mitochondrial genome. Chen *et al.* (2000) used the cytochrome oxidase II mitochondrial gene as the target and successfully amplified aphid prey DNA. This was followed by Agusti *et al.* (2003a) where the cytochrome oxidase I mitochondrial gene was used. These studies showed that targeting short mitochondrial sequences is a reliable method for investigating predator-prey interactions.

4.1.4 Predation by linyphiid spiders in winter wheat

The consumption of aphids by linyphiid spiders in winter wheat has been shown not to be adversely affected by the presence of Collembola, an alternative non-pest prey (Harwood *et al.* 2004). Linyphiid spiders have been shown to locate their webs in areas of high Collembola density (Harwood *et al.* 2001; Harwood *et al.* 2003b; chapter 2, this thesis) and Collembola are thought to be a staple food source early in the season (Harwood *et al.* 2004). Three common species of Collembola (*Isotoma anglicana*, *Entomobrya multifasciata* and *Lepidocyrtus cyaneus*) found in winter wheat have been shown to be predated upon at rates different to what was expected revealing a measure of selection (Agusti *et al.* 2003a). In this study, predation on these three Collembola is further examined and expanded to include predation

throughout the growing season of winter wheat. Population data on these Collembola is taken from the web sites of linyphiid spiders to give a direct measure of the availability of each species to the spiders. Predation on an additional common non-pest prey, *Lycoriella castanescens* (Diptera: Sciaridae) (chapter 2, this thesis), is also investigated as well as predation on the cereal aphid *Sitobion avenae*. Direct investigation of the diet of linyphiid spiders in the field will reveal the extent of predation on these non-pest prey and pest prey items indicating their relative importance as a resource for linyphiid spiders.

4.2 Methods

4.2.1 Field Site

The sampling sites were two fields of winter wheat (cv. 'Hereward') planted in predominantly sandy loamy soil at Horticulture Research International (HRI) in Wellsbourne, Warwickshire, UK (52°12.18'N, 1°36.00'W). The fields were managed according to standard farming practices without the use of pesticides.

4.2.2 Spider collection and potential prey density monitoring

Spiders that had their web sites sampled in Chapter 2 (this thesis) were immediately removed to a labelled Eppendorf tube and placed on ice before being stored at –80 °C within one hour of collection for later identification and gut content analysis. On each sampling occasion twenty eight spiders were collected from occupied webs in each field. For each sampling occasion, spiders were split into two groups according to whether spider web sites were sampled using sticky traps or quadrats. The density of each prey item at the web sites of spiders was taken by identifying prey from either the sticky trap or quadrat samples used in Chapter 2 (this thesis). Where spider web sites were sampled using sticky traps, spiders and their potential prey were further classified as either ground or aerial for further analysis.

4.2.3 Screening of spiders for target prey

Field caught spiders were identified prior to whole DNA extraction using the DNeasy® Tissue Kit. Individual whole organisms were placed in 1.5 ml Eppendorf tubes and 180 µl of Buffer ATL was added. The spider was then homogenized using a plastic pestle and the DNA was extracted as directed by the manufacturer using the

protocol "Isolation of Total DNA from Animal Tissues" from step 2. DNA extracts were then screened for the presence of the five potential prey using species specific primers for the Collembola *Isotoma anglicana*, *Lepidocyrtus cyaneus* and *Entomobrya multifasciata*, the Dipteran *Lycoriella castanescens* and the aphid *Sitobion avenae*. The PCR reaction volume was 12 µl containing 2 µl DNA extract, 1 X PCR Buffer, 2 mM $MgCl_2$, 0.05 mM each dNTP, 0.5 µM each primer, 0.625 units of *Taq* DNA Polymerase and made up to final volume with sterile water. The optimum reaction conditions were: initial denaturation for 2 minutes at 94 °C, then 35 cycles of denaturation for 1 minute 10 seconds at 94 °C, annealing for 1 minute 10 seconds at a temperature specific for the primer pair (see Table 4.1) and extension for 1 minute 10 seconds at 72 °C, then final extension for 5 minutes at 72 °C.

Table 4.1: List of primer pairs used to screen field caught spiders for potential prey

Target organism	Primer pair	Annealing temp. (°C)	Source
Lycoriella castanescens	L.castF1 L.castR1	66	Chapter 2 (this thesis)
Isotoma anglicana	Ia1F Ia4R	68	Agusti *et al.* (2003)
Lepidocyrtus cyaneus	Lc2F Lc4R	69	Agusti *et al.* (2003)
Entomobrya multifasctiata	Em1F Em3R	64	Agusti *et al.* (2003)
Sitobion avenae	EgaCOIIF1 EgaCOIIR	62	Chen *et al.* (2000)

4.2.4 Analysis of results

All target prey population data obtained using sticky traps and quadrats was converted to population per cm^2 and $\log_{10}(x + 1)$ transformed to normalize data and stabilize variances prior to analysis. Prey population data was compared between sticky traps and quadrats or ground sticky traps and aerial sticky traps using two sampled t-tests. Analysis of prey population variation over time was carried out using ANOVA. The statistical significance of apparent prey selection (positive or negative) by spiders was determined using the Monte Carlo methods of Agusti *et al.* (2003a). These estimate the probability that the observed prevalence of different prey DNA amplified from spider guts could have arisen by chance i.e. if spiders selected different prey species in proportion to their density sampled at spider web sites, rather than showing any preferences. A separate Monte Carlo test was carried out for each web site sampling method. The basis for each test was a simulated spider population equal in size to the number of individuals that tested positive for prey DNA from the field and with the same number of spiders testing positive for one prey species, two different prey species, up to the maximum of 5 prey species. Only the identity of the prey allocated to each spider differed from the field data. Primer positive results were then allocated randomly for the simulated spider population, with the probability of a particular prey item being allocated to an individual directly proportional to the respective prey item's density relative to the other prey items in the sticky traps or quadrats (simulating prey density in webs). This simulation was replicated 5000 times. The observed prevalence of the different prey items in field collected spiders was then compared to the prevalence of allocated prey items in the simulated population to calculate the statistical significance of apparent prey selection. If the observed prevalence was much higher than simulated, suggesting positive selection, the

proportion of simulations in which the defection of the prey was equal to, or greater than, the observed prevalence was calculated. This ratio was equivalent to the P – value for a conventional statistical test (Manly 1997). Similarly, if observed prevalence of a prey item was lower than expected, the P – value was the proportion of simulations in which the same, or lower, prevalence of that primer was observed.

4.3 Results

4.3.1 spider preferences from sticky traps and quadrats

DNA from the five prey items was successfully amplified from the guts of the spiders collected from winter wheat and the results for all of the spiders collected is shown in Table 4.2. The populations of each potential prey item was monitored at web sites using sticky traps or quadrats and the differences between the two methods in sampling the density of *Isotoma anglicana* ($t = 2.06$, $P = 0.038$), *Entomobrya multifasciata* ($t = 3.98$, $P < 0.000$) and *Lepidocyrtus cyaneus* ($t = 2.01$, $P = 0.048$) were found to be significant. As insufficient numbers of *Lycoriella castanescens* and *Sitobion avenae* were collected using quadrats they are not included in the comparison. The Positive or negative selection of prey items by linyphiid spiders is shown in Table 4.3.

Table 4.2: The number of each species of spider collected from winter wheat and screened for the presence of DNA from five species of potential prey. The number of individuals from which prey DNA was amplified are shown with the percentage in parentheses.

Spider species and sex	n	Number of spiders positive				
		Isotoma anglicana	*Entomobrya multifasciata*	*Lepidocyrtus cyaneus*	*Lycoriella castanescens*	*Sitobion avenae*
Tenuiphantes tenuis (male)	56	15	20	12	4	16
T. tenuis (female)	90	17	26	11	8	19
T. tenuis total	146	32 (21.9)	46 (31.5)	23 (15.8)	12 (8.2)	35 (24)
Erigone atra (male)	13	3	4	1	2	0
E. atra (female)	33	10	8	4	3	0
E. atra total	46	13 (28.3)	12 (26.1)	5 (10.9)	5 (10.9)	0 (0)
Erigone dentipalpis (male)	2	0	0	0	1	0
E. dentipalpis (female)	14	3	4	1	1	3
E. dentipalpis total	16	3 (18.8)	4 (25)	1 (6.3)	2 (12.5)	3 (18.8)
Bathyphantes gracillis (male)	14	2	7	2	1	1
B. gracillis (female)	50	4	17	9	4	7
B. gracillis total	64	6 (9.4)	24 (37.5)	11 (17.2)	5 (7.8)	8 (12.5)
Oedothorax sp. (male)	3	0	1	1	0	1
Oedothorax sp. (female)	10	3	2	2	0	2
Oedothorax sp. total	13	3 (23.1)	3 (23.1)	3 (23.1)	0 (0)	3 (23.1)
Erigoninae sp. (male)	14	2	3	5	1	0
Erigoninae sp. (female)	10	1	4	1	1	1
Erigoninae sp. total	24	3 (12.5)	7 (29.1)	6 (25)	2 (8.3)	1 (4.2)
Other male spiders	4	0	0	0	0	0
Other female spiders	12	2	2	0	1	1
Other spiders total	16	2 (12.5)	2 (12.5)	0 (0)	1 (6.25)	1 (6.25)
All male spiders	106	22 (20.8)	35 (33)	21 (19.8)	9 (8.5)	18 (17)
All female spiders	219	40 (18.3)	63 (28.8)	28 (12.8)	18 (8.2)	33 (15.1)
All spiders	325	62 (19.1)	98 (30.2)	49 (15.1)	27 (8.3)	51 (15.7)

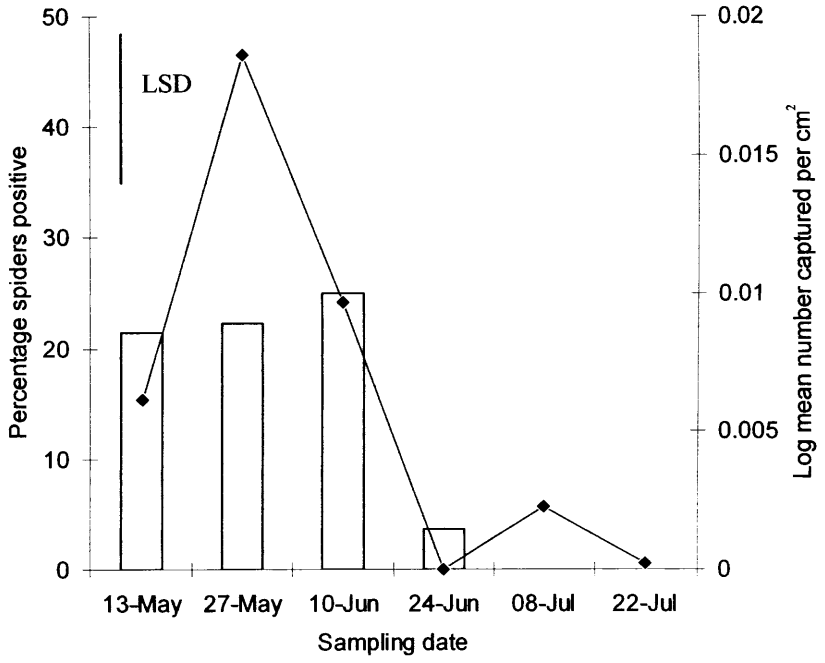
Table 4.3: Monte Carlo results showing selection for or against prey items when sampled using sticky traps and quadrats xxx = $p < 0.001$, xx = $P < 0.01$, x = $p < 0.05$ and ns = not significant.

Prey item	Total number captured	Number of positives expected from simulation	Number of positives observed	Evidence of selection	Significance (P)
a) Sticky traps					
Isotoma anglicana	28	10	20	For	xx
Entomobrya multifasciata	42	28	47	For	xxx
Lepidocyrtus cyaneus	42	35	22	Against	xx
Lycoriella castanescens	23	12	12	No selection	ns
Sitobion avenae	138	35	19	Against	xxx
b) Quadrats					
Isotoma anglicana	72	29	42	For	xx
Entomobrya multifasciata	44	19	50	For	xxx
Lepidocyrtus cyaneus	133	48	27	Against	xxx

4.3.1.1 Predation on *Isotoma anglicana*

Linyphiid spiders were shown to significantly select for *I. anglicana* under both sampling strategies (Table 4.3). Figure 4.1 shows density variation over time of *I. anglicana* and predation on *I. anglicana* by linyphiid spiders. The density of *I. anglicana* varied significantly when sampled with sticky traps ($F_{5,168}$ = 4.14, P = 0.001) and quadrats ($F_{5,168}$ = 8.22, P < 0.001). Peaks in the population density were observed in late May for sticky traps and early June and early July when sampled with quadrats. Linyphiid spiders were shown to have high rates of predation on *I. anglicana* from early May to early June.

a) Sticky traps



b) Quadrats

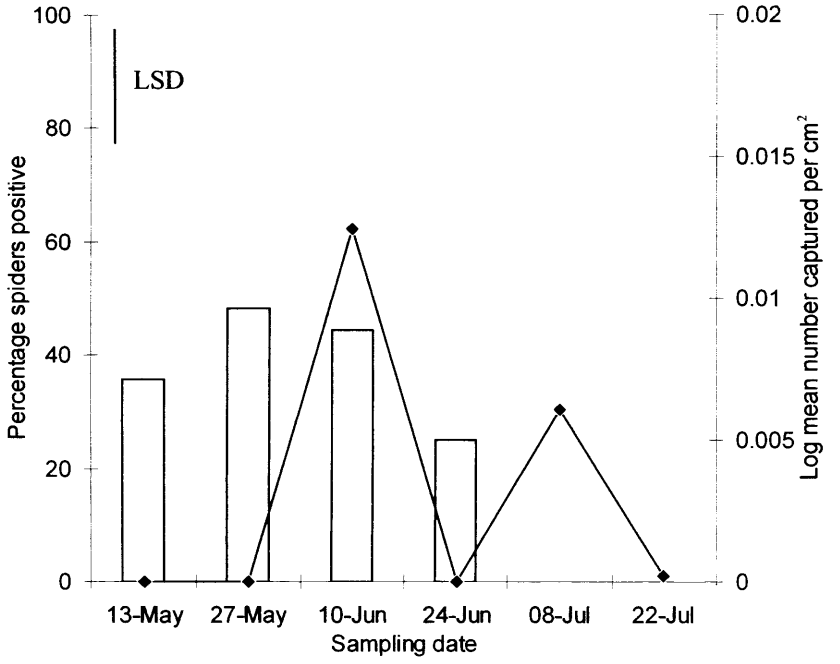


Figure 4.1: Bar charts showing the percentage of Linyphiidae spiders positive for *Isotoma anglicana* combined with line graphs showing the mean number *of Isotoma anglicana* per cm^2 (-♦-) captured at Linyphiidae spider web sites using a) sticky traps and b) quadrats.

4.3.1.2 Predation on *Entomobrya multifasciata*

Figure 4.2 shows the predation by Linyphiidae spiders on *Entomobrya multifasciata* with the population variation over time of *Entomobrya multifasciata*. The sticky traps samples showed no significant variation over time ($F_{5,168}$ = 0.23, P = 0.950) at web sites of Linyphiidae spiders with *E. multifasciata* present throughout sampling. When sampled with quadrats, the density variation of *E. multifasciata* was shown to be significant ($F_{5,168}$ = 2.72, P = 0.022) with a peak during late May to early June. The Linyphiidae spiders were shown to have significant selection for *E. multifasciata* with both prey sampling methods (Table 4.3).

4.3.1.3 Predation on *Lepidocyrtus cyaneus*

Figure 4.3 shows the predation by Linyphiidae spiders on *Lepidocyrtus cyaneus* with the population variation over time of *Lepidocyrtus cyaneus*. Sticky traps showed no significant variation over time ($F_{5,168}$ = 0.91, P = 0.475) however there was significant variation when sampled using quadrats ($F_{5,168}$ = 12.41, P < 0.001). Monte Carlo analysis showed that predation on *L. cyaneus* was avoided by spiders where web sites were sampled using both sticky traps and quadrats (Table 4.3).

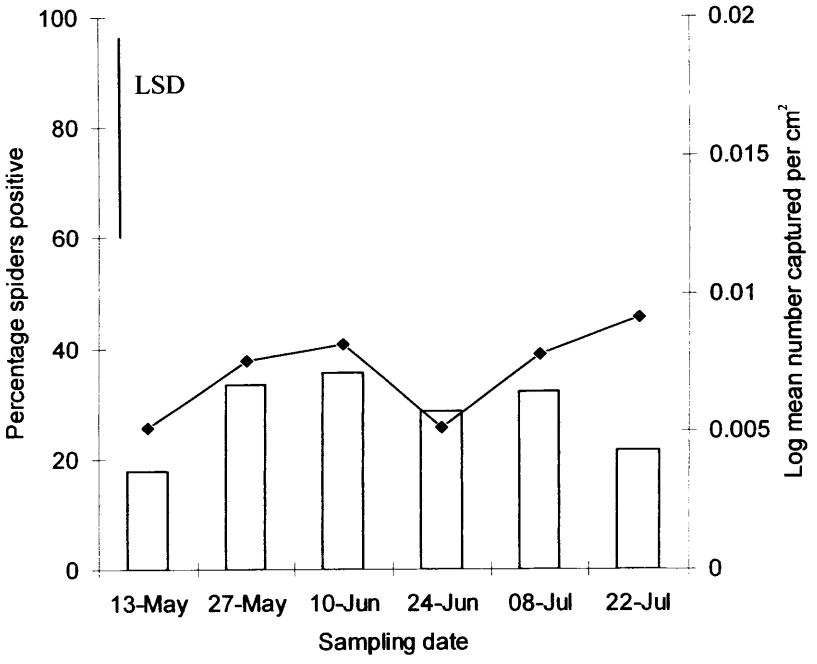
4.3.1.4 Predation on *Lycoriella castanescens*

Figure 4.4 shows the predation by Linyphiidae spiders on *Lycoriella castanescens* with the population variation over time of *Lycoriella castanescens* sampled at web sites using sticky traps. The population variation was shown to be significant ($F_{5,168}$ = 2.26, P = 0.041) with a peak in mid June. Predation by spiders was also highest in mid June however there was no significant selection for or against the prey item.

4.2.1.5 Predation on *Sitobion avenae*

Figure 4.5 shows the predation by Linyphiidae spiders on *Sitobion avenae* with the population variation over time of *Sitobion avenae* sampled at web sites using sticky traps. *S. avenae* population variation at web sites was shown to vary significantly ($F_{5,168} = 6.67$, $P < 0.001$) with few caught in May, increasing to peak in June before reducing in number in mid July. Linyphiidae spiders were found to avoid predating on *S. avenae* (Table 4.3).

a) Sticky traps



b) Quadrats

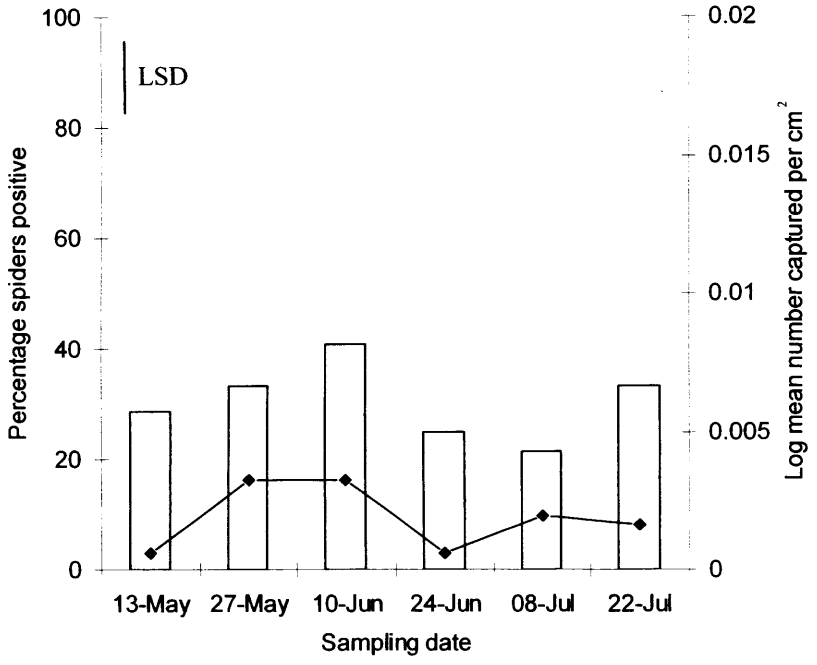
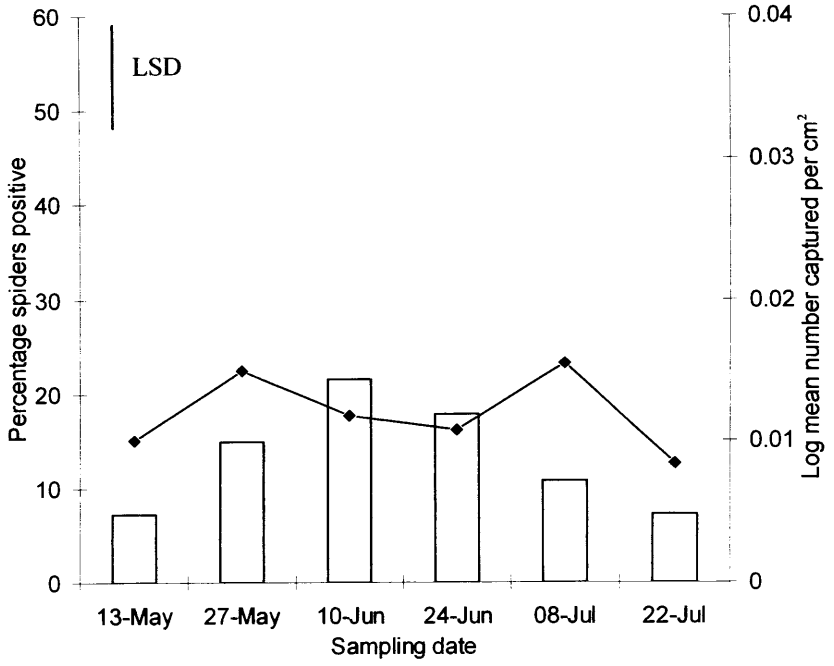


Figure 4.2: Bar charts showing the percentage of Linyphiidae spiders positive for *Entomobrya multifasciata* combined with line graphs showing the mean number of *Entomobrya multifasciata* per cm^2 (-♦-) captured at Linyphiidae spider web sites using a) sticky traps and b) quadrats

a) Sticky traps



b) Quadrats

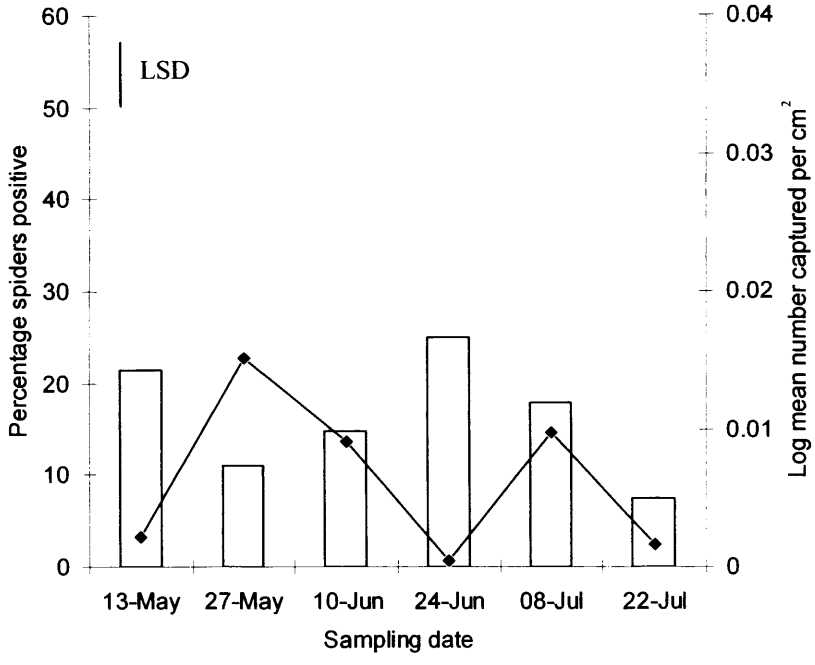


Figure 4.3: Bar charts showing the percentage of Linyphiidae spiders positive for *Lepidocyrtus cyaneus* combined with line graphs showing the mean number of *Lepidocyrtus cyaneus* per cm^2 (-♦-) captured at Linyphiidae spider web sites using a) sticky traps and b) quadrats

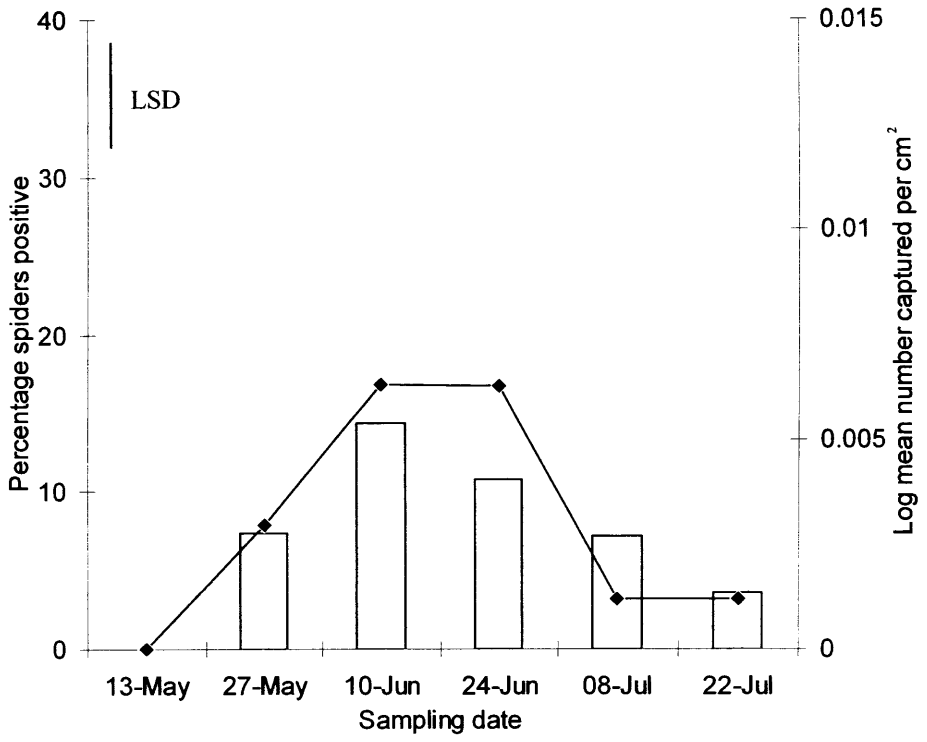


Figure 4.4: Bar charts showing the percentage of Linyphiidae spiders positive for *Lycoriella castanescens* combined with line graphs showing the mean number of *Lycoriella castanescens* per cm^2 (-♦-) captured at Linyphiidae spider web sites using sticky traps

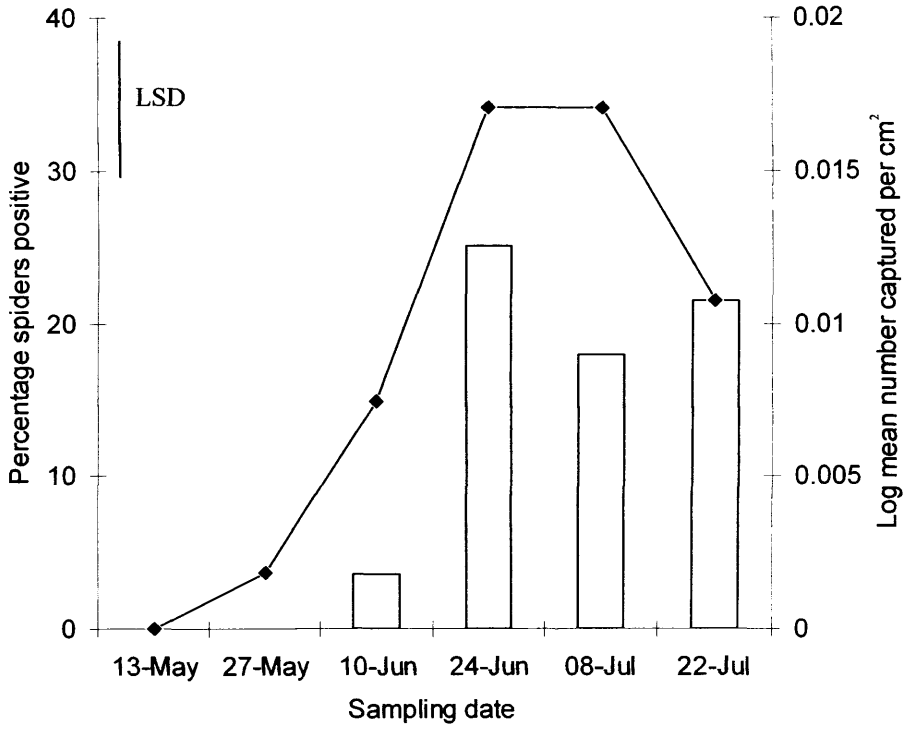


Figure 4.5: Bar charts showing the percentage of Linyphiidae spiders positive for *Sitobion avenae* combined with line graphs showing the mean number of *Sitobion avenae* per cm^2 (-♦-) captured at Linyphiidae spider web sites using sticky traps

4.3.2 Spider preferences for ground and aerial webs

Table 4.4 shows the number of spiders collected from ground webs that were found to contain DNA from each prey item and Table 4.5 shows the number of spiders collected from aerial webs from which prey DNA was successfully amplified. Differences were found in the population of each prey item between ground and aerial web sites. *Isotoma anglicana* ($t = 3.55$, $P = 0.006$), *Entomobrya multifasciata* ($t = 3.43$, $P = 0.001$) and *Lepidocyrtus cyaneus* ($t = 3.71$, $P < 0.001$) were all found in larger densities in ground web sites. The density of *Lycoriella castanescens* ($t = 0.17$, $P = 0.684$) was not significantly different in ground and aerial web sites, whereas *Sitobion avenae* ($t = 2.09$, $P = 0.038$) was found in higher densities at aerial web sites. Table 4.6 shows Monte Carlo simulation results testing the selection for or against the five prey items by Linyphiidae spiders from ground webs and aerial webs.

Table 4.4: The number of each species of spider collected from ground webs in winter wheat and screened for the presence of DNA from five species of potential prey. The number of individuals from which prey DNA was amplified are shown with the percentage in parentheses.

Spider species and sex	n	Number of spiders positive				
		Isotoma anglicana	*Entomobrya multifasciata*	*Lepidocyrtus cyaneus*	*Lycoriella castanescens*	*Sitobion avenae*
Tenuiphantes tenuis (male)	2	0	1	1	0	0
T. tenuis (female)	4	1	2	0	0	1
T. tenuis total	6	1 (16.7)	3 (50)	1 (16.7)	0 (0)	1 (16.7)
Erigone atra (male)	4	0	1	0	1	0
E. atra (female)	23	6	5	4	1	0
E. atra total	27	6 (22.2)	6 (22.2)	4 (14.8)	2 (7.4)	0 (0)
Erigone dentipalpis (male)	2	0	0	0	1	0
E. dentipalpis (female)	10	3	2	1	1	2
E. dentipalpis total	12	3 (25)	2 (16.7)	1 (8.3)	2 (16.7)	2 (16.7)
Bathyphantes gracillis (male)	2	0	0	0	0	0
B. gracillis (female)	4	1	0	0	0	0
B. gracillis total	6	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)
Oedothorax sp. (male)	3	0	0	1	0	0
Oedothorax sp. (female)	3	0	1	0	0	0
Oedothorax sp. total	6	0 (0)	1 (16.7)	1 (16.7)	0 (0)	0 (0)
Erigoninae sp. (male)	5	0	0	0	1	0
Erigoninae sp. (female)	2	0	1	1	0	1
Erigoninae sp. total	7	0 (0)	1 (14.3)	1 (14.3)	1 (14.3)	1 (14.3)
Other male spiders	0	0	0	0	0	0
Other female spiders	4	0	1	1	0	1
Other spiders total	4	0 (0)	1 (25)	1 (25)	0 (0)	1 (25)
All male spiders	18	0 (0)	2 (11.1)	2 (11.1)	3 (16.7)	0 (0)
All female spiders	50	11 (22)	12 (24)	7 (14)	2 (4)	5 (10)
All spiders	68	11 (16.2)	14 (20.6)	9 (13.2)	5 (7.4)	5 (7.4)

Table 4.5: The number of each species of spider collected from aerial webs in winter wheat and screened for the presence of DNA from five species of potential prey. The number of individuals from which prey DNA was amplified are shown with the percentage in parentheses.

Spider species and sex	n	Number of spiders positive				
		Isotoma anglicana	*Entomobrya multifasciata*	*Lepidocyrtus cyaneus*	*Lycoriella castanescens*	*Sitobion avenae*
Tenuiphantes tenuis (male)	22	4	6	4	0	6
T. tenuis (female)	37	3	11	4	3	5
T. tenuis total	59	7 (11.9)	17 (28.9)	8 (13.6)	3 (5.1)	11 (18.6)
Erigone atra (male)	0	0	0	0	0	0
E. atra (female)	0	0	0	0	0	0
E. atra total	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Erigone dentipalpis (male)	0	0	0	0	0	0
E. dentipalpis (female)	0	0	0	0	0	0
E. dentipalpis total	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Bathyphantes gracillis (male)	8	1	5	1	1	0
B. gracillis (female)	25	1	8	3	3	1
B. gracillis total	33	2 (6.1)	13 (39.1)	4 (12.1)	4 (12.1)	1 (3)
Oedothorax sp. (male)	1	1	0	1	0	1
Oedothorax sp. (female)	1	1	0	0	0	1
Oedothorax sp. total	2	2 (100)	0 (0)	1 (50)	0 (0)	2 (100)
Erigoninae sp. (male)	0	0	0	0	0	0
Erigoninae sp. (female)	1	0	1	0	0	0
Erigoninae sp. total	1	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)
Other male spiders	1	0	0	0	0	0
Other female spiders	2	0	1	0	0	0
Other spiders total	3	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)
All male spiders	32	6 (18.8)	11 (34.4)	6 (18.8)	1 (3.1)	7 (21.9)
All female spiders	66	5 (7.6)	21 (31.8)	7 (10.6)	6 (9)	7 (10.6)
All spiders	98	11 (11.2)	32 (32.6)	13 (13.3)	7 (7.1)	14 (14.3)

Table 4.6: Monte Carlo results showing selection for or against prey items of spiders collected from ground and aerial webs. xxx = $p < 0.001$, xx = $P < 0.01$, x = $p < 0.05$ and ns = not significant.

Prey item	Total number captured	Number of positives expected from simulation	Number of positives observed	Evidence of selection	Signifcance (P)
a) Ground sticky traps					
Isotoma anglicana	8	2	11	For	xxx
Entomobrya multifasciata	7	14	14	No selection	ns
Lepidocyrtus cyaneus	10	19	10	Against	xx
Lycoriella castanescens	3	4	5	For	ns
Sitobion avenae	24	5	5	No selection	ns
a) Aerial sticky traps					
Isotoma anglicana	20	10	9	Against	ns
Entomobrya multifasciata	35	13	33	For	xxx
Lepidocyrtus cyaneus	32	12	12	No selection	ns
Lycoriella castanescens	21	8	7	Against	ns
Sitobion avenae	114	33	14	Against	xxx

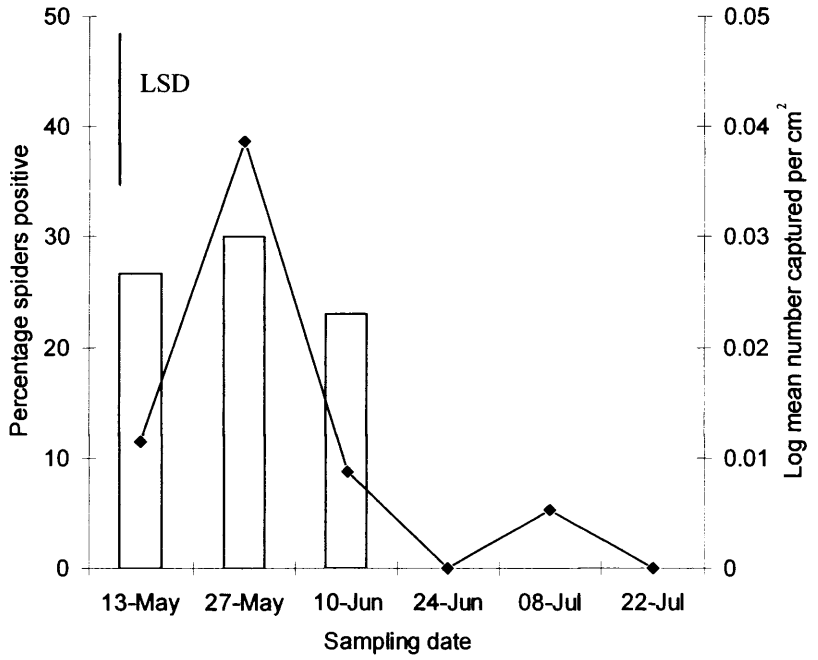
4.3.2.1 Predation on *Isotoma anglicana*

Figure 4.6 shows the variation over time of *I. anglicana* and predation on *I. anglicana* at ground web sites and aerial web sites. *I. anglicana* population peaks in late May at ground web sites and early June in aerial web sites with significant variation over time at both ground ($F_{5,69} = 3.20$, $P = 0.012$) and aerial ($F_{5,98} = 5.76$, $P < 0.001$) web sites. The highest amount of predation coincides with the population peak in both cases with spiders at ground web sites shown to select for *I. anglicana*. However, spiders at aerial web sites were not found to exhibit selection for or against the prey item.

4.3.2.2 Predation on *Entomobrya multifasciata*

Figure 4.7 shows the variation over time of *E. multifasciata* and predation on *E. multifasciata* at ground web sites and aerial web sites. No significant variation over time was shown at either ground web sites ($F_{5,69} = 0.21$, $P = 0.957$) or aerial web sites ($F_{5,98} = 1.17$, $P = 0.331$). Spiders collected from ground webs showed no significant selection for or avoidance of *E. multifasciata* but spiders from aerial webs showed significant selection for *E. multifasciata*.

a) Ground web sites



b) Aerial web sites

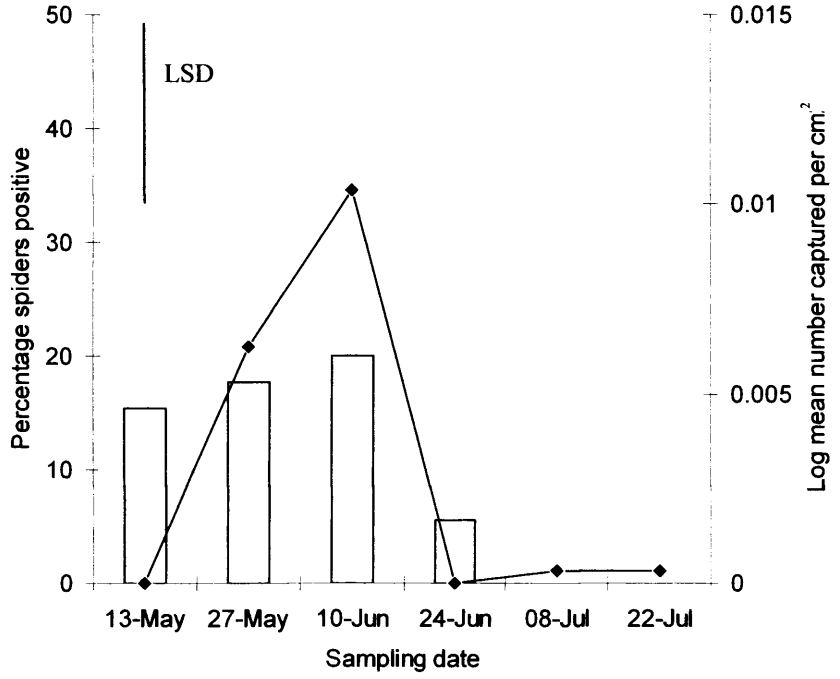
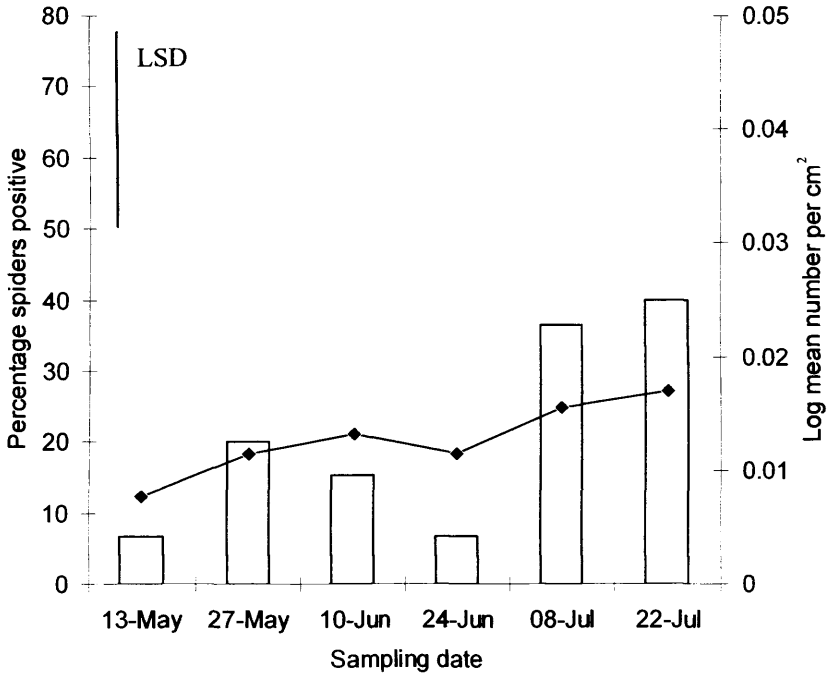


Figure 4.6: Bar charts showing the percentage of Linyphiidae spiders positive for *Isotoma anglicana* combined with line graphs showing the mean number *of Isotoma anglicana* per cm^2 (-♦-) captured at Linyphiidae spider web sites at a) ground web sites and b) aerial web sites

a) Ground web sites



b) Aerial web sites

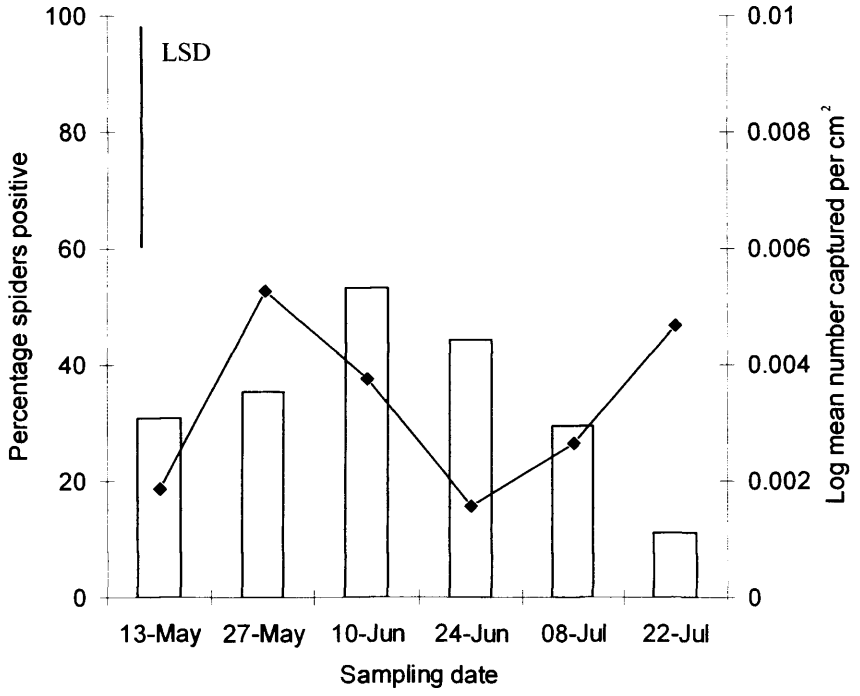


Figure 4.7: Bar charts showing the percentage of Linyphiidae spiders positive for *Entomobrya multifasciata* combined with line graphs showing the mean number of *Entomobrya multifasciata* per cm^2 (-♦-) captured at Linyphiidae spider web sites at a) ground web sites and b) aerial web sites

4.3.2.3 Predation on *Lepidocyrtus cyaneus*

Figure 4.8 shows the variation over time of *L. cyaneus* and predation on *L. cyaneus* at ground web sites and aerial web sites. For both ground ($F_{5,69}$ = 1.41, P = 0.235) and aerial ($F_{5,98}$ = 0.42, P = 0.830) web sites, *L. cyaneus* density was shown not to vary significantly. Similarly, spiders from aerial web sites predated *L. cyaneus* throughout the sampling period with no selection for or avoidance of *L. cyaneus*. Spiders at ground webs had the highest predation in early June, however, overall there was significant avoidance of *L. cyaneus* as a prey item (see Table 4.7).

4.3.2.4 Predation on *Lycoriella castanescens*

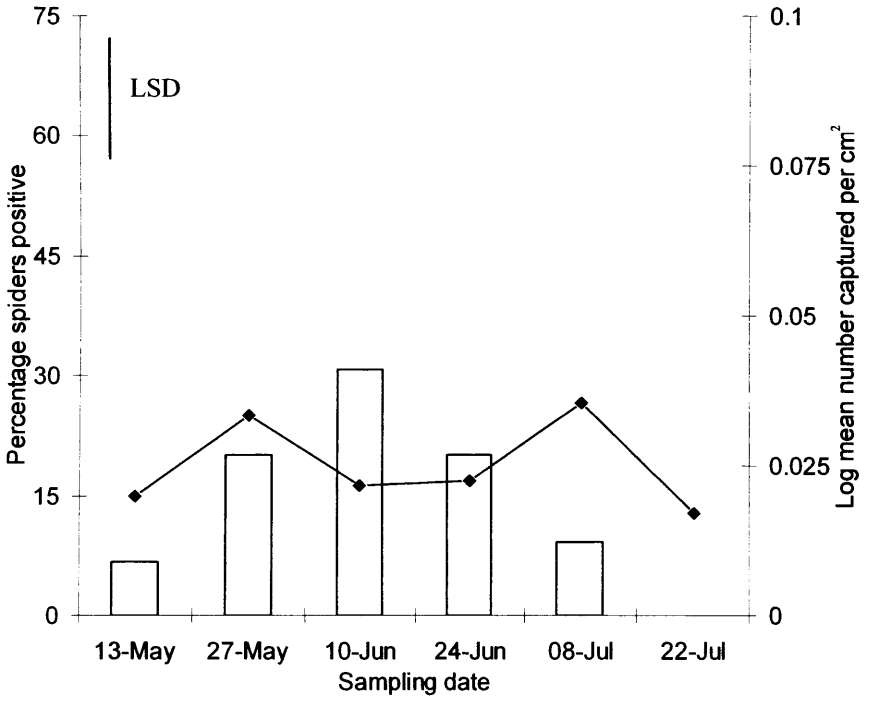
Figure 4.9 shows the variation over time of *L. castanescens* and predation on *L. castanescens* at ground web sites and aerial web sites. Significant variation of *L. castanescens* was shown to occur at both ground ($F_{5,69}$ = 2.11, P = 0.034) with a peak in early June and aerial ($F_{5,98}$ = 2.95, P = 0.029) web sites with a peak in late June. Predation by spiders collected from both ground and aerial web sites showed no selection for or against *L. castanescens*.

4.3.2.5 Predation on *Sitobion avenae*

Figure 4.10 shows the variation over time of *S. avenae* and predation on *S. avenae* at ground web sites and aerial web sites. At both ground ($F_{5,69}$ = 2.61, P = 0.033) and aerial ($F_{5,98}$ = 4.45, P = 0.001) web sites there was significant variation over time with *S. avenae* population increasing throughout the sampling period and falling from late and early July respectively. No selection was shown by spiders collected from ground webs with predation with the highest predation found late in July. For those spiders

collected from aerial webs, the greatest predation was at the peak of aphid density in late June but spiders showed significant avoidance of *S. avenae* as a prey item.

a) Ground web sites



b) Aerial web sites

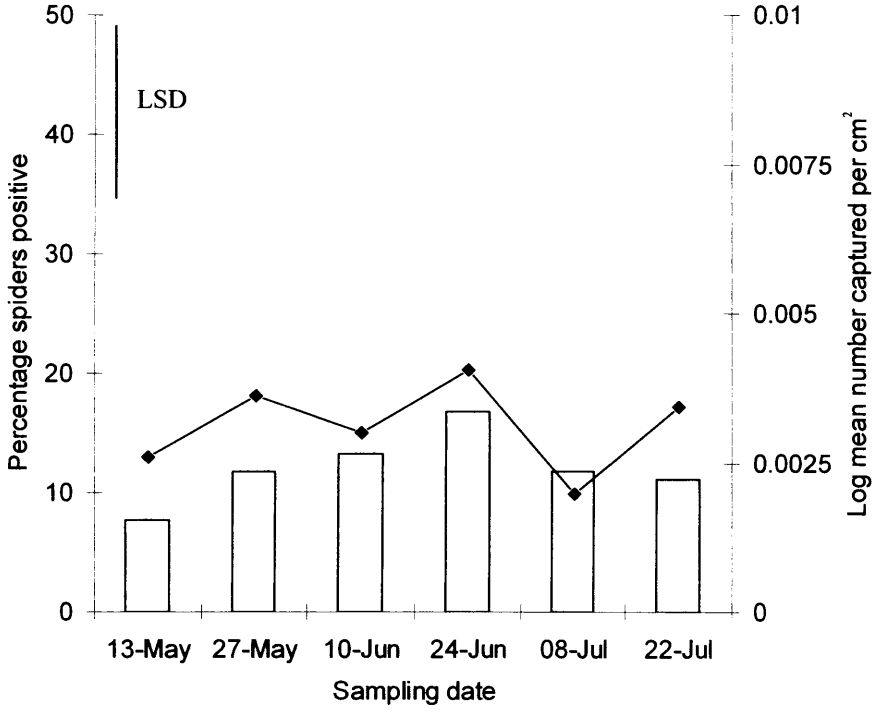
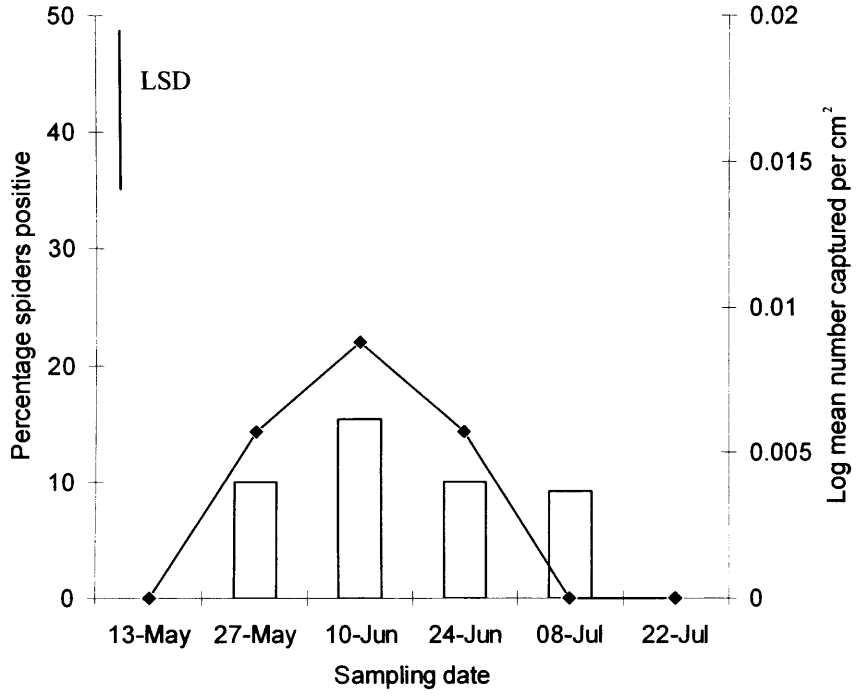


Figure 4.8: Bar charts showing the percentage of Linyphiidae spiders positive for *Lepidocyrtus cyaneus* combined with line graphs showing the mean number of *Lepidocyrtus cyaneus* per cm^2 (-♦-) captured at Linyphiidae spider web sites at a) ground web sites and b) aerial web sites

a) Ground web sites



b) Aerial web sites

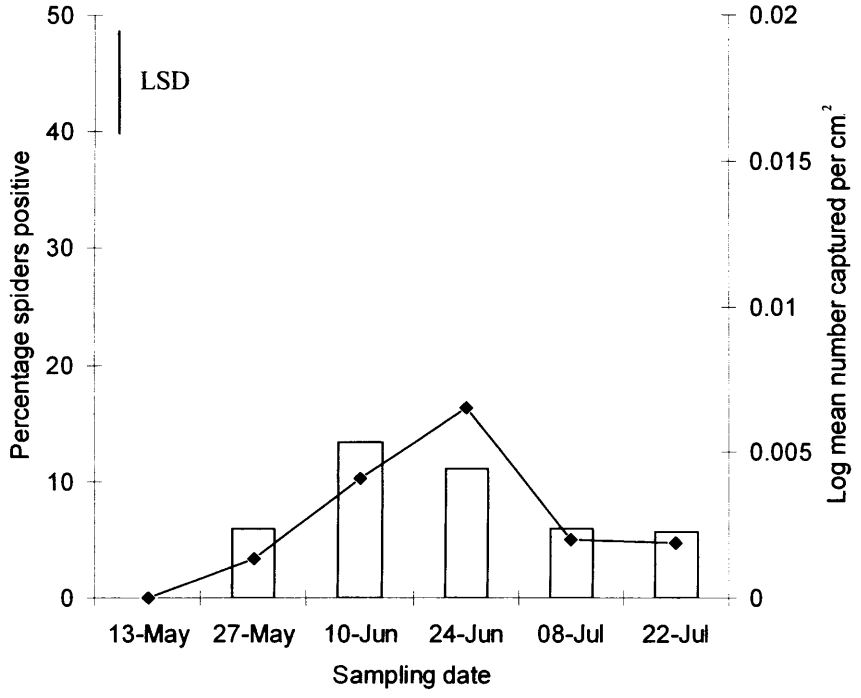
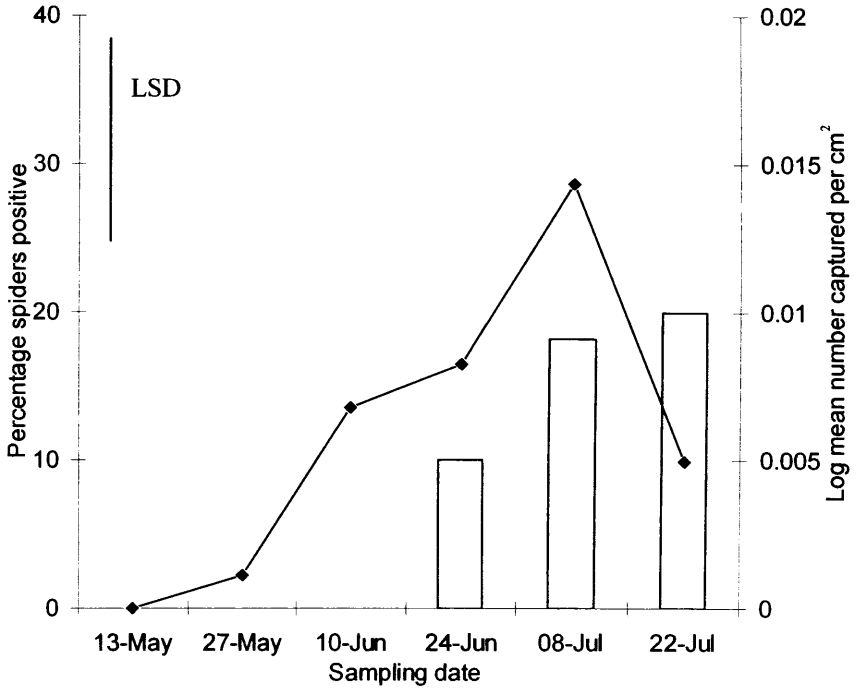


Figure 4.9: Bar charts showing the percentage of Linyphiidae spiders positive for *Lycoriella castanescens* combined with line graphs showing the mean number of *Lycoriella castanescens* per cm^2 (-♦-) captured at Linyphiidae spider web sites at a) ground web sites and b) aerial web sites

a) Ground web sites



b) Aerial web sites

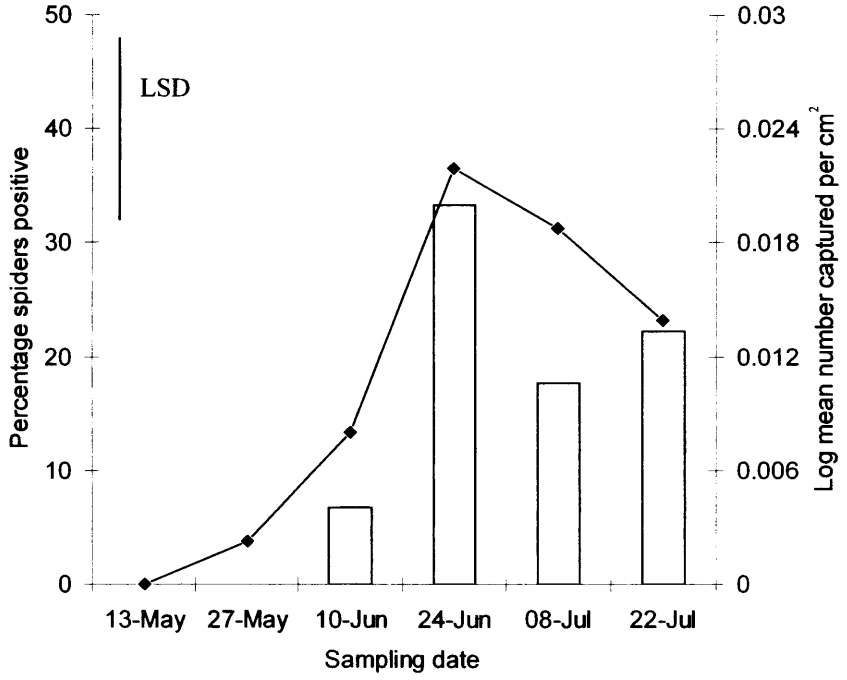


Figure 4.10: Bar charts showing the percentage of Linyphiidae spiders positive for *Sitobion avenae* combined with line graphs showing the mean number of *Sitobion avenae* per cm^2 (-♦-) captured at Linyphiidae spider web sites at a) ground web sites and b) aerial web sites

4.4 Discussion

This is the first DNA based gut analysis study to directly investigate predation of linyphiid spiders on such an extensive range of prey in the field. The combination of screening for the DNA of prey items with monitoring of their population at web sites allows an accurate depiction of linyphiid predatory behaviour in normal crop conditions. The densities of prey populations were different depending on the sampling strategy used. Insufficient densities of *L. castanescens* and *S. avenue* were recorded to facilitate Monte Carlo simulations to be run when these prey populations were sampled using quadrats. The activity of these prey is high so they are unlikely to remain in one area for any length of time, however, they were caught in high enough densities on sticky traps allowing Monte Carlo simulations to be run indicating that they are transient visitors to web sites either by flight or by falling from higher in the crop (Winder *et al.* 1994). Sampling of Collembola was also lower using quadrats but they were still caught in sufficient numbers for analysis. The same pattern of selection by linyphiid spiders of Collembola was shown regardless of prey sampling method. On occasion, predation was shown to occur at points when prey density was very low in both quadrats and sticky traps such as Figure 4.1 in late June. As linyphiid spiders are not entirely web dependent in capturing their prey (Sunderland *et al.* 1986a; Alderweireldt 1994b), this may be evidence of hunting away from the web.

Several prey items were shown to either be avoided or actively selected for. Overall, predation on Collembola revealed that *I. anglicana* and *E. multifasciata* were selected for whereas *L. cyaneus* were avoided. Predation on *I. anglicana* occurred between early May and early June when *I. anglicana* was most abundant. *Erigone atra*, when

fed exclusively on *I. anglicana*, had high levels of fecundity when compared to other prey diets indicating that for linyphiid spiders, *I. anglicana* is a high quality prey item (Marcussen *et al.* 1999). This is supported by Agusti *et al.* (2003) where it was shown that linyphiid spiders showed preferences for *I. anglicana* in the field. Linyphiid spiders have also been shown to have a preference towards larger Collembola (Alderweireldt 1994b) and the larger size of *I. anglicana* (4 mm) relative to the other Collembola (*E. multifaciata* adults are 1.5 mm and *L. cyaneus* adults are 1.2 mm) may make it a favourable prey item. *Entomobrya multifasciata* and *L. cyaneus* are both Entomobryidae and one of the identifying features of this family is the presence of scales (Hopkin, *in press*). These scales have a defensive function in reducing friction and allowing escape from predators (Bauer & Pfeiffer, 1991). However, as there was significant selection for *E. multifasciata* and it was predated upon throughout the season, the presence of scales alone may not deter predation by spiders. There was significant avoidance of predating *L. cyaneus* which indicates it may have further defence mechanisms. This supports findings by Agusti *et al.* (2003a) which also found less than expected predation on *L. cyaneus* by linyphiid spiders. It may be that *L. cyaneus* contains chemical defences similar to those found in some other Collembola (Dettner *et al.* 1996; Messer *et al.* 2000). Despite these defences, there was still some level of predation on *L. cyaneus* throughout the season. Through the inclusion of other high quality prey items, the detrimental effects of consuming *L. cyaneus* may be counteracted to some degree allowing spiders to include small numbers of *L. cyaneus* in their diet.

Overall predation on *L. castanescens* by linyphiid spiders occurred without active selection for or against between late May and early July. Feeding trials using other Sciaridae (Toft & Wise 1999) showed that although they were not toxic, they were low quality prey items resulting in reduced growth and survival. However, as with *L. cyaneus*, the presence of better quality prey items may offset the poor nutrient content of *L. castanescens* and counteract any negative effects. Predation on *S. avenae* also occurred when they were present in the crop though at a lower than expected level showing a measure of avoidance. This is consistent with toxicity studies where low level consumption can be tolerated by the inclusion of higher quality prey to counteract detrimental effects and provide a favourable nutrient balance (Greenstone 1979; Mayntz & Toft 2000). Predation on *S. avenae* was highest from the end of June which corresponds to the point from which *I. anglicana* population and predation becomes low. As the other two Collembola species were predated upon throughout the season and occurred at an approximate steady density, *I. anglicana* may be important in determining the level of inclusion of *S. avenae* within linyphiid spiders diets. This effect was shown by Harwood *et al.* (2004) where after a crash in Collembola population, there was a large increase in predation on aphids by linyphiid spiders.

The two different sticky trap types were shown to be effective at providing realistic target prey data. The patterns of predation appeared to be different for those spiders from ground webs (predominantly Erigoninae) and those from aerial webs (predominantly Linyphiinae). This is in support of (Harwood *et al.* 2003b; Harwood *et al.* 2004) where it was shown that the two groups of spiders were aggregating to different prey types with Erigoninae locating their webs in areas of high Collembola

density and Linyphiinae locating their webs in areas of high aphid density. This effect was also seen in chapter 3 (this thesis). Predation on *I. anglicana* was different at ground web sites and aerial web sites. At aerial web sites, although predation occurred when *I. anglicana* was most abundant from mid May to mid June, there was no selection and this may be an indication of the reliance of Linyphiinae on their webs as the primary mode of prey capture (Sunderland *et al.* 1986b; Alderweireldt 1994b). Conversely, at ground web sites there were high levels of predation on *I. anglicana*. Erigoninae are known to hunt away from their web (Sunderland *et al.* 1986b; Alderweireldt 1994b) which may explain why such a high preference for *I. anglicana* was exhibited at ground web sites. Also at ground web sites, no selection was found for predation on *E. mulitfasciata* and this may be attributed to the combination of active preferences for *I. anglicana* with the defence strategy of *E. multifasciata*. This is contrary to spiders from aerial web sites where a high rate of predation on *E. multifasciata* occurred. This suggests that the defence mechanisms of *E. multifasciata* are relatively ineffective against Linyphiinae spiders. The morphology of Linyphiinae spiders is different to Erigoninae spiders, for example Linyphiinae spiders have longer legs. This characteristic has been attributed to enabling spiders to more easily manipulate prey caught in webs (Henaut *et al.* 2001). The dependence of Linyphiinae on webs to capture their prey may also be a factor in *E. multifasciata* predation. Scales may be removed when *E. multifasciata* struggles to free itself from the web, although this would enable the Collembolan to escape quicker, it would also leave it relatively defenceless when attacked by the Linyphiinae spiders allowing for greater consumption of *E. multifasciata* from aerial webs. Similar mechanisms could influence predation on *L. cyaneus*. However, the additional possible chemical defences of *L. cyaneus* may be the reason why it is not actively selected for by

Linyphiinae whereas the combination of chemical defences with scales would make *L. cyaneus* undesirable for Erigoninae.

Lycoriella castanescens predation did not differ between spiders at aerial web sites, ground web sites and overall Linyphiidae predation. *S. avenae* showed marked difference in predation between the two groups. Although there is overall avoidance of predating on *S. avenae*, the Erigoninae at ground web sites appeared to tolerate *S. avenae* to a greater degree than the Linyphiinae at aerial web sites. However, the actual predation on *S. avenae* by Linyphiinae from aerial webs was higher than those spiders from ground webs. Aerial webs caught more *S. avenae* per cm^2 than ground webs allowing Linyphiinae to potentially consume larger numbers of *S. avenae*. However, the lower availability of high quality prey at aerial web sites could limit predation on *S. avenae* by imposing a point beyond which these spiders would be unable to tolerate the detrimental effects of *S. avenae* consumption and so they avoid predating on them. This is supported by the feeding study carried out by Mayntz & Toft (2000) showing that if spiders had a good nutrient balance they would consume more low quality prey such as aphids but would reject them if the spiders had a poor nutrient diet. To obtain a favourable nutrient balance at aerial web sites, web dependent spiders such as Linyphiinae would have to construct webs large enough to capture a wide diversity of prey and this was demonstrated in Chapter 3 (this thesis) where aerial webs were shown to capture a wider diversity of potential prey than ground webs.

Laboratory studies have previously shown that there are factors that affect predation by spiders on certain prey items such as nutrient balance (Toft 1997; Bilde & Toft

2000; Mayntz & Toft 2000) and prey defence (Bauer & Pfeiffer 1991; Dettner *et al.* 1996; Messer *et al.* 2000). It is clear from this study that spiders have complex strategies to counteract these problems in the field. At ground web sites, high quality prey items are available to spiders and those without defence mechanisms are preferentially predated upon. At aerial web sites, there is predominantly lower quality prey so a high diversity of prey is more important.

This underlines the importance of prey diversity to spiders and even a small increase in diversity could potentially result in a large increase in predation on aphids especially by those spiders from aerial webs. Previous studies have revealed effects of diversity of prey on predation of pests either by feeding studies (Bilde *et al.* 2000; Mayntz & Toft 2000) or by manipulative field studies (Chiverton 1986; Alderweireldt 1994a). However, this is the first study that has directly shown the interactions between Linyphiidae spiders with a pest prey and a group of non-pest prey directly revealing the mechanisms by which increased predation on the pest prey may operate in the field.

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Chapter 5:

The effect of compost augmentation in winter wheat on predation by Linyphiidae spiders

5.1 Introduction

5.1.1 Pest control background

Agricultural crops are under constant threat of attack by pests. For cereal crops such as winter wheat one of the main pests are cereal aphids. Aphids cause mechanical damage to plants (Butts *et al.* 1997) and can act as vectors for viruses that limit the growth of the crop (Oswald & Houston 1951; 1953). Populations of aphids have the capacity to increase rapidly by parthenogenesis (Vickerman & Wratten 1979) and can easily reach levels that have the potential to damage a crop (Fletcher & Bardner 1969; George & Gair 1979; Carter *et al.* 1989). Chemical pesticides can be used to reduce aphid numbers (Neil *et al.* 1997) and recently produced synthetic pesticides have become more selective in their effects reducing the chances of harming non-target organisms (Perrin 1997). However, environmental concerns (McDonald *et al.* 1999) and the possibility that pests can develop resistances to pesticides (Perez *et al.* 2000; Ripper 1944) has lead to research into alternative methods of pest control. One such alternative to chemical control is the use of biological control agents. Naturally occuring aphid predators are present in crop systems and these have the potential to control aphid numbers. Specialist aphid predators can be cultured for release in response to aphid infestations or to augment existing populations (Kehrli, Wyss & 2001; Michaud & Belliure 2001). An alternative is to use generalist predators by conservation biological control (Ehler, 1998). Although generalist predators consume a wide range of prey, feeding studies have shown that aphids can be included in the diet of generalist predators such as Carabidae beetles (Jorgensen, Toft, 1997; Kielty et al., 1999; Mundy et al., 2000), Staphyphinidae beetles (Petersen, 1998), Linyphiidae spiders (Beck, Toft, 2000; Sunderland et al., 1986b) and Lycosidae spiders (Mayntz,

Toft, 2000; Mayntz, Toft, 2001). In the field, selective removal of generalist predators can result in an increase of aphid numbers (Chambers et al., 1983; Chiverton, 1986; Dennis, Wratten, 1991; Duffield et al., 1996) showing that the aphid populations were suppressed by interactions involving the generalist predators. The mechanisms by which generalist predators suppress a pest population involve complex interactions with non-pest prey. As generalists are not dependent on the pest, they can survive in the absence of the pest by predating on non-pest prey. Early in the growing season of a crop, a population of generalist predators can increase by predating on non-pest prey resulting in a favourable predator to pest ratio when pests invade the crop and this predation pressure can be enough to suppress a pest population so preventing its establishment (Settle et al., 1996). The inclusion of non-pest prey in the generalist predator diet is an important factor in their ability to control pest populations. In feeding studies, aphids have been shown to be a poor quality food source. Carabidae beetles have low feeding rates on aphids (Bilde, Toft, 1997) indicating a reluctance to consume them even when starved. Similar mechanisms exist in spiders where they can aquire aversions to aphids (Mayntz, Toft, 2000; Toft, Wise, 1999) and the strength of the aversion can depend on the quality of the aphid prey (Toft, 1997). The poor quality of aphid prey can be seen where aphid exclusive diets adversly affect growth rates and fecundity (Beck, Toft, 2000; Bilde, Toft, 2000; Toft, 1995) of spiders. However, when a mixed diet is consumed spiders have been shown to grow and develop more quickly (Bilde, Toft, 2000; Sigsgaard et al., 2001). This strategy of predating a wide variety of prey maximizes the intake of essential amino acids (Greenstone, 1979). This stratetgy also allows for the tolerance of low quality prey in the diet and higher numbers of aphids can be consumed when spiders have a good nutrient balance (Mayntz, Toft, 2000).

5.1.2 Predation by Linyphiidae spiders

Linyphiid spiders occur in large numbers within agricultural crops including winter wheat (Alderweireldt, 1994b; Holland et al. 2004) and feeding studies have shown that they will readily consume aphids (Sunderland et al. 1986b; Beck & Toft 2000) especially when they are included in mixed diets (Bilde & Toft 2000). This has also been shown to occur in the field where spiders removed from winter wheat for gut content analysis have tested positive for the presence of aphid proteins (Sunderland et al. 1986b; Harwood et al. 2004) or for aphid DNA (Chapter 4). In laboratory studies, linyphiid spiders have been shown to consume a range of non-pest prey including Diptera (Toft 1995; Dinter 2004) and Collembola (Marcussen et al. 1999; Dinter 2004) which are considered as high quality prey items. This is reflected in field studies where linyphiid spiders are shown to aggregate to areas of high Collembola density (Harwood et al. 2001; Harwood et al. 2003; Chapter 2, this thesis) field caught spiders have been found to contain the remains of Diptera and several species of Collembola (Chapter 2, this thesis), (Agusti et al. 2003). This combination of pest and non-pest prey shows that linyphiid spiders have the potential to control aphids by predating on Collembola early in the growing season when they are most abudant allowing the spider population to increase which then predate on aphids when they invade the crop later in the season at the point when Collembola density is low. However, under standard farming practices Linyphiidae spiders in winter wheat were not found to actively select for aphids (Chapter 4, this thesis). This is thought to be due to the complex interactions between non-pest prey, pest prey and linyphiid spiders where non-pest prey quality and avilability are an influencing factor in the linyphiid spider's ability to tolerate aphids as low quality prey items (Chapter 4, this thesis). An increase in the non-pest prey populations may therefore lead to an increase

in predation pressure on aphids. Crop habitats can be manipulated to increase arthropod abundance. The addition of sawdust (Wardle et al. 1999) or compost (Mathews et al. 2002; Mathews et al. 2004) to crop ecosystems can result in large increases in soil detritivores and generalist predators. Spider populations have also been shown to be higher in weed patches (Bogya & Marko, 1999) and if grass cuttings are left to dry (Thorbeck 2004) which may provide increased opportunities for web attatchment. The importance of suitable web sites has been shown by Alderweireldt (1994a) and Samu *et al.* (1996) where holes made in the ground were rapidly colonized by Linyphiidae spiders and were actively competed for between individuals. The sheet webs of Linyphiidae spiders can also contribute to the control of aphids. Although many prey items caught in webs are not consumed by the spider (Alderweireldt 1994b) few prey items are able to escape the web (Nentwig 1982) and large numbers of aphids caught in Linyphiidae webs can be killed in this way (Sunderland et al. 1986b; Samu et al. 1996). As Linyphiidae spiders freqently abandon their webs intact (Samu et al. 1996; Sunderland 1999) this increases the potential of an individual spider to control aphids. Linyphiidae spiders are not totally web dependent and can hunt away from their web (Alderweireldt 1994b). The subfamily Erigoninae constructs small webs on the ground (Alderweireldt 1994b; Sunderland et al. 1986a) and will frequently hunt away from the web (Harwood et al.,2003) whereas Linyphiinae are more dependent on their web which is larger and situated above the ground. These different strategies are thought to be due to differences in the quality of prey available to the spiders in each niche (Chapter 2 and Chapter 4, this thesis).

5.1.3 The aims of this study

Linyphiidae spiders in winter wheat have been shown to have preferences for specific prey items that could provide a favourable nutrient balance (Chapter 4), (Agusti et al., 2003). Predation on low quality pest prey such as aphids may be limited where a diet of non-pest prey does not provide sufficient nutrients to facilitate the further consumption of low quality prey (Chapter 4), (Mayntz, Toft, 2000). In this study, the effect of compost on predation by Linyphiidae spiders on three Collembola (*Isotoma anglicana*, *Entomobrya multifasciata* and *Lepidocyrtus cyaneus*), a Dipteran (*Lycoriella castanescens*) and the pest aphid *Sitobion avenae* is investigated in winter wheat using techniques shown to be viable for use in the field (Chapter 4), (Agusti et al., 2003). This study is the first to directly investigate the preferences of Linyphiidae spiders in different crop management conditions and will give a clear indication of the mechanisms by which Linyphiidae spiders are able to suppress aphid pest populations.

5.2 Methods

5.2.1 Field Site

The sampling site was a field of winter wheat (cv. 'Hereward') planted in predominantly sandy loamy soil at Horticulture Research International (HRI) in Wellesbourne, Warwickshire, UK (52°12.18'N, 1°36.00'W). Twenty 4 m x 4 m plots were marked out eight meters apart and ten of the plots were treated with a 3 cm layer of 'Formula 3' spent mushroom compost (Noble et al., 1998) according to a chequerboard design on the 15th April. The field was subsequently managed according to standard farming practices without the use of pesticides.

5.2.2 Spider collection

Sampling was carried out every two weeks between 08:00 and 16:00 from mid May 2002 until late July 2002 just prior to harvest. On each sampling occasion 28 spiders were collected from occupied webs. 14 of the spiders were collected from compost treated plots and 14 were collected from the non-compost plots. At each sampling occasion, half of the spiders from a treatment were collected from aerial webs and half were collected from ground webs. Spiders were removed to individual Eppendorfs and immediately placed on ice. Within one hour of initial collection, spiders were frozen at –80 °C for later identification and gut content analysis.

5.2.3 Sampling of spider web sites

Potential prey populations were monitored using sticky trap sampling of web sites. Aerial web sites were sampled using aerial sticky traps and ground web sites were

sampled using ground sticky traps using the protocol outlined in Chapter 2 (this thesis).

5.2.4 Screening of spiders for target prey

Spiders were identified prior to whole DNA extraction using DNeasy® Tissue Kit. Individual spiders were placed in 1.5 ml Eppendorf tubes and 180 µl of Buffer ATL was added. The arthropod was then homogenized using a plastic pestle and the DNA was extracted as directed by the manufacturer using the protocol "Isolation of Total DNA from Animal Tissues" from step 2. DNA from five target prey (*Isotoma anglicana*, *Entomobrya multifasciata*, *Lepidocyrtus cyaneus*, *Lycoriella castanescens* and *Sitobion avenae*) was amplified using PCR with species specific primers. The PCR constituents were 2 µl DNA extract, 1 X PCR Buffer, 2 mM $MgCl_2$, 0.05 mM each dNTP, 0.5 µM each primer, 0.625 units of Taq DNA Polymerase and made up to final volume of 12 µl with sterile water. The reaction conditions were: initial denaturation for 2 minutes at 94 °C, then 35 cycles of denaturation for 1 minute 10 seconds at 94 °C, annealing for 1 minute 10 seconds at a temperature specific for the primer pair (see Table 5.1) and extension for 1 minute 10 seconds at 72 °C, then final extension for 5 minutes at 72 °C.

Table 5.1: List of primer pairs used to screen field caught spiders for potential prey

Target organism	Primer pair	Annealing temp. (°C)	Source
Lycoriella castanescens	L.castF1 L.castR1	66	Chapter 2 (this thesis)
Isotoma anglicana	Ia1F Ia4R	68	Agusti *et al.* (2003)
Lepidocyrtus cyaneus	Lc2F Lc4R	69	Agusti *et al.* (2003)
Entomobrya multifasctiata	Em1F Em3R	64	Agusti *et al.* (2003)
Sitobion avenae	EgaCOIIF1 EgaCOIIR	62	Chen *et al.* (2000)

5.2.5 Analysis of results

All arthropod population data obtained using sticky traps was converted to population per cm^2 and $\log_{10}(x+1)$ transformed to stabilize variances prior to analyses. Paired t-tests were used to compare the density of the most commonly represented taxa in each treatment and the density of the target prey items in each treatment. Diversity indices were calculated using the Shannon diversity index (H):

$$H = - \sum pi \ln pi$$

where *pi* is the proportion of total individuals found in the *i*th species. Calculated indices were to mean Shannon values per sampling occasion for comparisons of diversity between compost and non-compost data using t-tests. Population variation over time was analysed using One-Way Analysis of Variance (ANOVA). The predatory preferences of spiders was determined using Monte Carlo analysis as described in Chapter 4 (this thesis).

5.3 Results

5.3.1 Analysis of arthropods captured in non-compost and compost plots

A complete list of all the arthropods captured at web sites on the sticky traps in non-compost plots and compost plots is shown in Appendix 3. Table 5.2 shows the difference in mean number per cm^2 of each taxa between web sites sampled in non-compost plots and compost plots whereas Table 5.3 shows ANOVA results testing for the significance of variation over time each taxa at web sites in each treatment.

Table 5.2: Paired t-test results for the difference between five potential prey taxa at web sites of Linyphiidae spiders when sampled using sticky traps in non-compost or compost treated winter wheat. All data was $Log_{10}([\text{potential prey per } cm^2] + 1)$ transformed prior to analysis.

Variable	t	n	Mean per web site ± SE non-compost	Mean per non web site ± SE compost	P
All arthropods	0.64	6	0.145185 (±0.027416)	0.158675 (±0.022787)	0.534
Collembola	0.51	6	0.062659 (±0.027121)	0.070318 (±0.018945)	0.622
Diptera	3.79	6	0.015107 (±0.003606)	0.033915 (±0.005393)	0.003
Hemiptera	1.19	6	0.034187 (±0.013940)	0.026899 (±0.012898)	0.260
Hymenoptera	2.18	6	0.025818 (±0.006833)	0.033939 (±0.006274)	0.031
Coleoptera	0.38	6	0.006383 (±0.002925)	0.005078 (±0.001783)	0.554

Table 5.3: ANOVA showing the variation over time in the number captured per cm^2 of the most common taxa at web sites of Linyphiidae spiders in non-compost plots and compost plots.

Variable	d.f.	SS	F	P
a) Non-compost sticky traps				
Collembola	5, 84	0.030777	10.33	0.000
Diptera	5, 84	0.021151	5.98	0.003
Hemiptera	5, 84	0.048654	3.39	0.005
Hymenoptera	5, 84	0.008440	2.22	0.042
Coleoptera	5, 84	0.019311	0.64	0.451
b) Compost sticky traps				
Collembola	5, 84	0.028967	4.15	0.013
Diptera	5, 84	0.026948	3.47	0.007
Hemiptera	5, 84	0.14274	3.57	0.011
Hymenoptera	5, 84	0.034136	2.68	0.022
Coleoptera	5, 84	0.016936	1.03	0.322

The total number of arthropods captured at web sites in both non-compost and compost plots was not significantly different. The mean Shannon values calculated for each sampling occasion were compared using Paired t-tests and showed that the diversity was also not significantly different for all arthropods captured at web sites from each treatment ($t = 1.08$, $P = 0.331$).

5.3.1.1 Collembola analysis in compost and non-compost plots

Figure 5.1 shows the variation of Collembola in both treatments.The population of Collembola in non-compost plots was highest in late May and Collembola were captured at comparatively low levels throughout the rest of the sampling season. A different pattern can be seen in compost plots where two peaks are present. The first peak is in late May with another peak also present in early June. Despite these differences the mean density per cm^2 was not significantly different in each treatment (see Table 5.2) and the diversity of Collembola was also found to be not significantly different ($t = 1.03$, $P = 0.352$).

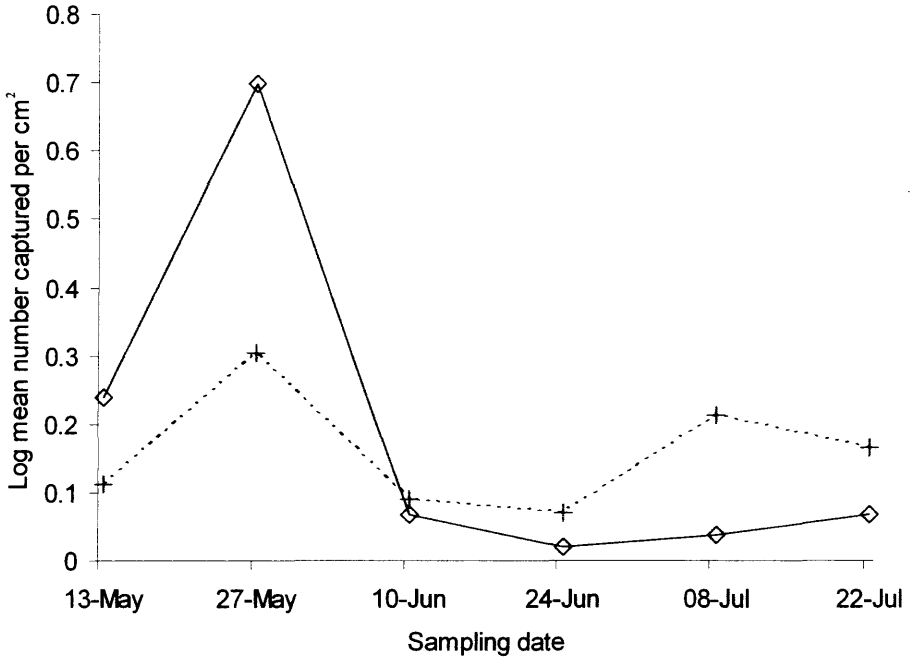


Figure 5.1: Line graphs of the mean log number per cm^2 of Collembola captured using sticky traps at web sites of Linyphiidae spiders in a) non-compost (◊) and b) compost (+) treated winter wheat.

5.3.1.2 Diptera analysis in compost and non-compost plots

In Figure 5.2 the variation of Diptera over time at non-compost and compost web sites is shown. In both treatments the variation was significant but with significantly higher density caught at compost web sites (Table 5.2) with the highest density for both treatments occurring throughout June. Even though compost web site sticky traps caught more Diptera, the diversity of Diptera was shown to be not significantly different to non-compost web sites ($t= 0.98$, $P = 0.373$).

5.3.1.3 Hemiptera analysis in compost and non-compost plots

Hemiptera variation over time in each treatment is shown in Figure 5.3 In compost plots, the highest density was captured during June after which the population density crashed resulting in a low density during July. For non-compost plots, the density of Hemiptera was highest in early July with a subsequent crash in late July. No significant difference was found in the mean population per cm^2 in each treatment (Table 5.2).

5.3.1.4 Hymenoptera analysis in compost and non-compost plots

Figure 5.4 shows variation in the mean density per cm^2 of Hymenoptera at web sites in each treatment. The density in both treatments varied significantly over time (Table 5.3) with a general increase in the trend until mid July after which the population crashed. In the compost treated winter wheat, significantly more Hymenoptera were caught during the sampling season than in the non-compost plots (Table 5.2).

5.3.1.5 Coleoptera analysis in compost and non-compost plots

The variation in the density of Coleoptera in each treatment is shown in Figure 5.5 The density of Coleoptera caught throughout the season did not vary significantly over time (Table 5.3) in either treatment. Also, there was no significant difference between the two treatments (Table 5.2).

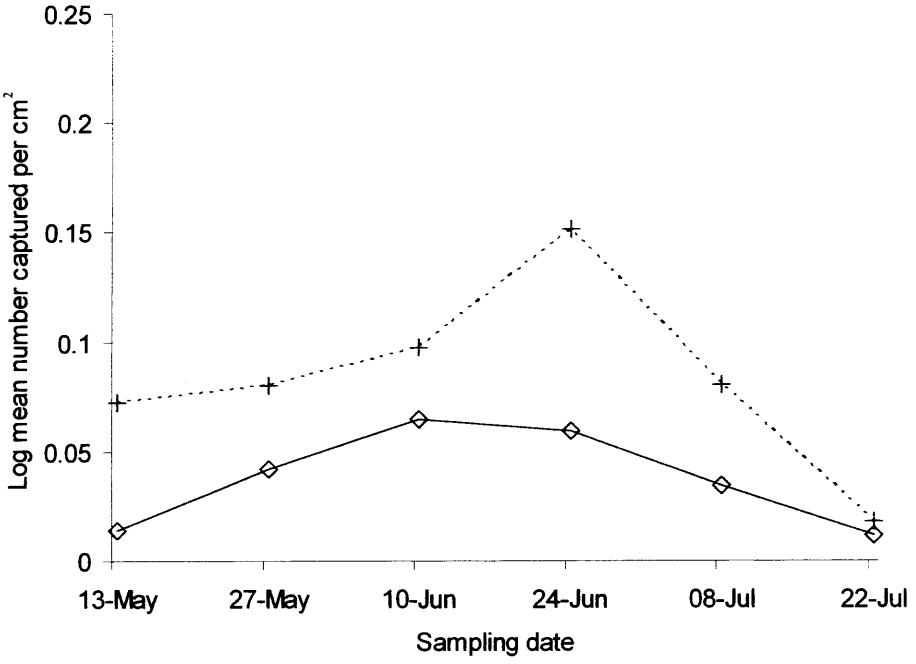


Figure 5.2: Line graphs of the mean number per cm^2 of Diptera captured using sticky traps at web sites of Linyphiidae spiders in a) non-compost (◊) and b) compost (+) treated winter wheat.

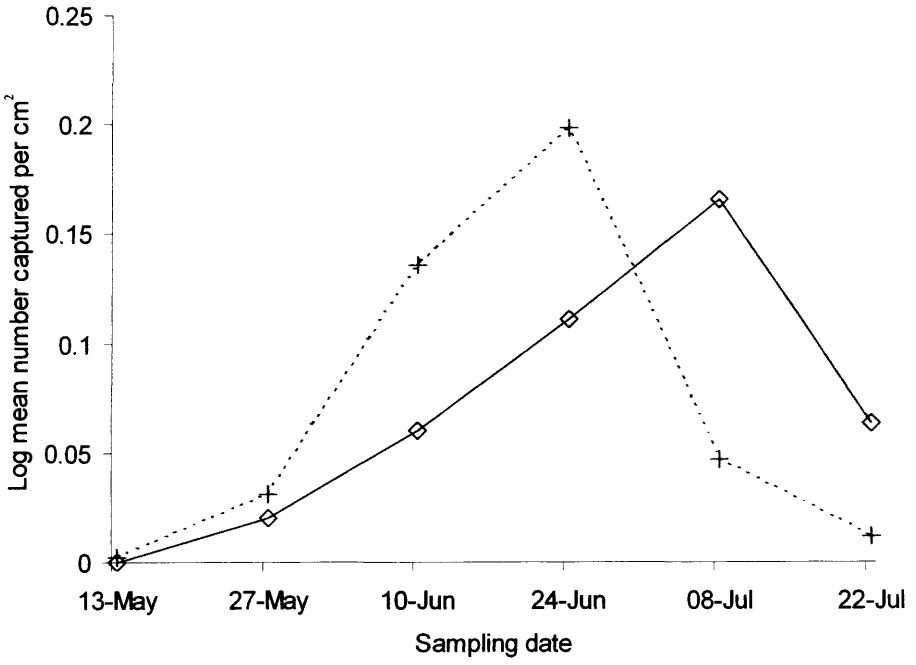


Figure 5.3: Line graphs of the mean number per cm^2 of Hemiptera captured using sticky traps at web sites of Linyphiidae spiders in a) non-compost (◊) and b) compost (+) treated winter wheat.

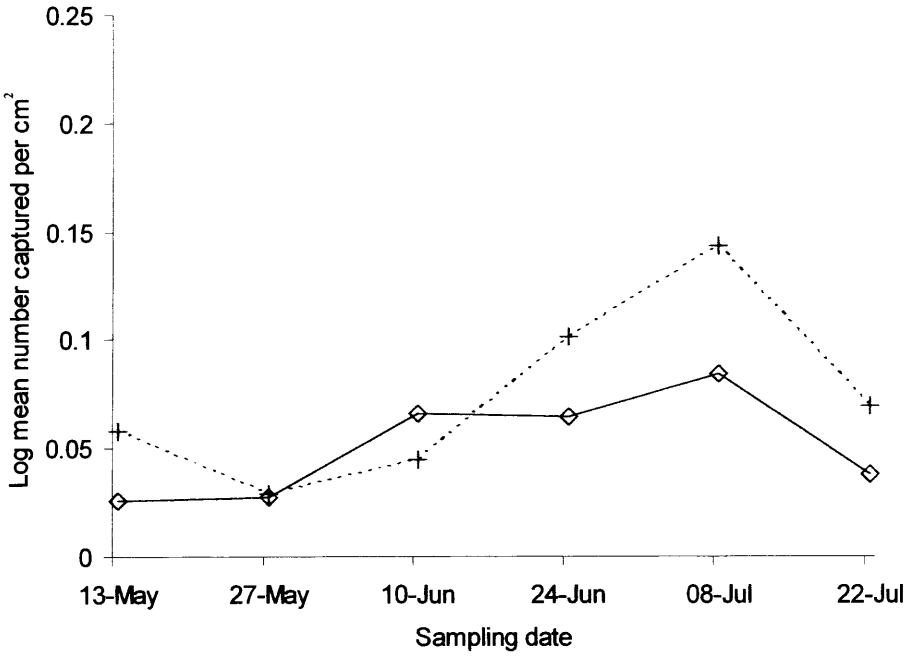


Figure 5.4: Line graphs of the mean number per cm^2 of Hymenoptera captured using sticky traps at web sites of Linyphiidae spiders in a) non-compost (◊) and b) compost (+) treated winter wheat.

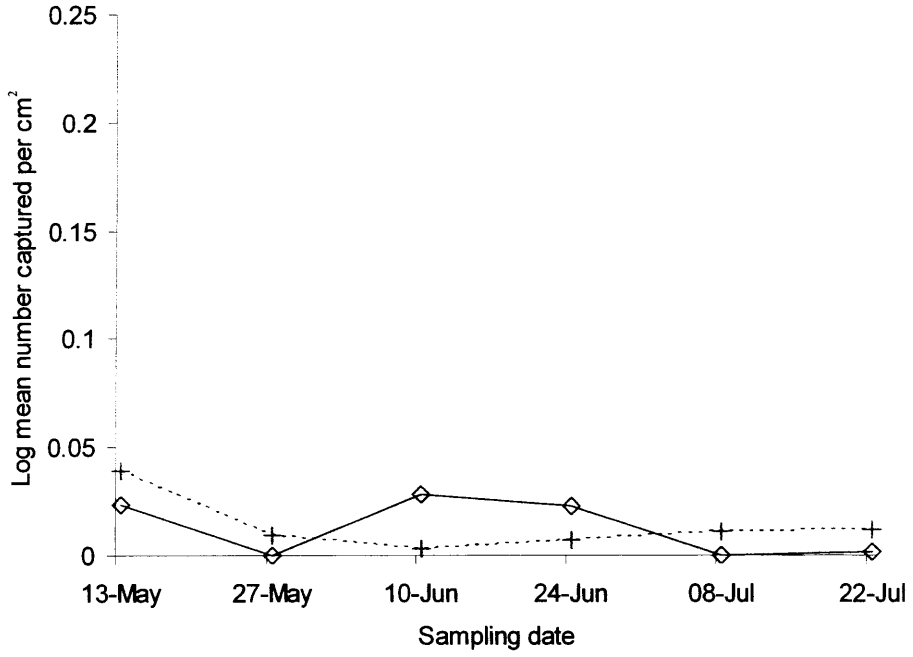


Figure 5.5: Line graphs of the mean number per cm^2 of Coleoptera captured using sticky traps at web sites of Linyphiidae spiders in a) non-compost (◊) and b) compost (+) treated winter wheat.

5.3.2 Results from linyphiid spider preferences analysis

DNA of the five potential prey items was successfully amplified from the guts of spiders collected from both treatments. Table 5.4 shows the spiders collected from web sites in non-compost plots and the number of spiders that were positive for each prey item. In Table 5.5, spiders that were collected from webs in compost plots are shown with the number of spiders that contained DNA of the potential prey items within their guts. The differences between non-compost and compost mean density per cm^2 of each prey item is shown in Table 5.6. The variation over time of the density of each prey item was also analysed and is shown in Table 5.7. The monitoring of the density of each prey item at web sites enabled Monte Carlo simulations to be used to determine spider preferences for or avoidance of consuming each prey item and the results of the analysis are shown in Table 5.8.

Table 5.4: The number of each species of spider collected from webs in non-compost plots of winter wheat and screened for the presence of DNA from five species of potential prey. The number of individuals from which prey DNA was amplified are shown with the percentage in paraetheses.

Spider species and sex	n	Number of spiders positive				
		Isotoma anglicana	Entomobrya multifasciata	Lepidocyrtus cyaneus	Lycoriella castanescens	Sitobion avenae
Tenuiphantes tenuis (male)	13	3	1	3	0	3
T. tenuis (female)	21	1	3	1	2	4
T. tenuis total	34	4 (11.8)	4 (11.8)	4 (11.8)	2 (5.9)	7 (20.6)
Erigone atra (male)	2	0	0	0	0	0
E. atra (female)	9	4	2	2	0	0
E. atra total	11	4 (36.4)	2 (18.2)	2 (18.2)	0	0
Erigone dentipalpis (male)	1	0	0	0	0	0
E. dentipalpis (female)	8	0	1	0	0	2
E. dentipalpis total	9	0	1 (11.1)	0	0	2 (22.2)
Bathyphantes gracilis (male)	6	0	1	0	2	0
B. gracilis (female)	13	1	1	1	0	0
B. gracilis total	19	1 (5.3)	2 (10.5)	1 (5.3)	2 (10.5)	0
Oedothorax sp. (male)	0	0	0	0	0	0
Oedothorax sp. (female)	0	0	0	0	0	0
Oedothorax sp. total	0	0	0	0	0	0
Erigoninae sp. (male)	2	0	0	1	1	0
Erigoninae sp. (female)	0	0	0	0	0	0
Erigoninae sp. total	2	0	0	1 (50)	1 (50)	0
Other male spiders	3	0	0	1	0	0
Other female spiders	6	0	0	0	0	0
Other spiders total	9	0	0	1 (11.1)	0	0
All male spiders	27	3 (11.1)	2 (7.4)	5 (18.5)	3 (11.1)	3 (11.1)
All female spiders	57	6 (10.5)	7 (12.3)	4 (7)	2 (3.5)	6 (10.5)
All spiders	84	9 (10.7)	9 (10.7)	9 (10.7)	5 (5.9)	9 (10.7)

Table 5.5: The number of each species of spider collected from webs in compost plots of winter wheat and screened for the presence of DNA from five species of potential prey. The number of individuals from which prey DNA was amplified are shown with the percentage in paraetheses.

Spider species and sex	n	Number of spiders positive				
		Isotoma anglicana	Entomobrya multifasciata	Lepidocyrtus cyaneus	Lycoriella castanescens	Sitobion avenae
Tenuiphantes tenuis (male)	12	0	4	0	1	0
T. tenuis (female)	29	1	5	1	0	3
T. tenuis total	41	1 (2.4)	9 (30)	1 (2.4)	1 (2.4)	3 (7.3)
Erigone atra (male)	1	0	0	0	0	0
E. atra (female)	9	2	0	0	0	1
E. atra total	10	2 (20)	0	0	0	1 (10)
Erigone dentipalpis (male)	0	0	0	0	0	0
E. dentipalpis (female)	7	1	0	1	1	1
E. dentipalpis total	7	1 (14.3)	0 (0)	1 (14.3)	1 (14.3)	1 (14.3)
Bathyphantes gracilis (male)	2	0	1	0	1	0
B. gracilis (female)	6	0	0	1	0	0
B. gracilis total	8	0 (0)	1 (12.5)	1 (12.5)	1 (12.5)	0 (0)
Oedothorax sp. (male)	1	0	1	0	1	0
Oedothorax sp. (female)	6	0	1	1	1	2
Oedothorax sp. total	7	0 (0)	2 (28.6)	1 (14.3)	2 (28.6)	2 (28.6)
Erigoninae sp. (male)	0	0	0	0	0	0
Erigoninae sp. (female)	2	0	1	0	0	0
Erigoninae sp. total	2	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)
Other male spiders	0	0	0	0	1	0
Other female spiders	3	0	2	1	0	0
Other spiders total	3	0 (0)	2 (66.7)	1 (33.3)	1 (33.3)	0 (0)
All male spiders	16	0 (0)	6 (37.5)	0 (0)	4 (25)	0 (0)
All female spiders	62	4 (6.5)	9 (14.5)	5 (8.1)	2 (3.2)	7 (11.3)
All spiders	78	4 (5.1)	15 (19.2)	5 (6.4)	6 (7.7)	7 (9)

Table 5.6: Paired t-test results for the difference between the mean density per cm^2 of five potential prey items at web sites of Linyphiidae spiders when sampled using sticky traps in non-compost or compost treated winter wheat. All data was Log_{10}([potential prey per cm^2] + 1) transformed prior to analysis.

Potential prey item	t	n	Mean per non-compost web site ± SE	Mean per compost web site ± SE	P
Isotoma anglicana	3.02	6	0.00617 (±0.00348)	0.00388 (±0.00310)	0.039
Entomobrya multifasciata	2.74	6	0.00506 (±0.00140)	0.00654 (±0.00271)	0.025
Lepidocyrtus cyaneus	3.39	6	0.00997 (±0.00252)	0.02271 (±0.01072)	0.005
Lycoriella castanescens	3.97	6	0.00353 (±0.00114)	0.00992 (±0.00405)	0.040
Sitobion avenae	1.07	6	0.00808 (±0.00295)	0.00665 (±0.00297)	0.128

Table 5.7: ANOVA output showing the variation over time of each prey item at web sites of Linyphiidae spiders in a) non-compost plots and b) compost plots.

Prey item	df	SS	F	P
a) Non-compost sticky traps				
Isotoma anglicana	5, 84	0.030777	2.85	0.020
Entomobrya multifasciata	5, 84	0.021151	0.79	0.101
Lepidocyrtus cyaneus	5, 84	0.048654	0.86	0.513
Lycoriella castanescens	5, 84	0.008440	1.05	0.395
Sitobion avenae	5, 84	0.019311	3.52	0.006
b) Compost sticky traps				
Isotoma anglicana	5, 84	0.028967	2.32	0.041
Entomobrya multifasciata	5, 84	0.026948	2.55	0.029
Lepidocyrtus cyaneus	5, 84	0.14274	6.79	0.000
Lycoriella castanescens	5, 84	0.034136	3.38	0.008
Sitobion avenae	5, 84	0.016936	3.80	0.004

Table 5.8: Monte Carlo results showing selection for or against prey items at web sites of Linyphiidae spiders in a) non-compost plots and b) compost plots. xxx = $p < 0.001$, xx = $P < 0.01$, x = $p < 0.05$ and ns = not significant.

Prey item	Total number captured	Number of positives expected from simulation	Number of positives observed	Evidence of selection	Significance (P)
a) non-compost					
Isotoma anglicana	5	3	9	For	xx
Entomobrya multifasciata	10	3	9	For	xx
Lepidocyrtus cyaneus	15	13	9	Against	ns
Lycoriella castanescens	12	9	5	Against	ns
Sitobion avenae	42	12	9	Against	ns
a) compost					
Isotoma anglicana	6	4	4	No selection	ns
Entomobrya multifasciata	25	6	15	For	xxx
Lepidocyrtus cyaneus	78	17	5	Against	xxx
Lycoriella castanescens	52	6	6	No selection	ns
Sitobion avenae	33	3	7	For	x

5.3.2.1 Predation on *Isotoma anglicana* in non-compost and compost plots

Figure 5.6 shows the variation in density over time for *Isotoma anglicana* at web sites with the predation by Linyphiidae spiders in each treatment. Predation on *I. anglicana* in both non-compost and compost plots occurred early in growing season when *I. anglicana* was found to be most abundant. More predation occurred in the non-compost plots where *I. anglicana* was actively selected for (see Table 5.8) whereas in the compost plots there was no selection. No predation occurred later than mid June when the density of *I. anglicana* was shown to be low.

5.3.2.2 Predation on *Entomobrya multifasciata*

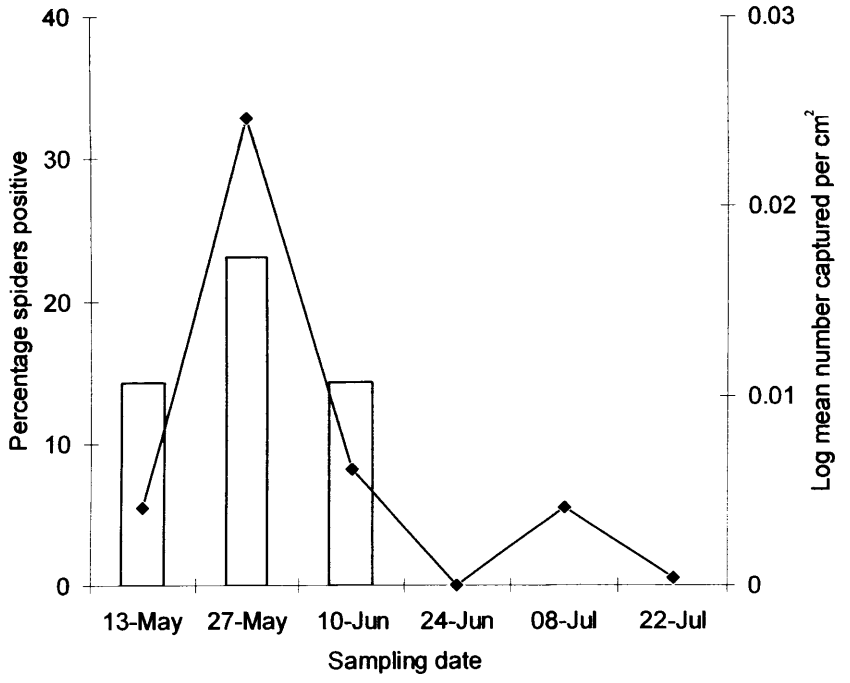
Figure 5.7 shows density variation in the population of *Entomobrya multifasciata* at web sites in non-compost and compost treated winter wheat plots and shows predation on *Entomobrya multifasciata* by Linyphiidae spiders in each treatment. In non-compost plots, there was evidence of selection for *Entomobrya multifasciata* with predation occurring thoughout the sampling season except early May and with no significant variation in the density of *E. multifasciata* over time. Predation in the compost plots showed that E. multifasciata was actively selected for more strongly (see Table 5.8) within this treatment where the density of *E. multifasciata* increased from mid June with predation also showing an increase throughout the season.

5.3.2.3 Predation on *Lepidocyrtus cyaneus*

Figure 5.8 shows predation on *Lepidocyrtus cyaneus* by Linyphiidae spiders in each treatment and the density over time of *L. cyaneus* in each treatment. Predation in non-compost plots occurred throughout the season without selection and there was no significant variation in the density of *L. cyaneus*. In the compost plots, there was

active avoidance of predating *L. cyaneus* with the highest predation in mid May but no predation from mid June onwards. The density of *L. cyaneus* was shown to increase significantly from mid June until late July.

a) Non-compost



b) Compost

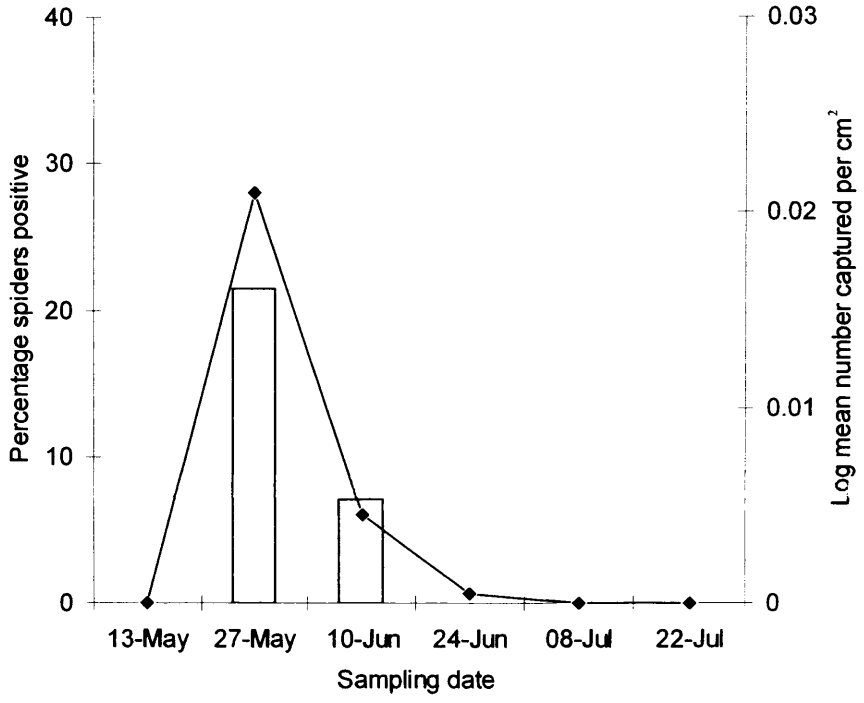
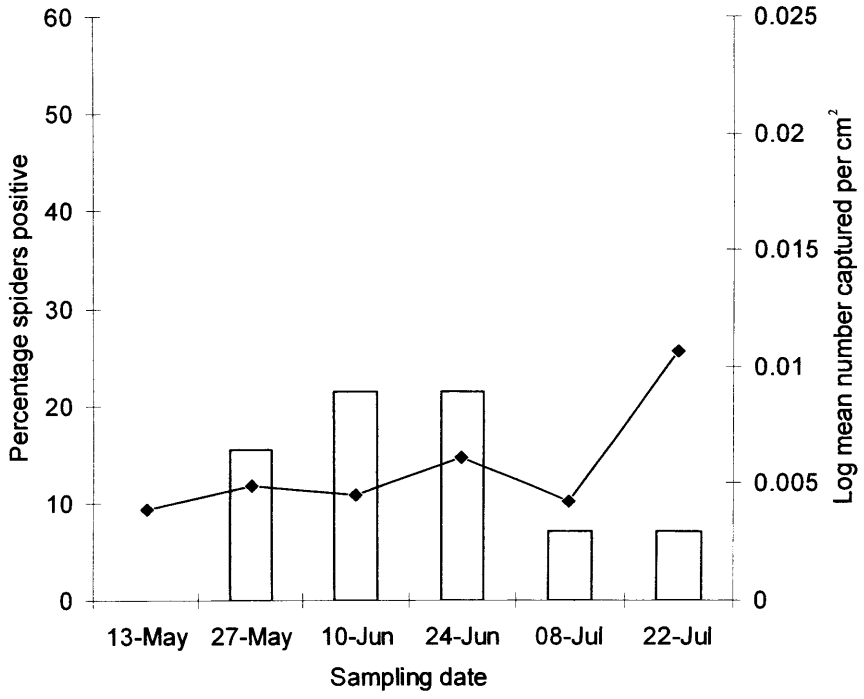


Figure 5.6: Bar charts showing the percentage of Linyphiidae spiders positive for *Isotoma anglicana* combined with line graphs showing the mean number *of Isotoma anglicana* per cm^2 (-♦-) captured at Linyphiidae spider web sites from a) non-compost and b) compost treated winter wheat

a) Non-compost



b) Compost

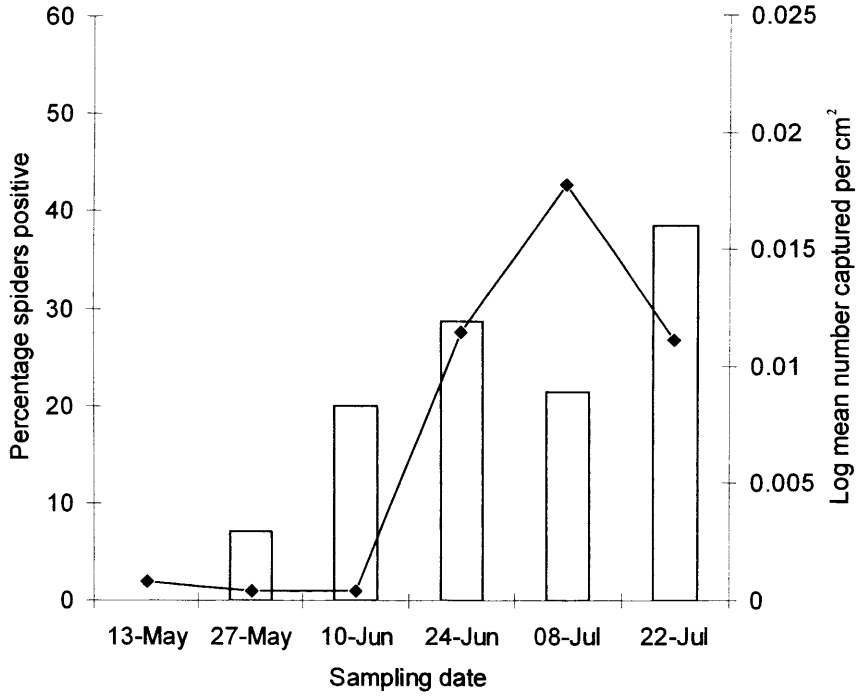
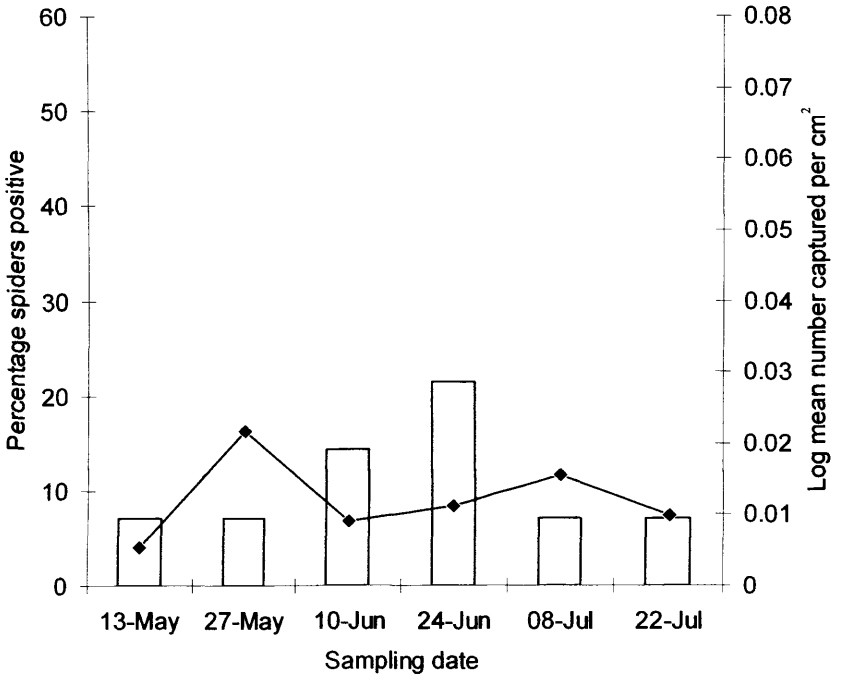


Figure 5.7: Bar charts showing the percentage of Linyphiidae spiders positive for *Entomobrya multifasciata* combined with line graphs showing the mean number of *Entomobrya multifasciata* per cm^2 (-♦-) captured at Linyphiidae spider web sites from a) non-compost and b) compost treated winter wheat

a) Non-compost



b) Compost

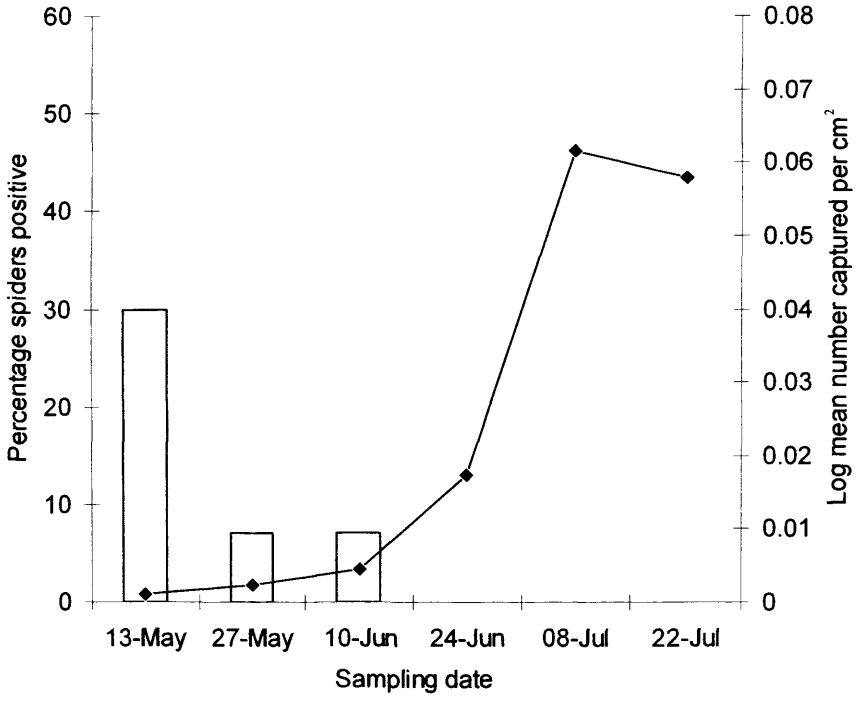


Figure 5.8: Bar charts showing the percentage of Linyphiidae spiders positive for *Lepidocyrtus cyaneus* combined with line graphs showing the mean number of *Lepidocyrtus cyaneus* per cm^2 (-♦-) captured at Linyphiidae spider web sites from a) non-compost and b) compost treated winter wheat

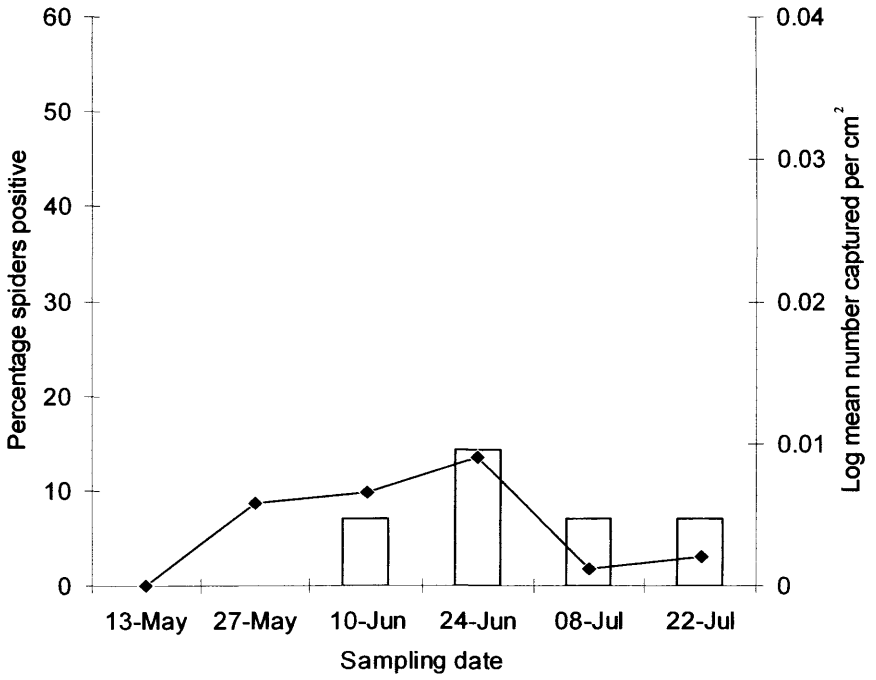
5.3.2.4 Predation on *Lycoriella castanescens*

Figure 5.9 shows predation by Linyphiidae spiders on *Lycoriella castanescens* in non-compost plots and compost plots with the density of *L. castanescens* also shown from each treatment. No significant variation over time was found in the density of *L. castanescens* in non-compost plots and predation was also shown to occur without significant selection from early June. In compost plots, *L. castanescens* varied significantly with a peak in mid June. Predation was not selective with the highest rate also occurring in mid June.

5.3.2.5 Predation on *Sitobion avenae*

Figure 5.10 shows the variation over time of *Sitobion avenae* at Linyphiidae spider web sites in non-compost plots and compost plots. Predation by Linyphiidae spiders in each treatment is also shown. In non-compost plots, *S. avenae* increased significantly from early June to peak in early July and a fall in density in late July. Predation on *S. avenae* was not selective and occurred increasingly from late June. In compost plots, a similar significant increase in *S. avenae* density was shown to peak in late June followed by a population crash. Predation occurred earlier than in the non-compost plots at early June with no predation shown once the density of *S. avenae* had reached a low level. Linyphiidae spiders in compost plots were shown to actively select for *S. avenae*.

a) Non-compost



b) Compost

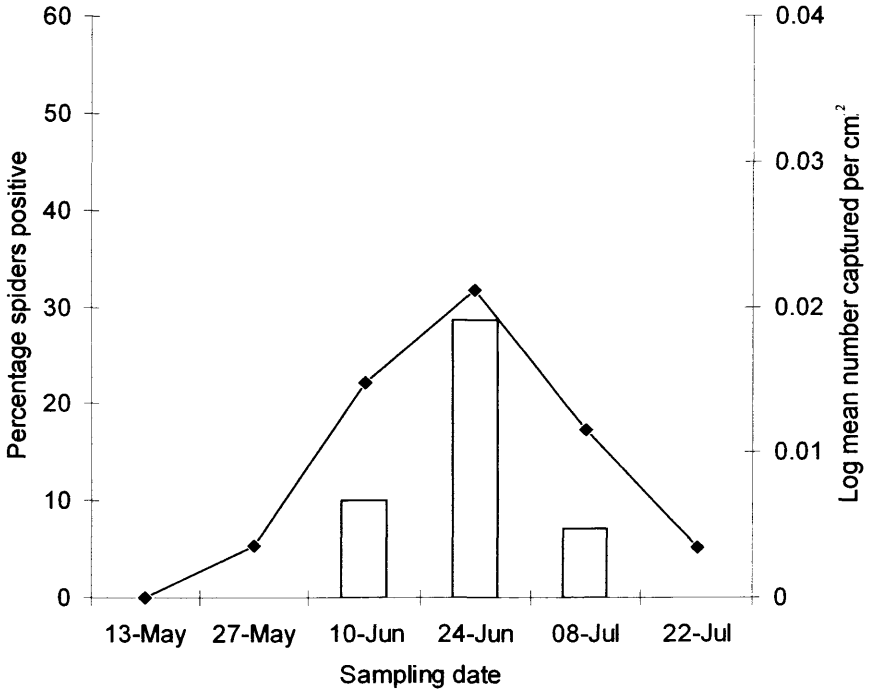
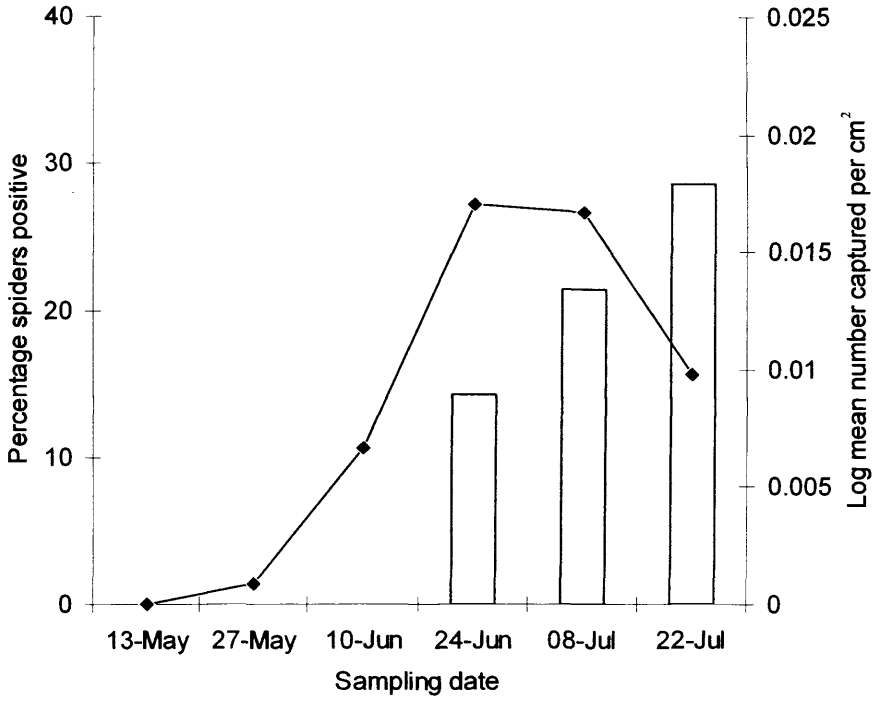


Figure 5.9: Bar charts showing the percentage of Linyphiidae spiders positive for *Lycoriella castanescens* combined with line graphs showing the mean number of *Lycoriella castanescens* per cm^2 (-♦-) captured at Linyphiidae spider web sites from a) non-compost and b) compost treated winter wheat

a) Non-compost



b) Compost

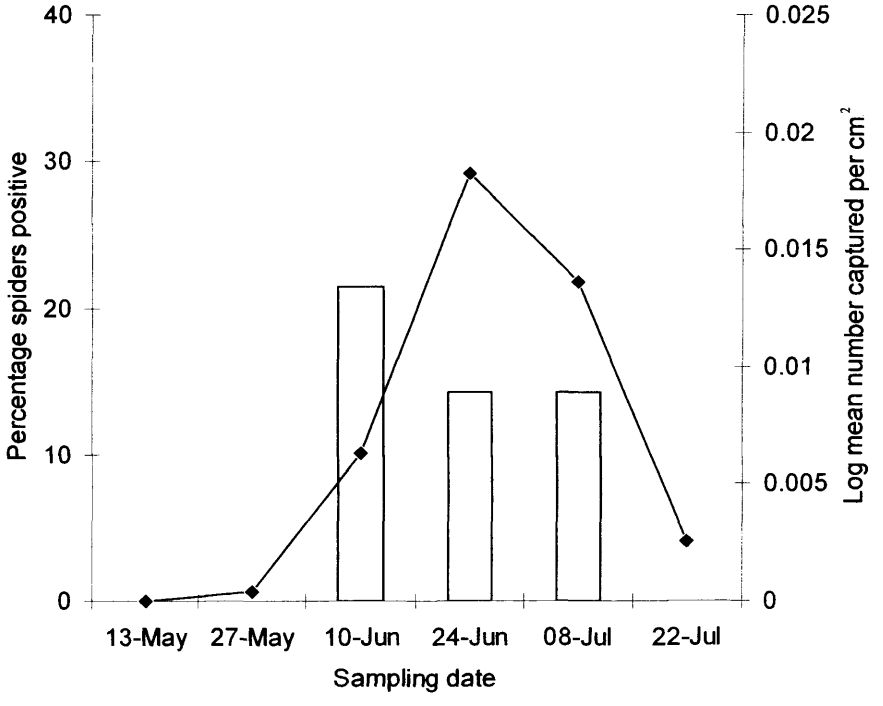


Figure 5.10: Bar charts showing the percentage of Linyphiidae spiders positive for *Sitobion avenae* combined with line graphs showing the mean number *of Sitobion avenae* per cm^2 (-♦-) captured at Linyphiidae spider web sites from a) non-compost and b) compost treated winter wheat

5.4 Discussion

This study gives an insight into some of the mechanisms by which Linyphiidae spiders can suppress aphid populations in winter wheat. The addition of compost to the crop affected the populations of non-pest prey and the predation of Linyphiidae spiders on them. Although there was no significant change in the diversity of arthropods captured at Linyphiidae web sites, the abundance of various taxa had increased. Diptera and Hymenoptera were significantly more abundant in the compost treated winter wheat. The increased numbers of Diptera (mainly Sciaridae and Cecidomyiidae) could be a food source for spiders, especially Linyphiinae that construct aerial sheet webs, and may serve to attract spiders to compost plots. The higher numbers of Hymenoptera in compost plots include Braconidae, a family which includes aphid parasitoids (Legrand *et al.*, 2004), and Scelionidae. Although the potential exists for the Linyphiidae spiders to predate upon Braconidae and disrupt any control the Braconidae may have on *Sitobion avenae*, there is some evidence to suggest that Linyphiidae spiders find parasitoids inedible (Nentwig, 1983). Also, some studies have found that there is no adverse effects of the presence of generalist predators on the efficiency of parasitoid pest suppression (Schmidt *et al.*, 2003) and this could be due to the immobility of mummified aphids when parasitized which would minimize interference by Linyphiidae spiders. Many Scelionidae are parasitoids of eggs and in winter wheat *Baeus sp.* is known to parasitize the eggs of *Erigone atra* (Vanbaarlen *et al.*, 1994). The higher number of Scelionidae caught in compost plots could be an indication of larger numbers of Linyphiidae spiders. However, it has been shown that spider parasitoids will increase their searching behaviour in the presence of silk from the host spider (vanBaarlen *et al.*, 1996). As the

physical structure of compost may provide an increase in suitable web attachment points (Rypstra *et al.*, 1999), the parastoids could be responding to a higher presence of silk.

Predation by Linyphiidae spiders was different in non-compost and compost plots. Although the diversity and abundance of Collembola was not different between the two treatments, the density of *Isotoma anglicana* was lower in compost plots whereas density of *Entomobrya multifasciata* and *Lepidocyrtus cyaneus* was higher. In both compost and non-compost plots the higher density of *I. anglicana* early in the season may provide an incentive for colonizing spiders to remain in the crop. This response was shown by (Weyman & Jepson, 1994) where Linyphiidae spiders arriving in an area where prey had been removed were more likely to leave the area than if prey was present.

Isotoma anglicana is thought to be a high quality prey item (Marcussen *et al.*, 1999) and preferential predation on them was found in the non-compost plots. This was also found in Chapter 4, especially for those Linyphiidae that contruct ground webs (Erigoninae). However, no selection by Linyphiidae spiders was found in the compost plots and significantly lower numbers were caught at web sites. As there was no preferential feeding on *Isotoma anglicana*, the reduction in their density at web sites is unlikely to have been a result of increased predation pressure by Linyphiidae spiders. Although it is possible that this was caused by predation from another predator, for example Staphylinidae beetles or Carabidae beetles, there was no significant difference between Coleoptera density at web sites in non-compost and compost plots with their density remaining at low levels throughout the year in both

treatments. Alternatively, as *Isotoma anglicana* preferentially occurs below the soil horizon where the microclimate contains more moisture (Simonsen *et al.*, 1999), the addition of compost may have created a more favorable microclimate for *Isotoma anglicana* to inhabit at the boundary between the soil and the compost. This would have rendered *Isotoma anglicana* relatively inaccessable to Linyphiidae spiders and so limit predation. This possible behavioural response by *Isotoma anglicana* could explain their lack of phyical or chemical defences.

The scales of *Entomobrya multifasciata* are thought to be a form of defence to facilitate escape from predators (Chapter 4)(Bauer, Pfeiffer, 1991; Hopkin, *in press*). In non-compost plots, there was preferential selection for *Entomobrya multifasciata* with predation occuring throughout the year and the density of *E. multifasciata* remaining approximately constant throughout the year. This is thought to be due to the increased capability of Linyphiinae spiders (from aerial webs) to handle *E. multifasciata* (Chapter 4). In compost plots there was also high selection for *E. multifasciata* with predation increasing throughout the season in response to increasing density of *E. multifasciata* at web sites. In May, when the density of *E. multifasciata* was lowest at web sites, this collembolan is likely to occur in greater densities within the compost substrate where Linyphiidae spiders are relatively unable to predate them. As the population increases, competition for resources either within the species or with other species of Collembola could force the increasing population of *E. multifasciata* to inhabit higher strata where they are more likely to come into contact with Linyphiidae spiders. Similar mechanisms may take place with *Lepidocyrtus cyaneus*. A similar increase in the density of *L. cyaneus* was found in the compost plots with low levels occuring early in the season followed by a rapid

increase in their population at web sites. However, this increase was much more pronounced than for *E. multifasciata*. Although overall there was avoidance of *L. cyaneus* as a prey item, at the beginning of the season there was a high percentage of spiders postive for *L. cyaneus* in compost plots. Here, although the population was low, other prey may not have been available for Linyphiidae spiders to consume. *L. cyaneus* may be unable to compete with the other Collembola at lower levels in the compost so more may venture to the surface where spiders would be able to predate them. Later in the season when the overall population of Collembola below the compost reaches a high level, more alternative prey would become available, such as *E. multifasciata*, and these may be preferentially predated. Although *E. multifasciata* is thought to have defences against predation, the defences of *L. cyaneus* may be more potent which is reflected in the overall avoidence of consuming *L. cyaneus* by Linyphiidae spiders here and in other studies (Agusti *et al.*, 2003)(Chapter 4). In Bilde & Toft (2000), a study of the quality of *L. cyaneus* as a prey item for a Carabidae beetle showed that dead *L. cyaneus* were of high quality but when the Carabidae was fed a diet of live *L. cyaneus* there was lower egg production indicating *L. cyaneus* was a low quality prey item. Although observations showed a possible handling cost of live *L. cyaneus* due to the presence of scales (Bauer, Pfeiffer, 1991), the change in food conversion efficiency from live to dead *L. cyaneus* was also attributed to possible changes in nutrient composition during freezing to kill the *L. cyaneus*. Collembola from other families are known to have chemical defences to deter predation (Dettner *et al.*, 1996; Messer *et al.*, 2000). It is possible that *L. cyaneus* could also have chemical defences and if these defences have to be actively maintained then they could rapidly break down once the Collembolan dies, raising its quality as a prey item. However, if these chemical defences exist, they would incur a

metabolic cost that could inhibit *L. cyaneus*'s ability to compete with other Collembola whereby *L. cyaneus* would be forced to remain on the surface of the compost where it could be easily predated by the Linyphiidae spiders. However, once a high enough density of *E. multifasciata* are present above the compost, the additional possible chemical defences of *L. cyaneus* would deter predators in favor of *E. multifasciata* and allow the population of *L. cyaneus* to increase logarithmically until July when the density of *L. cyaneus* is self-limiting. These possible interactions between *L. cyaneus*, *E. multifasciata* and Linyphidae spiders are strong evidence for predator mediated apparent competition. This supports evidence that apparent competition can alter the structure of ecological assemblages (Bonsall, Hassell, 1997). However, the strength of effects shown here contradict the theory that generalist predators exhert a weak effect (Holt, 1997).

Predation on *Lycoriella castanescens* was found to be non-selective in both non-compost plots and compost plots. This was also found in Chapter 4 for both Linyphiinae at aerial web sites and Erigoninae at ground web sites. It is curious that predation on a prey item can be so consistent regardless of its overall density (*L. castanescens* density was higher in compost plots) or its vertical distribution. The value of *L. castanescens* as a prey item is thought to be of medium quality (similar to the sciarid used in feeding studies by (Toft, 1995)) and so no selection for or against is expected. However, it is unlikely that the fluctuating predatory preferences of Linyphiidae spiders for the other prey items under different conditions would have no effect on predation of *L. castanescens*. This lack of selection is therefore possibly due to an ability of *L. castanescens* to avoid predation through behavioural responses. Diptera have well developed directional motion vision (Sinakevitch & Strausfeld,

2004), presumably a requirement for rapid flight through structurally complex environments, which may allow Diptera to detect the approach of a predator from a distance and this could induce an early escape response. Also, other prey items have no control when falling from higher strata into the sheet webs of Linyphiidae spiders but the high degree of control Diptera have during flight could allow *L. castanescens* to have a greater measure of avoiding becoming trapped upon encountering a sheet web.

The density of *S. avenae* was not significantly different between non-compost and compost plots. However, this density was measured at web sites of Linyphiidae spiders so only those aphids that were walking between plants or had dropped from the wheat would have been sampled. Both behaviours are known to frequently occur in response to encountering predators and parasitoids foraging in the areas where aphid colonies reside (Losey, Denno, 1998; Villagra *et al.*, 2002). Linyphiidae spiders could therefore increase pressure on aphid populations that are under attack from other predators. These aphid behaviours also occur in the absence of external influence. (Sopp *et al.*, 1987) showed that aphids will drop of their own accord and that dropping behaviour was higher when aphid densities were lower. This could indicate that even if the population of aphids in compost plots was lower than in non-compost plots, the density of aphids caught at web sites would still be high. Also, aphids have been observed to quickly climb plants after they had fallen from higher in the crop (Winder *et al.*, 1994). In compost plots, the increased structural complexity of the ground may increase the time taken for aphids to locate plants to climb and would increase the risk of predation by Linyphiidae spiders.

Even though *S. avenae* is considered a low quality prey item (Beck, Toft, 2000; Bilde, Toft, 2000; Toft, 1995), Linyphiidae spiders in compost plots were found to change their predation habits to select for *S. avenae*. In Chapter 4, the ability of Linyphiidae spiders to predate *S. avenae* was shown to be determined by the overall quality of the non-pest prey in the spider diet where the consumption of a low quality diet would limit the predation on *S. avenae*. *Entomobrya multifasciata* is considered to be high in nutrient content but its low density and defences in non-compost plots could limit its inclusion into the Linyphiidae spider diet even with preferential predation on it. In compost plots, there was a higher density of *E. multifasciata* increasing the opportunity of predation by Linyphiidae spiders and allowing more *E. multifasciata* to be included in the spider diet despite *E. multifasciata*'s defences. This would have given Linyphiidae spiders a high enough nutrient balance to tolerate the inclusion of higher numbers of *S. avenae* in their diet. In addition, the lack of physical defences of *S. avenae* would mean *S. avenae* would have a lower handling cost compared to *E. multifasciata* and so further increase predation rate on the aphid.

Predation on *S. avenae* occurred earlier in compost plots than in non-compost plots and also was highest at this point. Here, Collembola would have been relatively unavailable due to the physical structure of compost. However, low numbers of the high quality prey item *I. anglicana* may have been available in early June providing a limited intake of a high nutrient diet. Although a high rate of predation on such a high quality prey item would have resulted in a lack of interest in other prey, the low availability of *I. anglicana* may have left spiders in a starved state but with a nutrient balance that allowed for the intake of low quality prey resulting in higher predation on *S. avenae*. In late July in the compost plots there was no predation on *S. avenae*. At

this point predation on *E. multifasciata* was highest and corresponded with a reduction in denstiy. This may be an indication of low availability of *S. avenae* forcing the Linyphiid spiders to rely on *E. multifasciata* as their primary food source. In non-compost plots predation on *S. avenae* was at its highest in late July with comparatively low predation on *E. multifasciata* despite a rise in the density of *E. multifasciata*. (Topping, Sunderland, 1998) showed that at this point in the seaon there was a large increase in the number of hatchlings of *Tenuiphantes tenuis* (*Lephthyphantes tenuis*). The energetic cost of reproduction may lead to a low level of fitness of the spider population and this could reduce the ability of the spiders to handle prey. The defences of *E. multifasciata* may incur handling costs that are too high for spiders of low fitness and this combined with the high availablity / low handling cost of aphids could result in higher predation on aphids to recover the energy deficit. Alternatively, low spider fitness may be the result of the long term effects of a low quality diet. Mayntz *et al.* (2005) showed that generalist predators will select prey on the basis of their lipid and protein content relative to the lipid : protein ratio of their preceeding diet to obtain a balance between the two. This provides a further possible explanation for changes in the preferences of linyphiid spiders. A high rate of predation on prey rich in, for example, lipids, would offset the balance of proteins and lipids within the spider. Spiders would then be more likely to be averse to further consumption of this prey item in favour of those that have a higher protein content in order to restore the balance in nutrients.

Although studies in the field have previously shown that spiders can suppress prey populations (Chiverton, 1986; Schmidt *et al.*, 2004), by detecting the DNA of prey within the gut of Linyphiidae spiders this study has provided direct evidence to show how a simple crop treatment can alter predator-prey interactions with non-pests to facilitate preferential feeding on a pest.

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6.1 Conclusions:

A number of methods have been employed in this study to investigate predation by linyphiid spiders on pest and non-pest prey. The combination of monitoring the spiders' potential prey with direct identification of prey DNA within the gut of the spiders revealed that complex interactions occur between sub-families of spiders, their non-pest prey and pest prey and showed how the diversity and abundance of alternative prey affect predation by linyphiid spiders on cereal aphids. The main conclusions from this project are:

- Combining the identification of prey DNA in the guts of spiders, the monitoring of prey density and Monte Carlo simulations to determine predator preferences is a powerful approach for investigating predator–prey interactions

- Linyphiid spiders locate their webs in areas where there is a high abundance of potential prey

- Erigoninae at ground web sites have small webs and are not web dependent due to the abundance of high quality prey that can be caught away from their webs

- Linyphiinae at aerial web sites invest in larger webs and are web dependent as a strategy to obtain the diversity of prey necessary to gain a favourable balance of amino acids in their diet due to the lower density of high quality prey

- Linyphiid spiders in the field show preferences for certain prey items and aversions to other prey items, supporting laboratory feeding studies
- Predation on cereal aphids by linyphiid spiders occurs earlier in the season in compost enhanced crop conditions
- Under compost enhanced crop conditions, predation on *S. avenae* by linyphiid spiders was increased through the increased availability of suitable alternative prey such as the Collembolan, *Entombrya multifaciata*. Predation on alternative prey offsets the detrimental effects of consuming low quality pest prey by restoring the balance of nutrients.

Appendix 1: List of arthropods collected in 2001 using web sites sticky traps (WS), non-web site sticky traps (NWS), web site quadrats (WQ) and non-web site quadrats (NWQ). Part 1.

Species	WS	NWS	WQ	NWQ
Neuroptera				
Chrysopidae larva	1	0	0	0
Collembola				
Unidentifiable Entomobyramorpha	4	1	82	65
Isotomidae				
Isotoma anglicana	0	0	23	31
Isotomurus palustis	8	3	0	0
Entomobryidae				
Entomobrya multifaciata	4	0	42	34
Lepidocyrtus cyanus	18	11	128	36
Unidentifed Sminthuridae	2	5	7	12
Sminthurus niger	1	2	0	1
Sminthurus elegans	0	1	5	13
Sminthurus aureus	6	3	10	2
Tomoceridae				
Tomocerus longicornis	0	0	1	0
Poduridae	0	0	0	2
Total collembolan	43	26	298	196
Diptera				
Nematocera				
Sciaridae	11	3	0	1
Lycoriella castanescens	7	5	1	0
Cecidomyiidae	16	13	29	15
Psychodidae	1	0	0	0
Mycetophylidae	3	3	1	0
Brachycera				
Dolichopodidae	3	6	0	0
Empididae	1	0	0	1
Phoridae	30	15	0	0
Cyclorrapha Aschiza				
Syrphidae	0	2	0	0
Cyclorrapha Schizophora: Acalypterates				
Chloropidae	1	1	0	0
Lonchopteridae	0	1	0	0
Cyclorrapha Schizophora: Calypterates				
Muscidae	1	3	0	0
Total Diptera	74	52	32	18
Hemiptera				
Homoptera				
Auchenorrhyncha				
Cicadellidae	8	8	8	5
Cercopidae	1	0	0	0
Delphacidae	2	2	2	
Sternorrhyncha				
Aphididae	1	0	0	0
Sitobion avenae	68	52	21	11
Metopolophium dirhodum	8	12	4	8
Heteroptera				
Nabidae	1	1	0	0
Total Hemiptera	89	75	35	24

Appendix 1: List of arthropods collected in 2001 using web sites sticky traps (WS), non-web site sticky traps (NWS), web site quadrats (WQ) and non-web site quadrats (NWQ). Part 2.

Species	WS	NWS	WQ	NWQ
Hymenoptera				
Cynipoidea				
Cynipidae	0	0	1	3
Chrysidoidea				
Dyrinidae	14	22	0	0
Chalcidoidea				
Chalcididae	8	17	1	1
Encyrtidae	2	1	1	1
Proctotrupoidea				
Diapriidae	5	1	0	0
Scelionidae	9	11	0	0
Platygasteridae	12	15	6	1
Proctotrupidae	4	3	0	0
Ceraphronoidea				
Ceraphronidae	7	9	1	1
Ichneumonoidea				
Ichneumonidae	2	2	0	0
Braconidae	6	2	0	0
Total hymenoptera	69	83	10	7
Coleoptera				
Brachyelytra				
Staphylinidae larva	1	0	12	3
Staphylinus sp.	0	0	0	1
Tachyporus sp.	2	1	5	0
Stenuis sp.	1	3	0	0
Aleocharini	1	0	5	2
Carabidae	0	0	0	0
Pterostichus madidus	1	1	0	0
Bembidion sp.	0	0	1	0
Trechus sp.	0	0	0	0
Chrysomelidae	2	1	0	0
Cantharidae	1	0	0	0
Elateridae	0	0	1	0
Clavicornia				
Enicmus sp.	0	7	9	4
Coccinellidae	0	0	0	0
Coccinellidae larva	0	0	2	1
Byrridae	0	0	0	1
Unidentified beetle larva	8	0	14	8
Total coleopteran	17	20	49	29
Thysanoptera	29	38	47	59
Acari	36	22	76	80
Chilopoda				
Lamyctes fulvicornis	0	1	2	0

Appendix 1: List of arthropods collected in 2001 using web sites sticky traps (WS), non-web site sticky traps (NWS), web site quadrats (WQ) and non-web site quadrats (NWQ). Part 3.

Species	WS	NWS	WQ	NWQ
Araneae				
Linyphiidae spiderling	14	1	22	9
Meioneta rurestris ♂	3	2	3	0
Meioneta rurestris ♀	0	0	1	0
Erigoninae subadult ♂	3	0	3	0
Erigoninae subadult ♀	4	0	5	0
Erigone atra ♂	7	0	5	0
Erigone atra ♀	3	0	6	0
Erigone dentipalpis ♂	1	1	0	0
Oedothorax apicatus ♂	0	0	1	0
Oedothorax sp. ♀	7	2	13	0
Oedothorax sp. subadult ♂	1	0	3	0
Tenuiphantes tenuis ♂	3	0	4	0
Tenuiphantes tenuis ♀	6	0	8	0
Tenuiphantes tenuis subadult ♂	5	0	8	0
Tenuiphantes tenuis subadult ♀	11	0	14	0
Bathyphantes gracilis ♂	2	1	1	0
Bathyphantes gracilis ♀	3	0	1	0
Bathyphantes gracilis subadult ♂	2	0	1	0
Bathyphantes gracilis subadult ♀	3	0	1	0
Pachygnatha degeeri ♀	7	0	2	0
Microlinyphia pusilla ♂	0	0	1	0
Total Araneae	83	7	103	9
Total Arthropods captured	438	324	652	422

Appendix 2: List of arthropods collected in 2002 using web sites sticky traps (WS), non-web site sticky traps (NWS), web site quadrats (WQ) and non-web site quadrats (NWQ). Part 1.

Species	WS	NWS	WQ	NWQ
Neuroptera				
Chrysopidae larva	0	0	1	0
Collembola				
Unidentifiable Entomobyramorpha	124	96	108	97
Isotomidae				
Isotoma anglicana	41	13	96	53
Isotomurus palustis	114	73	536	337
Entomobryidae				
Entomobrya multifaciata	69	49	56	41
Lepidocyrtus cyanus	79	53	193	97
Unidentified Sminthuridae	14	26	1	0
Sminthurus niger	0	1	0	0
Sminthurus elegans	0	0	4	4
Sminthurus aureus	10	6	0	0
Tomoceridae				
Tomocerus longicornis	2	1	0	0
Unidentified Poduridae	15	9	0	0
Total collembolan	468	327	994	629
Diptera				
Nematocera				
Sciaridae	29	45	1	1
Lycoriella castanescens	21	19	0	0
Tipulidae	0	2	0	0
Cecidomyiidae	29	30	2	2
Mycetophylidae	10	9	0	0
Brachycera				
Dolichopodidae	39	22	0	0
Empididae	7	10	0	0
Phoridae	73	58	0	0
Cyclorrapha Aschiza				
Syrphidae	1	0	0	0
Cyclorrapha Schizophora: Acalypterates				
Sphaeroceridae	1	0	0	0
Agromyzidae	3	4	0	0
Chloropidae	4	3	0	0
Lonchopteridae	12	6	0	0
Anthromyzidae	0	2	0	0
Cyclorrapha Schizophora: Calypterates				
Muscidae	9	12	0	0
Total Diptera	238	222	3	3
Hemiptera				
Homoptera				
Auchenorrhyncha				
Cicadellidae	15	10	0	1
Cercopidae	0	0	0	0
Delphacidae	11	5	0	0
Sternorrhyncha				
Aphididae	0	0	0	0
Sitobion avenae	278	125	12	8
Metopolophium dirhodum	1	2	2	0
Heteroptera				
Nabidae	0	1	1	1
Total Hemiptera	305	143	15	9

Appendix 2: List of arthropods collected in 2002 using web sites sticky traps (WS), non-web site sticky traps (NWS), web site quadrats (WQ) and non-web site quadrats (NWQ). Part 2.

Species	WS	NWS	WQ	NWQ
Hymenoptera				
Cynipoidea				
Cynipidae	4	4	0	0
Chrysidoidea				
Dyrinidae	39	37	0	0
Chalcidoidea				
Chalcididae	5	11	3	4
Encyrtidae	7	7	0	0
Proctotrupoidea				
Diapriidae	4	0	0	0
Scelionidae	29	42	0	0
Platygasteridae	38	56	1	0
Proctotrupidae	27	38	0	2
Codrus sp.	13	45	0	0
Ceraphronoidea				
Ceraphronidae	7	4	1	2
Megaspilidae	16	8	0	0
Ichneumonoidea				
Ichneumonidae	8	1	0	0
Braconidae	13	51	1	0
Total hymenoptera	210	304	6	8
Coleoptera				
Brachyelytra				
Staphylinidae larva	1	3	4	3
Staphylinus sp.	0	0	0	1
Tachyporus nitidulus	0	0	2	0
Tachyporus sp.	5	5	1	0
Stenuis sp.	0	0	9	6
Aleocharini	6	8	1	1
Anthribidae	0	1	0	0
Carabidae	0	0	0	0
Pterostichus madidus	0	1	0	0
Bembidion sp.	0	0	8	1
Demetrias sp.	0	1	0	0
Trechus sp.	1	1	4	2
Carabididae larva	5	0	1	2
Tachys sp.	1	0	0	0
Phytophaga				
Chrysomelidae	5	2	0	0
Malacadermata				
Cantharidae	1	1	0	0
Sternoxia				
Elateridae	1	0	1	0
Clavicornia				
Enicmus sp.	5	9	3	0
Coccinellidae	0	0	0	0
Coccinellidae larva	0	0	1	0
Micrapis sedecempuctata	1	0	1	0
Byrridae	3	3	0	0
Unidentified beetle larva	2	3	0	0
Total coleopteran	37	38	36	18

Appendix 2: List of arthropods collected in 2002 using web sites sticky traps (WS), non-web site sticky traps (NWS), web site quadrats (WQ) and non-web site quadrats (NWQ). Part 3.

Species	WS	NWS	WQ	NWQ
Thysanoptera	240	156	12	5
Acari	65	35	23	9
Araneae				
Linyphiidae spiderling	10	3	4	1
Meioneta rurestris ♂	1	0	1	0
Meioneta rurestris ♀	6	0	2	0
Pelecopsis parallela ♂	1	0	0	0
Erigoninae subadult ♂	7	0	10	0
Erigoninae subadult ♀	4	0	7	0
Erigone atra ♂	6	0	10	0
Erigone atra ♀	20	0	10	0
Erigone dentipalpis ♂	3	0	0	0
Erigone dentipalpis ♀	8	0	3	0
Oedothorax apicatus ♂	2	0	0	0
Oedothorax fuscus ♂	3	0	1	0
Oedothorax retusus ♂	1	0	0	0
Oedothorax sp. ♀	4	0	6	0
Oedothorax sp. subadult ♂	1	0	0	0
Tenuiphantes tenuis ♂	15	0	19	1
Tenuiphantes tenuis ♀	26	0	28	1
Tenuiphantes tenuis subadult ♂	14	1	25	0
Tenuiphantes tenuis subadult ♀	12	0	23	0
Bathyphantes gracilis ♂	15	3	6	1
Bathyphantes gracilis ♀	23	0	16	0
Bathyphantes gracilis subadult ♂	2	1	0	0
Bathyphantes gracilis subadult ♀	6	0	7	0
Pachygnatha degeeri ♀	0	0	3	0
Savignya frontata ♂	0	0	1	0
Savignya frontata ♀	0	0	1	0
Opiliones	0	0	1	0
Total Araneae	190	8	184	4
Total Arthropods captured	1753	1233	1274	685

Appendix 3: List of arthropods collected in 2002 using sticky traps at Linyphiidae web sites in two treatments of winter wheat. Part 1.

Species	Non-compost web sites	Compost web sites
Collembola		
Unidentifiable Entomobyramorpha	45	105
Isotomidae		
Isotoma anglicana	6	7
Isotomurus palustis	69	25
Entomobryidae		
Entomobrya multifaciata	21	39
Lepidocyrtus cyanus	35	165
Unidentified Sminthuridae	8	5
Sminthurus niger	0	1
Sminthurus aureus	9	1
Tomoceridae		
Tomocerus longicornis	2	1
Unidentified Poduridae	8	1
Total collembolan	203	350
Diptera		
Nematocera		
Sciaridae	20	110
Lycoriella castanescens	14	82
Tipulidae	0	2
Cecidomyiidae	15	57
Mycetophylidae	5	3
Brachycera		
Dolichopodidae	29	27
Empididae	4	3
Phoridae	32	30
Cyclorrapha Schizophora: Acalypterates		
Sphaeroceridae	0	10
Agromyzidae	3	2
Chloropidae	4	2
Lonchopteridae	10	5
Anthromyzidae	0	1
Cyclorrapha Schizophora: Calypterates		
Muscidae	6	16
Total Diptera	142	350
Hemiptera		
Homoptera		
Auchenorrhyncha		
Cicadellidae	9	9
Cercopidae	0	0
Delphacidae	4	8
Sternorrhyncha		
Aphididae		
Sitobion avenae	131	105
Metopolophium dirhodum	0	1
Heteroptera		
Nabidae	0	0
Total Hemiptera	144	124

Appendix 3: List of arthropods collected in 2002 using sticky traps at Linyphiidae web sites in two treatments of winter wheat. Part 2.

Species	Non-compost web sites	Compost web sites
Hymenoptera		
Cynipoidea		
Cynipidae	1	0
Chrysidoidea		
Dyrinidae	22	20
Chalcidoidea		
Chalcididae	3	5
Encyrtidae	5	6
Proctotrupoidea		
Diapriidae	1	0
Scelionidae	19	66
Platygasteridae	9	21
Proctotrupidae	8	20
Ceraphronoidea		
Ceraphronidae	5	3
Megaspilidae	16	28
Ichneumonoidea		
Ichneumonidae	1	3
Braconidae	17	44
Total hymenoptera	107	216
Coleoptera		
Brachyelytra		
Staphylinidae larva	0	2
Staphylinus sp.	0	1
Tachyporus sp.	4	2
Stenuis sp.	0	2
Aleocharini	6	17
Carabidae		
Trechus sp.	0	1
Carabididae larva	1	1
Tachys sp.	1	1
Phytophaga		
Chrysomelidae	3	0
Sternoxia		
Elateridae	1	0
Clavicornia		
Enicmus sp.	2	10
Coccellinidae		
Micrapis sedecempuctata	0	1
Byrridae	3	1
Unidentified beetle larva	2	9
Total coleopteran	23	48

Appendix 3: List of arthropods collected in 2002 using sticky traps at Linyphiidae web sites in two treatments of winter wheat. Part 3.

Species	Non-compost web sites	Compost web sites
Thysanoptera	138	75
Acari	28	35
Araneae		
Linyphiidae spiderling	4	12
Meioneta rurestris ♂	1	0
Meioneta rurestris ♀	3	0
Pelecopsis parallela ♂	1	0
Erigoninae subadult ♂	2	1
Erigoninae subadult ♀	1	1
Erigone atra ♂	4	1
Erigone atra ♀	7	8
Erigone dentipalpis ♂	1	0
Erigone dentipalpis ♀	4	3
Oedothorax apicatus ♂	1	0
Oedothorax fuscus ♂	2	1
Oedothorax retusus ♂	0	3
Oedothorax sp. ♀	2	7
Oedothorax sp. subadult ♂	0	1
Tenuiphantes tenuis ♂	8	7
Tenuiphantes tenuis ♀	11	26
Tenuiphantes tenuis subadult ♂	9	3
Tenuiphantes tenuis subadult ♀	7	4
Bathyphantes gracilis ♂	9	2
Bathyphantes gracilis ♀	15	8
Bathyphantes gracilis subadult ♂	1	0
Bathyphantes gracilis subadult ♀	1	2
Pachygnatha degeeri ♀	0	1
Opiliones	0	1
Total Araneae	94	92
Total Arthropods captured	879	1292

Appendix 4: Gel pictures from the cross-reactivity testing and the feeding experiments. Lane numbers are indicated at the top of each lane.

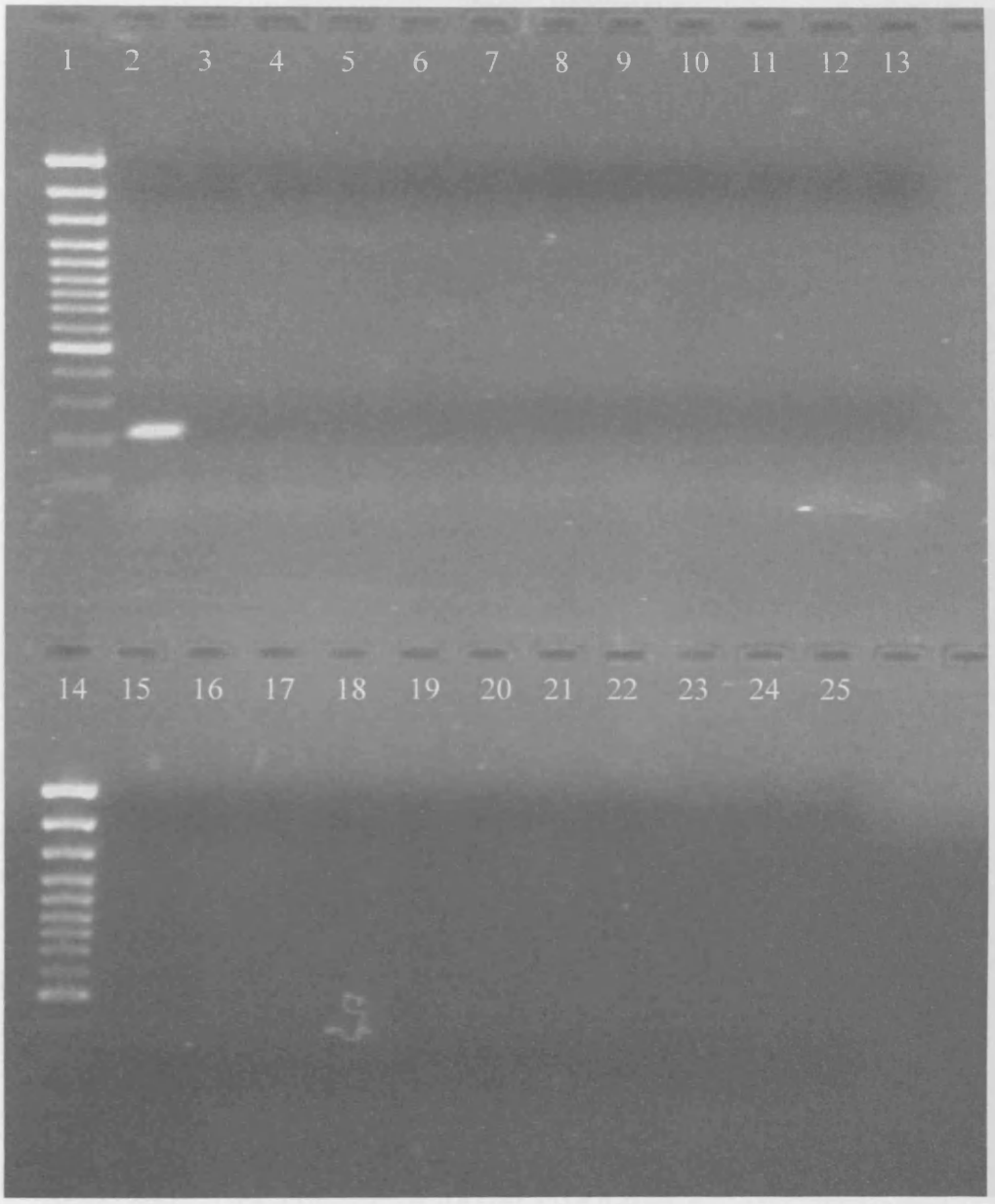

Figure 1: Agarose cross reactivity gel showing the amplification of DNA using primers L. castF1 and L.cast R1 optimized to amplify *Lycoriella castanescens*. Lane 1: 100 bp ladder, lane 2: *Lycoriella castanescens*, lane 3: *Bradysia confines*, lane 4: *B. triseriata*, lane 5: *Clinodoplosis* sp., lane 6: *Mayetola* sp., lane 7: *Putoniella* sp., lane 8: *Resseliella* sp., lane 9: *Peromyia* sp., lane 10: *Sitodiplosis mosellana*, lane 11: *Pteremis fenestralis*, lane 12: *Megaselia* sp., lane 13: *Campsicnemus curvipes*, lane 14: 100 bp ladder, lane 15: *Isotoma anglicana*, lane 16: *Lepidocyrtus cyanus*, lane 17: *Entomobrya multifasciata*, lane 18: *Sminthurus elegans*, lane 19: *Sitobion avenae*, lane 20: *Rhopalosiphum padi*, lane 21: *Metopolosiphum dirhodum*, lane 22: *Erigone atra*, lane 23: *E. dentipalpis*, lane 24 *Tenuiphantes tenuis*, lane 25: Blank (no DNA)

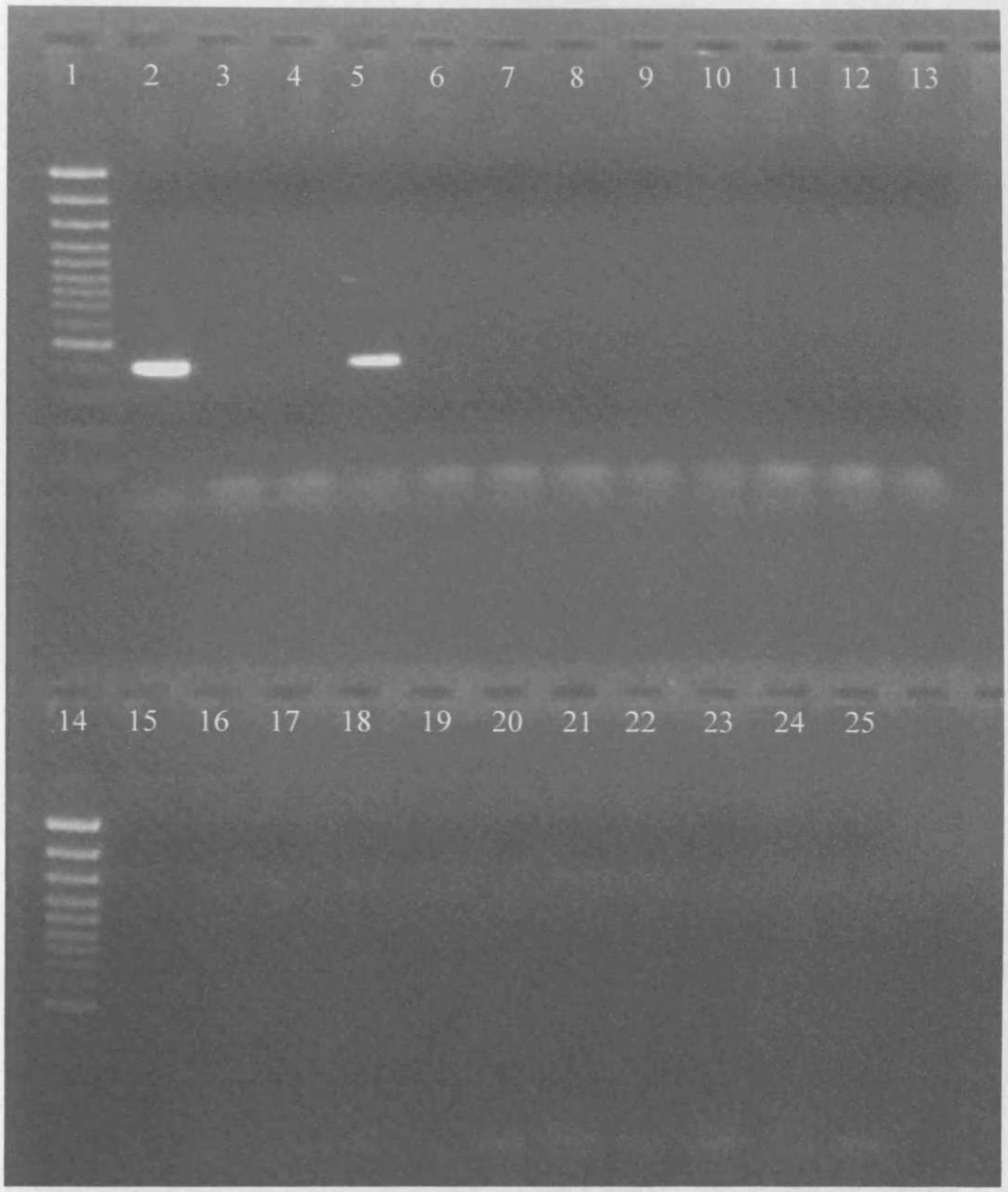

Figure 2: Agarose cross reactivity gel showing the amplification of DNA using primers CecidF1 and Cecid R1 optimized to amplify Cecidomyiidae.
Lane 1: 100 bp ladder, lane 2: *Sitodiplosis mosellana*, lane 3: *Clinodoplosis* sp., lane 4: *Mayetola* sp., lane 5: *Putoniella* sp., lane 6: *Resseliella* sp., lane 7: *Peromyia* sp., lane 8: *Lycoriella castanescens*, lane 9: *Bradysia confines*, lane 10: *B. triseriata*, lane 11: *Pteremis fenestralis*, lane 12: *Megaselia* sp., lane 13: *Campsicnemus curvipes*, lane 14: 100 bp ladder, lane 15: *Isotoma anglicana*, lane 16: *Lepidocyrtus cyanus*, lane 17: *Entomobrya multifasciata*, lane 18: *Sminthurus elegans*, lane 19: *Sitobion avenae*, lane 20: *Rhopalosiphum padi*, lane 21: *Metopolosiphum dirhodum*, lane 22: *Erigone atra*, lane 23: *E. dentipalpis*, lane 24 *Tenuiphantes tenuis*, lane 25: Blank (no DNA)

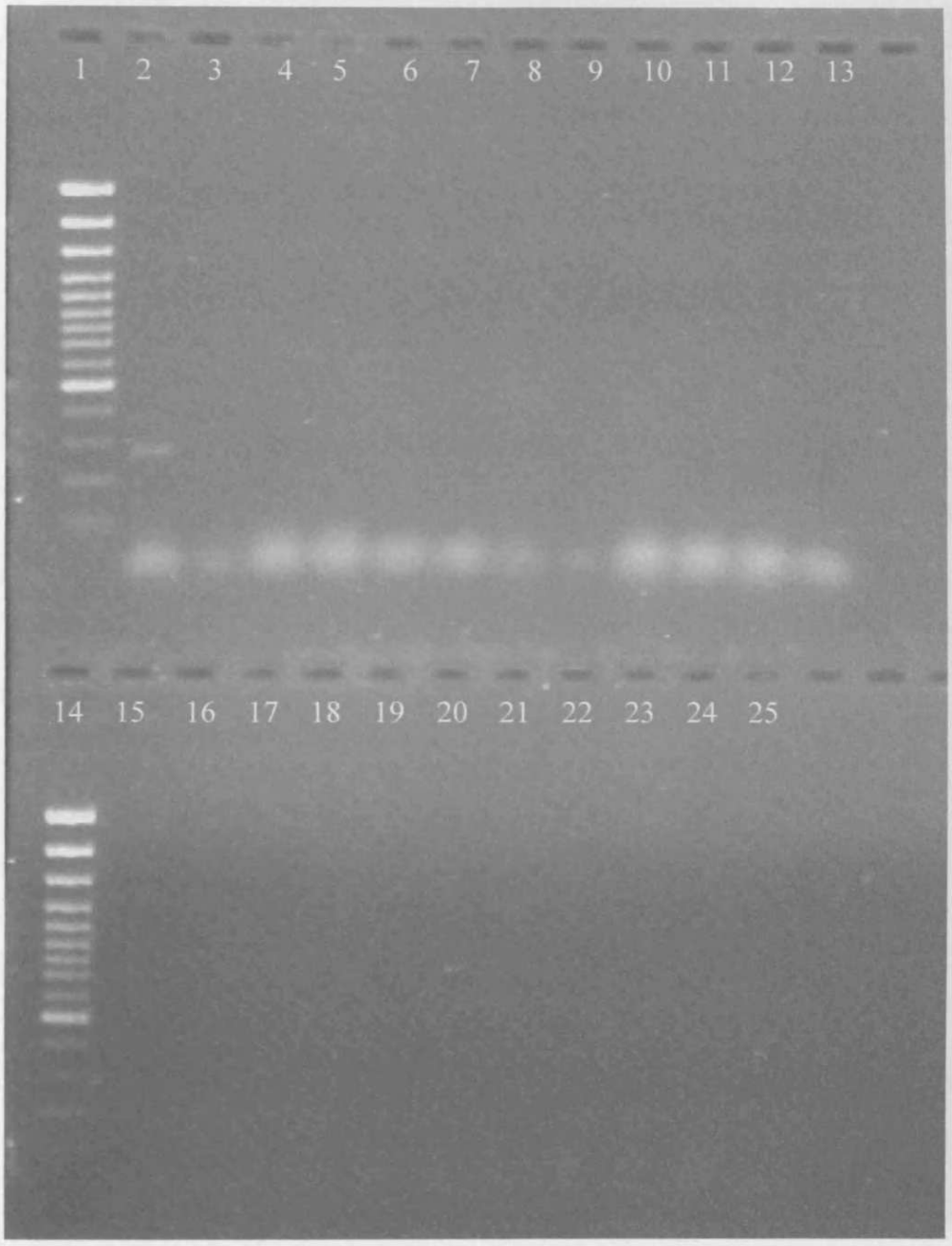

Figure 3: Agarose cross reactivity gel showing the amplification of DNA using primers Ia1F and Ia4R optimized to amplify *Isotoma anglicana*.
Lane 1: 100 bp ladder, lane 2: *Isotoma anglicana*, lane 3: *Lepidocyrtus cyanus*, lane 4: *Entomobrya multifasciata*, lane 5: *Sminthurus elegans*, lane 6: *Lycoriella castanescens*, lane 7: *Bradysia confines*, lane 8: *B. triseriata*, lane 9: *Clinodoplosis* sp., lane 10: *Mayetola* sp., lane 11: *Putoniella* sp., lane 12: *Resseliella* sp., lane 13: *Peromyia* sp., lane 14: 100 bp ladder, lane 15: *Sitodiplosis mosellana*, lane 16: *Pteremis fenestralis*, lane 17: *Megaselia* sp., lane 18: *Campsicnemus curvipes*, lane 19: *Sitobion avenae*, lane 20: *Rhopalosiphum padi*, lane 21: *Metopolosiphum dirhodum*, lane 22: *Erigone atra*, lane 23: *E. dentipalpis*, lane 24 *Tenuiphantes tenuis*, lane 25: Blank (no DNA)

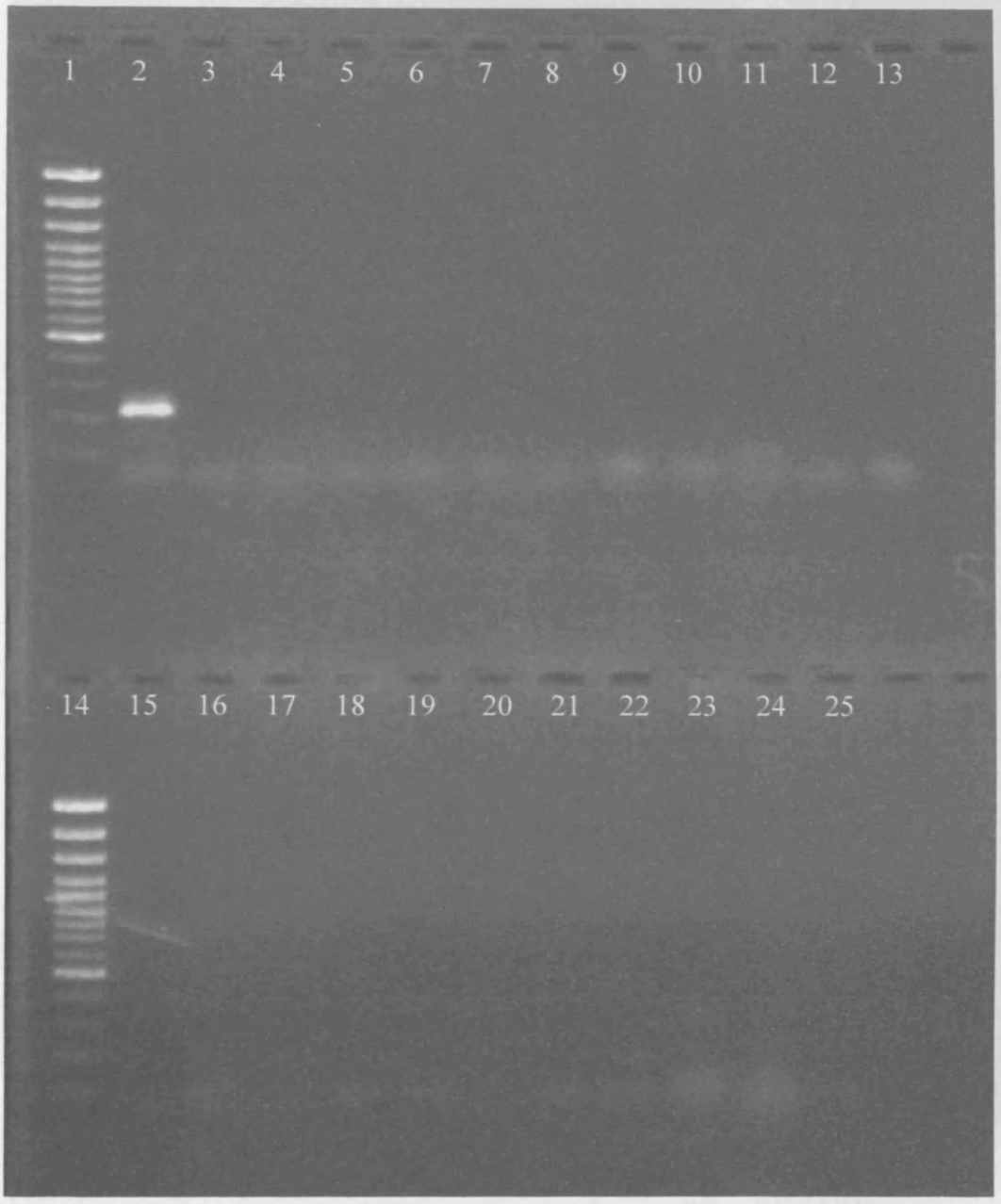

Figure 4: Agarose cross reactivity gel showing the amplification of DNA using primers Lc2F and Lc4R optimized to amplify *Lepidocyrtus cyanus*.
Lane 1: 100 bp ladder, lane 2: *Lepidocyrtus cyanus*, lane 3: *Isotoma* anglicana, lane 4: *Entomobrya multifasciata*, lane 5: *Sminthurus elegans*, lane 6: *Lycoriella castanescens*, lane 7: *Bradysia confines*, lane 8: *B. triseriata*, lane 9: *Clinodoplosis* sp., lane 10: *Mayetola* sp., lane 11: *Putoniella* sp., lane 12: *Resseliella* sp., lane 13: *Peromyia* sp., lane 14: 100 bp ladder, lane 15: *Sitodiplosis mosellana*, lane 16: *Pteremis fenestralis*, lane 17: *Megaselia* sp., lane 18: *Campsicnemus curvipes*, lane 19: *Sitobion avenae*, lane 20: *Rhopalosiphum padi*, lane 21: *Metopolosiphum dirhodum*, lane 22: *Erigone atra*, lane 23: *E. dentipalpis*, lane 24 *Tenuiphantes tenuis*, lane 25: Blank (no DNA)

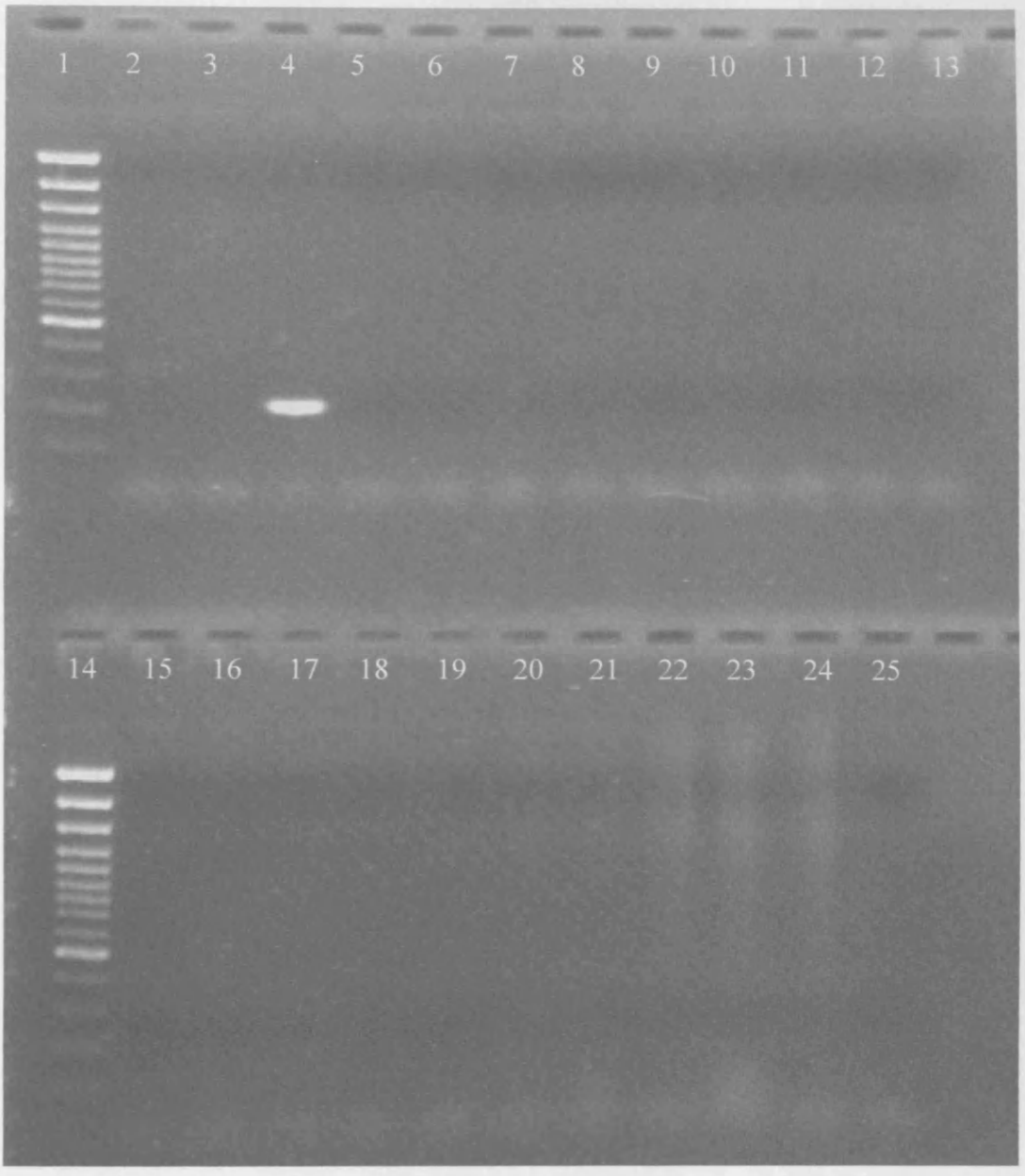

Figure 5: Agarose cross reactivity gel showing the amplification of DNA using primers Em1F and Em 3R optimized to amplify *Entomobrya multifasciata*..
Lane 1: 100 bp ladder, lane 2: *Lepidocyrtus cyanus*, lane 3: *Isotoma* anglicana, lane 4: *Entomobrya multifasciata*, lane 5: *Sminthurus elegans*, lane 6: *Lycoriella castanescens*, lane 7: *Bradysia confines*, lane 8: *B. triseriata*, lane 9: *Clinodoplosis* sp., lane 10: *Mayetola* sp., lane 11: *Putoniella* sp., lane 12: *Resseliella* sp., lane 13: *Peromyia* sp., lane 14: 100 bp ladder, lane 15: *Sitodiplosis mosellana*, lane 16: *Pteremis fenestralis*, lane 17: *Megaselia* sp., lane 18: *Campsicnemus curvipes*, lane 19: *Sitobion avenae*, lane 20: *Rhopalosiphum padi*, lane 21: *Metopolosiphum dirhodum*, lane 22: *Erigone atra*, lane 23: *E. dentipalpis*, lane 24 *Tenuiphantes tenuis*, lane 25: Blank (no DNA)

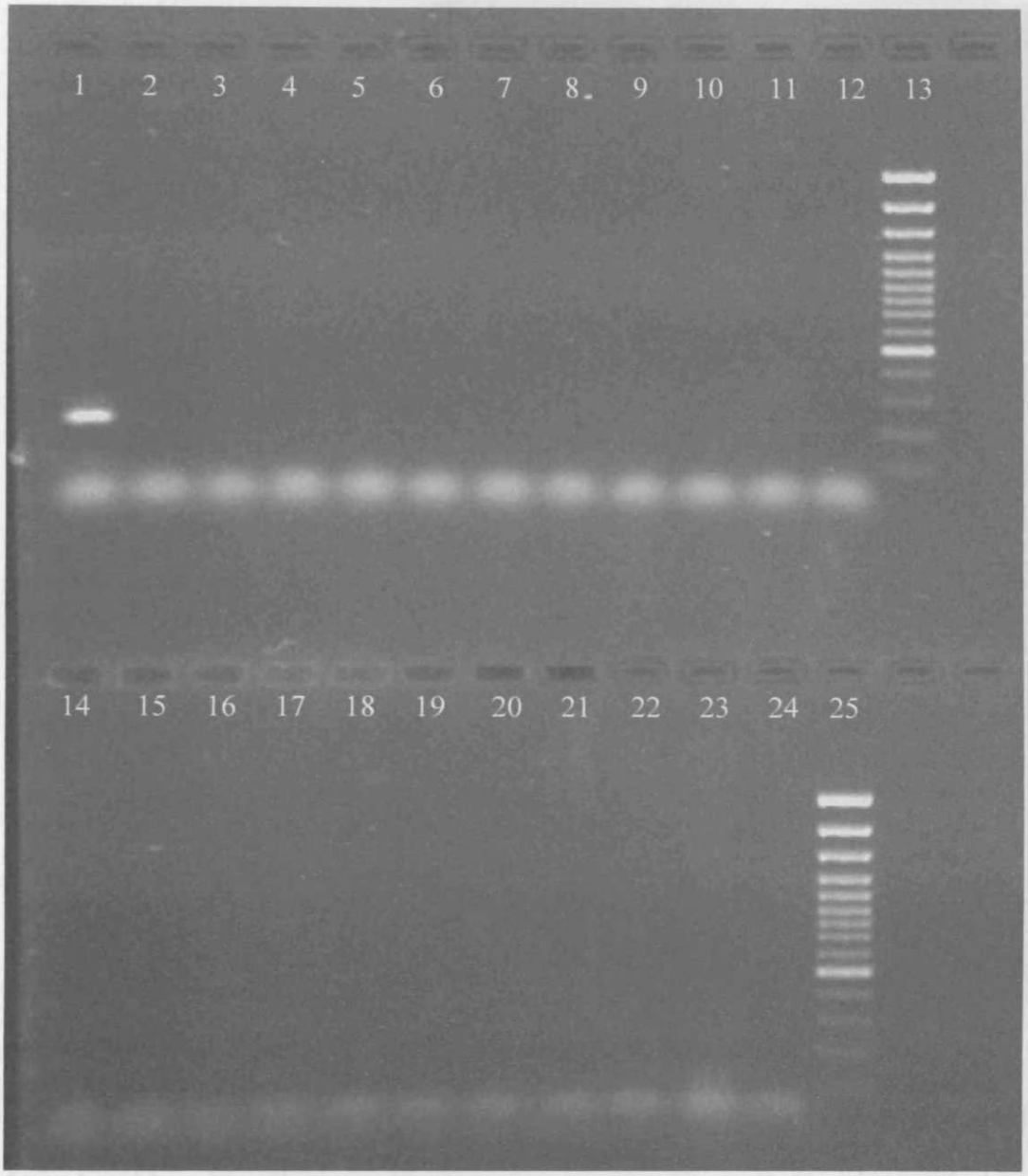

Figure 6: Agarose cross reactivity gel showing the amplification of DNA using primers EgaCOIIF1 and EgaCOIIR optimized to amplify *Sitobion avenae*. Lane 1: *Sitobion avenae*, lane 2: *Rhopalosiphum padi*, lane 3: *Metopolosiphum dirhodum,* lane 4: *Isotoma anglicana*, lane 5: *Lepidocyrtus cyanus*, lane 6: *Entomobrya multifasciata*, lane 7: *Sminthurus elegans*, lane 8: *Lycoriella castanescens*, lane 9: *Bradysia confines*, lane 10: *B. triseriata*, lane 11: *Clinodoplosis* sp., lane 12: *Mayetoloa* sp., lane 13: 100 bp ladder, lane 14: *Putoniella* sp., lane 15: *Resseliella* sp., lane 16: *Peromyia* sp., lane 17: *Sitodiplosis mosellana*, lane 18: *Pteremis fenestralis*, lane 19: *Megaselia* sp., lane 20: *Campsicnemus curvipes*, lane 21: *Erigone atra*, lane 22: *E. dentipalpis*, lane 23 *Tenuiphantes tenuis*, lane 24: Blank (no DNA), lane 25: 100 bp ladder

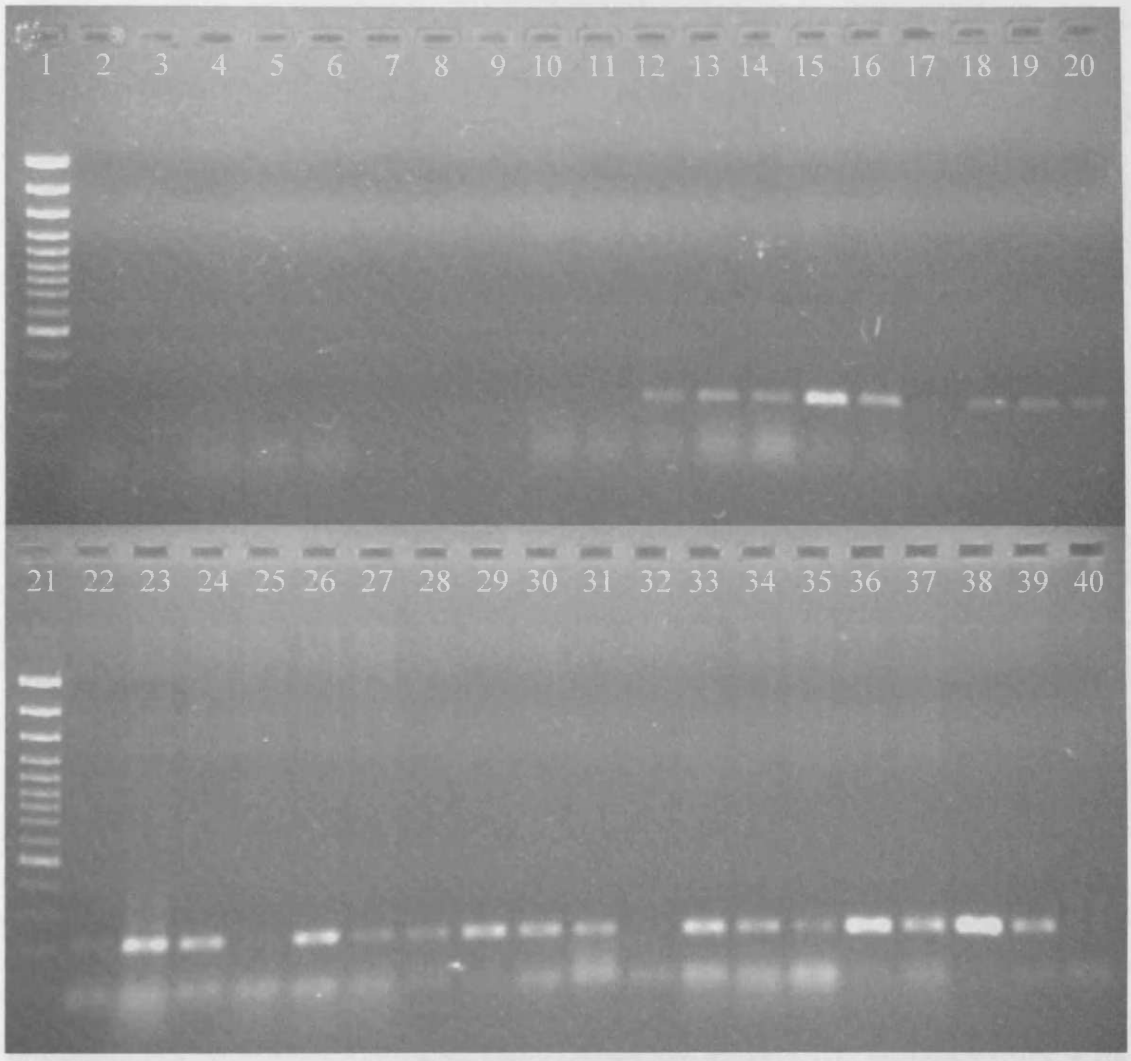

Figure 7 (part 1): Agarose gel showing the results of the *Lycoriella castanescens* DNA decay rate experiment. Each lane represents DNA extracts from individual *Tenuiphantes tenuis* from which prey DNA was amplified using the primers L.castF1 and L.castR1. Time since ingestion is stated in hours.
Lane 1 and lane 21: 100 bp ladder, lanes 2-6: starved female *T. tenuis* controls, lanes 7-11: starved male *T. tenuis* controls, lanes 12-16: 0 hrs female *T. tenuis*, lanes 17-20 and 22: 0 hrs male *T. tenuis*, lanes 23-27: 2 hrs female *T. tenuis*, lanes 28-32: 2 hrs male *T. tenuis*, lanes 33-37: 4 hrs female *T. tenuis*, lanes 38-40: 4 hrs male *T. tenuis*.

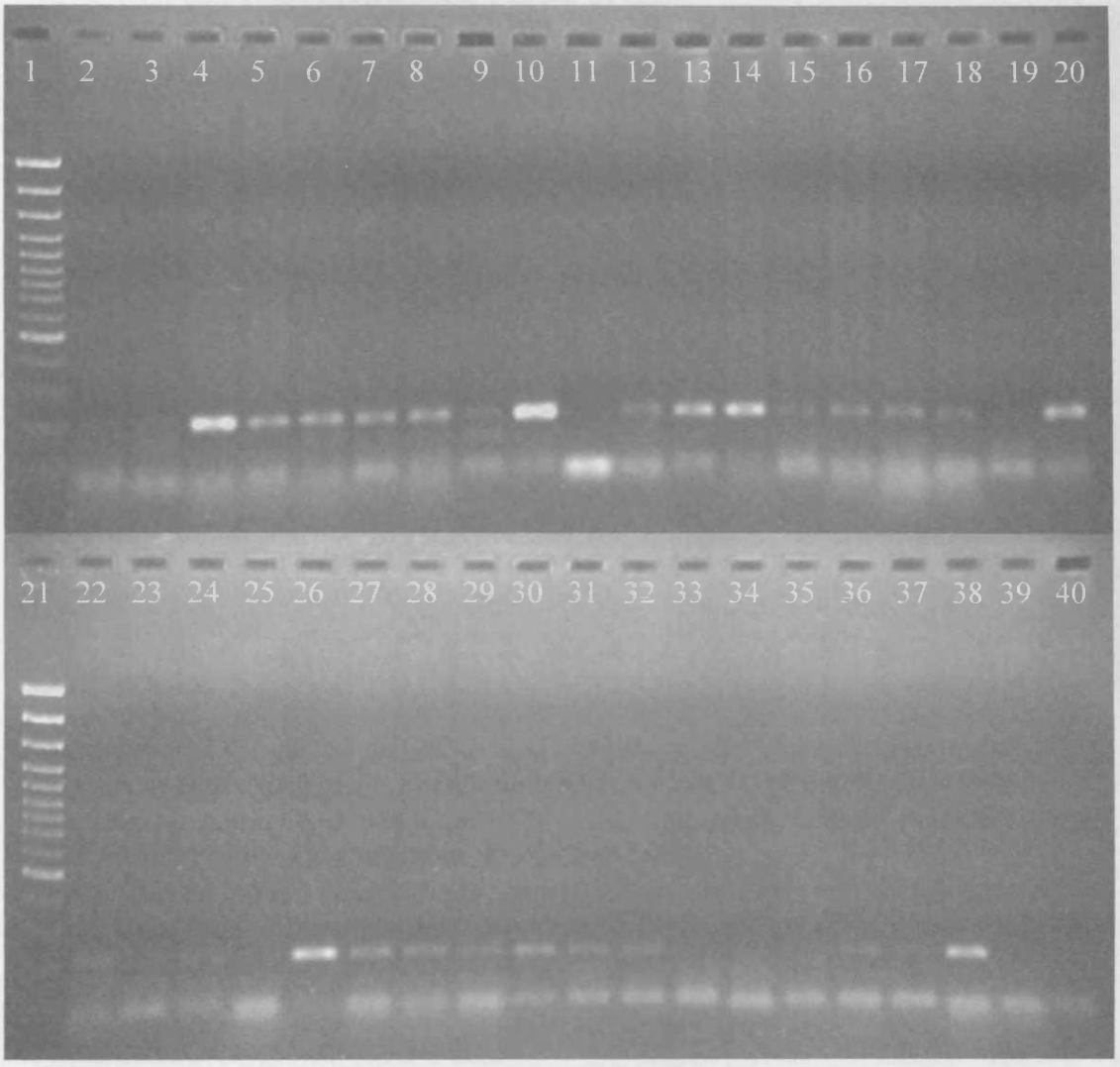

Figure 7 (part 2): Agarose gel showing the results of the *Lycoriella castanescens* DNA decay rate experiment. Each lane represents DNA extracts from individual *Tenuiphantes tenuis* from which prey DNA was amplified using the primers L.castF1 and L.castR1. Time since ingestion is stated in hours.
Lane 1 and lane 21: 100 bp ladder, lanes 2-3: 4 hrs male *T. tenuis* controls, lanes 4-8: 8 hrs female *T. tenuis* controls, lanes 9-13: 8 hrs male *T. tenuis*, lanes 14-18: 12 hrs female *T. tenuis*, lanes 19-20 and 22-24: 12 hrs male *T. tenuis*, lanes 25-29: 24 hrs female *T. tenuis*, lanes 30-34: 24 hrs male *T. tenuis*, lanes 35-39: 36 hrs female *T. tenuis*, lane 40: 36 hrs male *T. tenuis*.

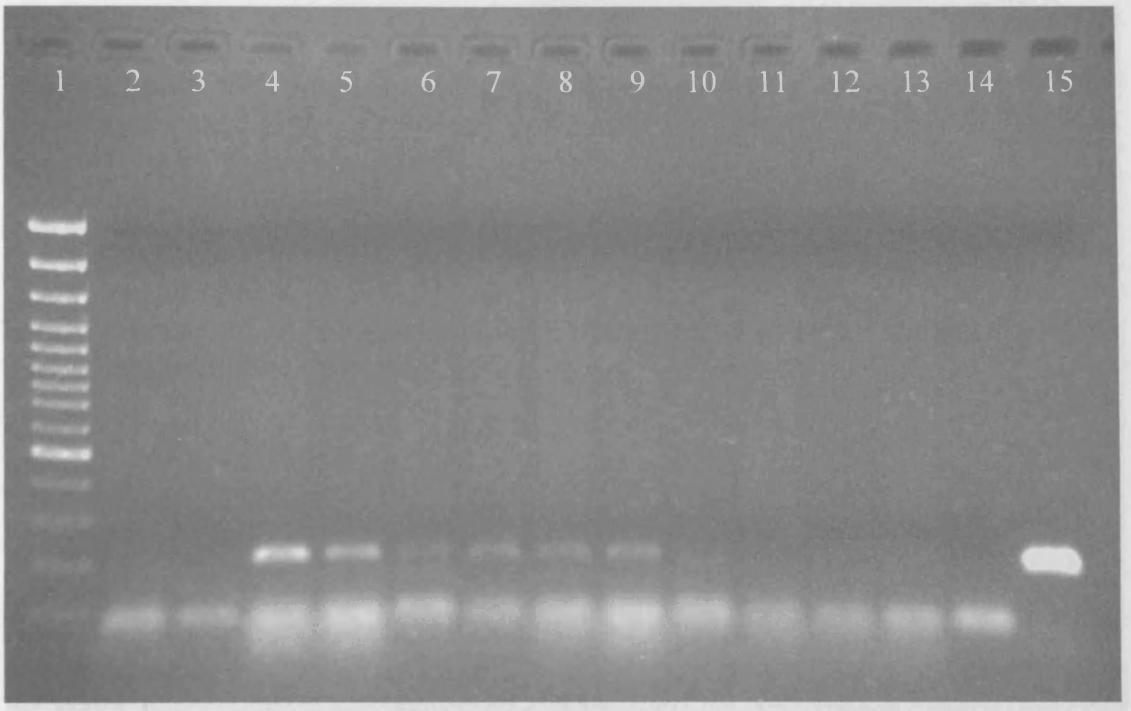

Figure 7 (part 3): Agarose gel showing the results of the *Lycoriella castanescens* DNA decay rate experiment. Each lane represents DNA extracts from individual *Tenuiphantes tenuis* from which prey DNA was amplified using the primers L.castF1 and L.castR1. Time since ingestion is stated in hours.
Lane 1: 100 bp ladder, lanes 2-3: 36 hrs male *T. tenuis*, lanes 4-8: 48 hrs female *T. tenuis*, lanes 9-13: 72 hrs female *T. tenuis*, lane 14: blank (no DNA), lane 15: positive control (*L. castanescens* DNA extract).

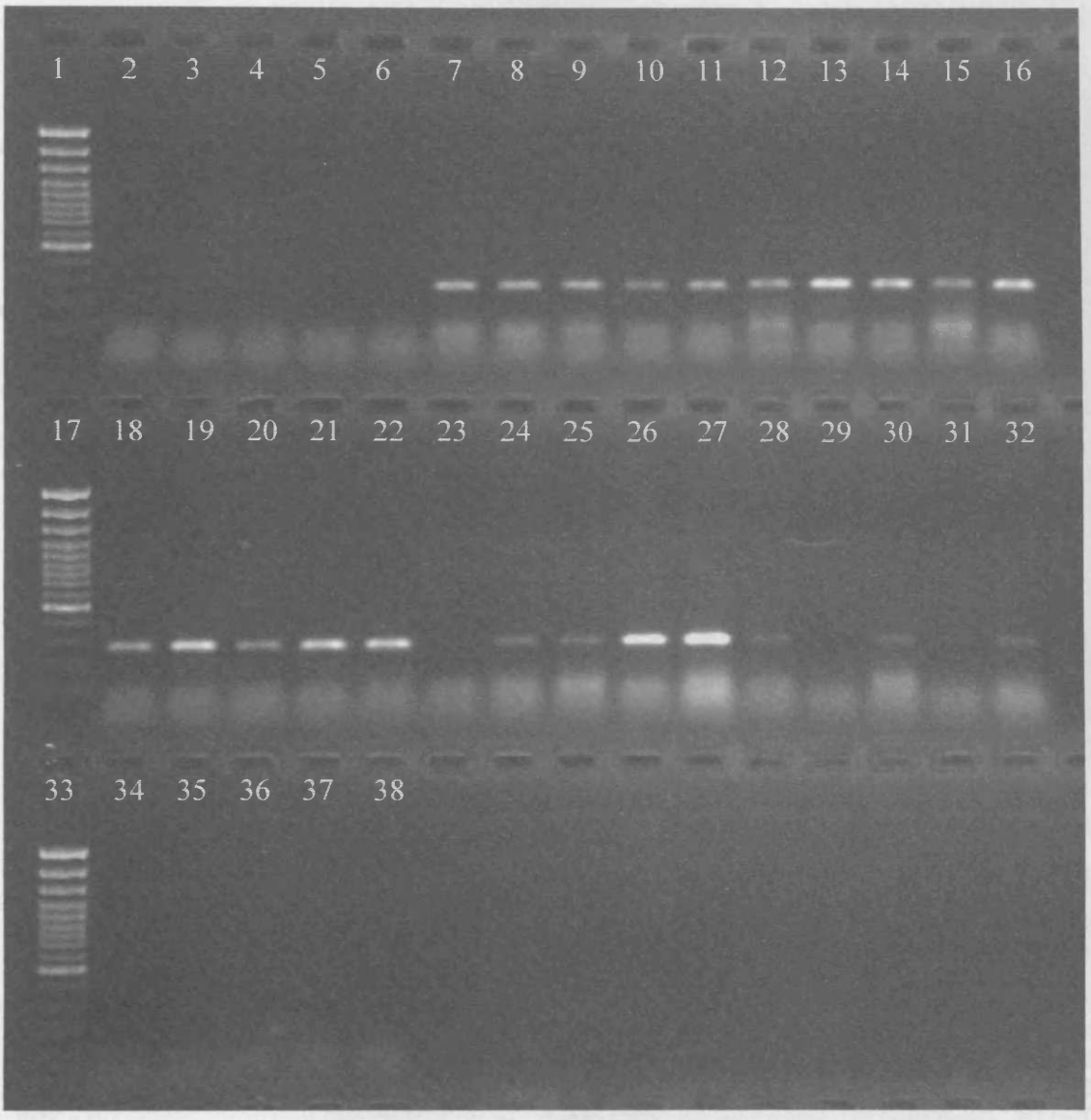

Figure 8: Agarose gel showing the results of the *Isotoma anglicana* DNA decay rate experiment. Each lane represents DNA extracts from individual female *Erigone atra* from which prey DNA was amplified using the primers Ia1F and Ia4R. Time since ingestion is stated in hours.
Lanes 1, 17 and 33: 100 bp ladder, lanes 2-6: starved *E. atra* controls, lanes 7-11: 0 hrs since ingestion, lanes 12-16: 12 hrs since ingestion, lanes 18-22: 24 hrs since ingestion, lanes 23-27: 36 hrs since ingestion, lanes 28-32: 48 hrs since ingestion, lanes 34-38: 72 hrs since ingestion.

Appendix 5: Cytochrome Oxidase I sequence alignment data. Primer binding sites are shown ir bold red type.

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                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                   10         20         30         40         50
Tenuiphantes tenuis       --GGAGGATT TGGAAATTGA TTAGTTCCTT TAATATTAGG GGCTCCAGAT
Erigone dentipalpis       --GGAGGATT TGGAAATTGA TTAGTTCCTT TAATATTAGG GGCTCCTGAT
E. atra                   --GGAGGATT TGGAAATTGA TTAGTTCCTT TAATGTTAGG GGCTCCTGAT
Sitobion avenae           --GGAGGATT TGGAAATTGA TTAGTTCCTA TAATAATAGG ATGTCCTGAT
Metopolosiphum dirhodum   ---------- ---------- ----TTCCTA TAATAATAGG TTGCCCTGAT
Rhopalosiphum padi        ----AGGATT TGGAAATTGA TTAGTTCCTA TAATAATAGG ATGCCCTGAT
Isotoma anglicana         --------TT TGGAAATTGA TTAGTTCCTT TAATAATTGG AGCGCCGGAT
Campsicnemus curvipes     ---------- TGGAAATTGA TTAGTTCCTT TAATACTAGG AGCCCCTGAC
Pteremis fenestralis      --GGAGGATT TGGAAATTGA TTAGTTCCCC TAATACTAGG AGCCCCAGAT
Megaselia sp.             -------ATT TGGAAATTGA TTAGTTCCTT TAATATTAGG AGCTCCTGAT
Sitodiplosis mosellana    ---------- -GGAAATTGA TTAGTTCCTA TTATACTAGG AGCC**CCAGAT**
Putoniella sp.            ---------- ---------- ---------- -----CTAGG AGCC**CCAGAT**
Unidentified Cecidomyiidae ---------- -GGAAATTGA TTAGTTCCAT TAATATTAGG AGCC**CCCGAT**
Bradysia difformis        TGGAGGA-TT TGGAAATTGA TTAGTTCCTT TAATATTATC GGCCCCTGAT
Lycoriella castanecens    TGGAGGAATT TGGAAATTGA TTAGTTCCAT TAATATTAAG AGCCC**CAGAT**
L.castF1                  ---------- ---------- ---------- ---------- -----CAGAT
L.castF2                  ---------- ---------- ---------- ---------- ----------
L.castR1                  ---------- ---------- ---------- ---------- ----------
L.castR2                  ---------- ---------- ---------- ---------- ----------
L.castR3                  ---------- ---------- ---------- ---------- ----------
CecidF1                   ---------- ---------- ---------- ---------- ----CCCGAT
CecidF4                   ---------- ---------- ---------- ---------- ----------
CecidR2                   ---------- ---------- ---------- ---------- ----------
CecidR3                   ---------- ---------- ---------- ---------- ----------


                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                   60         70         80         90        100
Tenuiphantes tenuis       ATAGCTTTTC CTCGAATGAA TAATTTAAGA TTTTGGTTAC TTCCTCCTTC
Erigone dentipalpis       ATAGCTTTTC CTCGTATAAA TAATTTAAGA TTTTGATTAT TACCCCCTTC
E. atra                   ATGGCATTTC CTCGTATAAA TAATTTGAGA TTTTGGCTAT TACCTCCTTC
Sitobion avenae           ATATCTTTCC CACGATTAAA TAACATTAGA TTCTGATTAT TACCACCCTC
Metopolosiphum dirhodum   ATATCATTCC CACGTTTAAA TAACATTAGA TTTTGATTAT TACCCCCATC
Rhopalosiphum padi        ATATCATTTC CACGATTAAA TAATATTAGA TTTTGACTAT TACCCCCTTC
Isotoma anglicana         ATGGCCTTCC CCCGAATAAA TAATATAAGA TTTTGACTTC TTCCCCCGTC
Campsicnemus curvipes     ATAGCCTTCC CTCGAATAAA TAATATAAGT TTTTGAATAT TACCCCCTTC
Pteremis fenestralis      ATAGCCTTTC CTCGAATAAA TAATATAAGA TTTTGATTAC TGCCTCCTTC
Megaselia sp.             ATAGCATTTC CTCGAATAAA TAATATAAGT TTTTGAATAC TTCCTCCTTC
Sitodiplosis mosellana    **ATAGCATTTC CACG**AATAAA TAATATAAGA TTTTGATTAT TACCTCCATC
Putoniella sp.            **ATAGCATTTC CACG**AATAAA TAATATAAGA TTTTGATTAT TACCCCCATC
Unidentified Cecidomyiidae **ATAGCATTTC CACG**AATAAA TAACATAAGA TTTTGATTGT TACCCCCATC
Bradysia difformis        ATAGCATTCC CACGACTAAA TAATATAAGA TTTTGATTAT TGCCACCCTC
Lycoriella castanecens    **ATAGCATTCC CCCGTT**TAAA TAATATAAGA TTTTGACTTC TACCTCCGTC
L.castF1                  ATAGCATTCC CCCGTT---- ---------- ---------- ----------
L.castF2                  ---------- ---------- ---------- ---------- ----------
L.castR1                  ---------- ---------- ---------- ---------- ----------
L.castR2                  ---------- ---------- ---------- ---------- ----------
L.castR3                  ---------- ---------- ---------- ---------- ----------
CecidF1                   ATAGCATTTC CACG------ ---------- ---------- ----------
CecidF4                   ---------- ---------- ---------- ---------- ----------
CecidR2                   ---------- ---------- ---------- ---------- ----------
CecidR3                   ---------- ---------- ---------- ---------- ----------
```

Appendix 5 (part 2)

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                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                        110        120        130        140        150
Tenuiphantes tenuis             ATTATTAATA TTATTTATTT CTTCAATAGT AGAAATAGGA GTTGGAGCAG
Erigone dentipalpis             TTTATTATTA TTATTTATCT CTAGAATAGA TGAAATAGGT GTAGGGGCGG
E. atra                         TTTATTTTTA CTTTTTATTT CTAGAATAGA TGAGATAGGA GTAGGTACTG
Sitobion avenae                 ATTAATAATA ATAATTTGTA GTTTTTTAAT TAATAATGGA ACAGGAACAG
Metopolosiphum dirhodum         ATTAATAATA ATAATTTGTA GTTTTTTAAT TAATAATGGA ACAGGAACAG
Rhopalosiphum padi              ATTAATAATA ATAATTTGTA GTTTTATAAT TAATAACGGA ACAGGAACAG
Isotoma anglicana               TTTAACTCTT CTATTGGCCG GAGGACTTGT TGAAAGAGGA GCAGGAACAG
Campsicnemus curvipes           AATTACTCTT TTATTAGCTA GAAGAATAGT AGAAAACGGA GCTGGAACAG
Pteremis fenestralis            CCTTACTTTA CTTTTAGTGA GCAGTATAGT GGAAAATGGA GCTGGTACAG
Megaselia sp.                   TTTAACTCTT TTATTAGCCA GTAGTATAGT AGAAAATGGA GCTGGAACTG
Sitodiplosis mosellana          TTTATCTTTA TTATTAATTA GAAGAATAGT AGAAACTGGA ACCGGAACTG
Putoniella sp.                  TTTATCTTTA TTATTAATTA GAAGAATAGT AGAAACTGGA ACAGGAACTG
Unidentified Cecidomyiidae      TCTCTCCTTA TTATTAATAA GAGGCTTAGT AGAATCAGGA ACAGGAACTG
Bradysia difformis              TTTAACACTT TTATTAACTA GAAGGTTAGT AGAAAGAGGT ACAGGTACTG
Lycoriella castanecens          TCTTACCCTT TTATTAACTA GAAGAATAGT AGAAAGAGGT ACAGGAACAG
L.castF1                        ---------- ---------- ---------- ---------- ----------
L.castF2                        ---------- ---------- ---------- ---------- ----------
L.castR1                        ---------- ---------- ---------- ---------- ----------
L.castR2                        ---------- ---------- ---------- ---------- ----------
L.castR3                        ---------- ---------- ---------- ---------- ----------
CecidF1                         ---------- ---------- ---------- ---------- ----------
CecidF4                         ---------- ---------- ---------- ---------- ----------
CecidR2                         ---------- ---------- ---------- ---------- ----------
CecidR3                         ---------- ---------- ---------- ---------- ----------


                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                        160        170        180        190        200
Tenuiphantes tenuis             GTTGAACTGT TTATCCTCCT TTAGCTTCTT TAGAAGGGCA TTCAGGGAGA
Erigone dentipalpis             GATGAACAAT TTATCCTCCT CTAGCTTCTT TAGAGGGTCA TTCTGGTAGT
E. atra                         GGTGAACTGT TTATCCTCCT ATTGCTTCTT TAGAAGGTCA TGCTGGTAGA
Sitobion avenae                 GATGAACTAT TTACCCACCC TTATCAAATA ATATTGCACA TAATAATATT
Metopolosiphum dirhodum         GATGAACTAT TTATCCACCT TTATCAAATA ACATTGCACA TAACAATATT
Rhopalosiphum padi              GATGAACAAT TTATCCTCCT TTATCTAATA ATATTGCTCA TAATAATATT
Isotoma anglicana               GATGAACAGT GTACCCTCCG CTTTCTTCAG GTATCGCGCA TGCTGGAGCA
Campsicnemus curvipes           GATGAACTGT TTATCCACCT TTATCTGCAG GAATTGCTCA TGGAGGAGCT
Pteremis fenestralis            GATGAACTGT TTACCCTCCT CTATCCTCTG GCATTGCACA TGGAGGAGCT
Megaselia sp.                   GTTGAACAGT TTACCCTCCT CTTTCTTCTA GTATTGCTCA TAGTGGAGCC
Sitodiplosis mosellana          GTTGAACAGT TTATCCACCC CTTTCATCTA TTATTGCTCA TACAGGATCA
Putoniella sp.                  GTTGAACAGT TTATCCACCC CTTTCATCTA TTATTGCTCA TACAGGATCA
Unidentified Cecidomyiidae      GGTGAACTGT CTATCCCCCA CTATCTTCCA CAATTGCTCA TAGAGGAGCA
Bradysia difformis              GATGAACTGT TTATCCTCCA TTATCATCAA CAATTGCTCA TTCAGGGGCC
Lycoriella castanecens          GATGAACAGT TTATCCTCCC CTATCTTCTA CTTTAGCTCA TTCAGGGGCT
L.castF1                        ---------- ---------- ---------- ---------- ----------
L.castF2                        ---GAACAGT TTATCCTCCC CTATC----- ---------- ----------
L.castR1                        ---------- ---------- ---------- ---------- ----------
L.castR2                        ---------- ---------- ---------- ---------- ----------
L.castR3                        ---------- ---------- ---------- ---------- ----------
CecidF1                         ---------- ---------- ---------- ---------- ----------
CecidF4                         ---------- ---------- ---------- --------CA TACAGGATCA
CecidR2                         ---------- ---------- ---------- ---------- ----------
CecidR3                         ---------- ---------- ---------- ---------- ----------
```

Appendix 5 (part 3)

```
                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                        210        220        230        240        250
Tenuiphantes tenuis             TCTGTGGATT TTGCTATTTT TTCTTTACAT TTAGCTGGGG CTTCGTCAAT
Erigone dentipalpis             TCTGTTGATT TTGCAATTTT CTCTTTACAC TTAGCTGGTG CTTCTTCTAT
E. atra                         TCTGTTGATT TTGCTATTTT TTCTTTACAT TTAGCTGGTG CTTCATCAAT
Sitobion avenae                 TCAGTTGATT TAACTATTTT TTCATTACAT TTAGCAGGAA TTTCATCAAT
Metopolosiphum dirhodum         TCAGTTGATT TAACTATTTT TTCATTACAT TTAGCAGGAA TCTCCTCAAT
Rhopalosiphum padi              TCAGTTGATT TAACAATTTT TTCTCTACAT TTAGCAGGAA TCTCATCAAT
Isotoma anglicana               TCTGTGGACT TATCTATTTT TAGTTTACAT TTAGCAGGAG CGTCTTCTAT
Campsicnemus curvipes           TCTGTTGATT TAGCAATTTT TTCTCTTCAT CTTGCCGGAA TTTCTTCTAT
Pteremis fenestralis            TCAGTAGATT TAGCTATTTT TTCTTTACAT TTAGCTGGAA TTTCTTCTAT
Megaselia sp.                   TCAGTTGATT TAGCTATTTT CTCCCTTCAT CTAGCTGGTA TTTCTTCAAT
Sitodiplosis mosellana          TCAGTAGATT TTTCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCTTCAAT
Putoniella sp.                  TCAGTAGATT TTTCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCTTCAAT
Unidentified Cecidomyiidae      TCTGTGGATT TGTCTATTTT TTCTTTGCAT TTAGCAGGAA TTTCTTCTAT
Bradysia difformis              TCTGTTGATC TATCAATTTT TTCTCTTCAT TTAGCAGGAA TTTCTTCAAT
Lycoriella castanecens          TCCGTAGATT TATCTATTTT TTCTTTACAT TTAGCGGGTA TTTCCTCAAT
L.castF1                        ---------- ---------- ---------- ---------- ----------
L.castF2                        ---------- ---------- ---------- ---------- ----------
L.castR1                        ---------- ---------- ---------- -----GGGTA TTTCCTCAAT
L.castR2                        ---------- ---------- ---------- ---------- ---CCTCAAT
L.castR3                        ---------- ---------- ---------- ---------- ----------
CecidF1                         ---------- ---------- ---------- ---------- ----------
CecidF4                         TCAGTAGA-- ---------- ---------- ---------- ----------
CecidR2                         ---------- ---------- ---------- ---------- ----------
CecidR3                         ---------- ---------- ---------- ---------- ----------

                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                        260        270        280        290        300
Tenuiphantes tenuis             TATAGGGGCT ATTAATTTTA TTTCGACAAT TATAAATATA CGAGCTTATG
Erigone dentipalpis             TATAGGGGCT ATTAATTTTA TTTCTACAAT TTTAAATATG CGTGGGTATG
E. atra                         TATGGGGGCT ATTAATTTTA TTTCTACTAT TTTAAATATA CGTGGTTATG
Sitobion avenae                 TTTAGGAGCA ATTAATTTTA TTTGTACAAT CTTAAATATA ATACCAAACA
Metopolosiphum dirhodum         TTTAGGAGCA ATTAACTTTA TTTGTACAAT TCTTAATATA ATACCAAATA
Rhopalosiphum padi              TTTAGGGGCA ATTAATTTTA TTTGTACAAT TTTAAATATA ATACCTAATA
Isotoma anglicana               TTTAGGGGCT GTAAACTTTA TTACAACAAT TATTAATATA CGAGCCGTTG
Campsicnemus curvipes           TTTAGGAGCT GTAAATTTTA TTACAACAGT TATTAATATA CGATCAACAG
Pteremis fenestralis            TTTAGGTGCC GTAAATTTCA TTACAACAGT AATTAATATA CGCTCTACTG
Megaselia sp.                   TTTAGGAGCT GTAAATTTTA TTACAACAAT CATTAATATA CGGTCATCTG
Sitodiplosis mosellana          TTTAGGAGCT ATTAATTTTA TTTCAACTAT ATTAAATATA AAAATTAAAT
Putoniella sp.                  TTTAGGAGCT ATTAATTTTA TTTCAACTAT ATTAAATATA AAAATTAAAT
Unidentified Cecidomyiidae      TTTAGGAGCA ATTAATTTTA TTACAACAAT AATCAATATA CGTGTTAAAA
Bradysia difformis              TTTAGGTGCA GTAAATTTTA TTTCTACAAT TATTAATATA CGAGCCCCAG
Lycoriella castanecens          CTTGGGGGCA GTAAATTTTA TTTCCACTAT TATTAATATA CGAGCCCCTG
L.castF1                        ---------- ---------- ---------- ---------- ----------
L.castF2                        ---------- ---------- ---------- ---------- ----------
L.castR1                        CTTGGG---- ---------- ---------- ---------- ----------
L.castR2                        CTTGGGGGCA GTAA------ ---------- ---------- ----------
L.castR3                        ---------- ---------- ---------- ---------- ----------
CecidF1                         ---------- ---------- ---------- ---------- ----------
CecidF4                         ---------- ---------- ---------- ---------- ----------
CecidR2                         ---------- ---------- ---------- ---------- ----------
CecidR3                         ---------- ---------- ---------- ---------- ----------
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Appendix 5 (part 4)

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                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                  310        320        330        340        350
Tenuiphantes tenuis       ATATCAGAAT AGAAAAGGTT TCTTTATTTG TTTGATCAGT ATTGATTACT
Erigone dentipalpis       GGATAACTAT AGAAAAAGTT CCTTTATTTG TATGGTCTGT TTTAATTACA
E. atra                   GAATAACTAT AGAAAAGGTT CCTTTATTTG TTTGATCTGT GCTTATTACA
Sitobion avenae           ATATAAAATT AAACCAAATC CCTTTATTTC CATGATCAAT TTTAATTACA
Metopolosiphum dirhodum   ATATAAAATT AAATCAAATC CCTCTTTTCC CTTGATCAAT TTTAATTACA
Rhopalosiphum padi        ATATAAAATT AAACCAAATT CCATTATTCC CTTGATCAAT TTTAATTACA
Isotoma anglicana         GAATATCATG AGATCGAACC CCTTTATTTG TGTGGTCAGT ATTTTTAACA
Campsicnemus curvipes     GAATTACTTT TGATCGAATA CCATTATTTG TTTGATCTGT TGTTATTACA
Pteremis fenestralis      GAATTACTTT TGACCGAATA CCTTTATTTG TTTGATCAGT AGTAATTACA
Megaselia sp.             GAATTACATT TGATCGAATA CCTTTATTTG TTTGATCAGT AGGAATCACT
Sitodiplosis mosellana    TTTTAAAATT TGATCAAATT TCATTATTTG TTTGATCAAT TTTAATTACA
Putoniella sp.            TTTTAAAATT TGATCAAATT TCATTATTTG TTTGATCAAT TTTAATTACA
Unidentified Cecidomyiidae TAATTAAATT TGATCAAATA CCTTTATTTT CTTGATCTGT ATTAATTACT
Bradysia difformis        GAATATCTTT TGATAAATTA CCTTTATTTA CTTGATCTGT TTTAATTACA
Lycoriella castanecens    GAATATCTTT TGATAAAATA CCATTATTTA TTTGATCAGT TTTAATTACT
L.castF1                  ---------- ---------- ---------- ---------- ----------
L.castF2                  ---------- ---------- ---------- ---------- ----------
L.castR1                  ---------- ---------- ---------- ---------- ----------
L.castR2                  ---------- ---------- ---------- ---------- ----------
L.castR3                  ---------- ---------- ---------- ---------- ----------
CecidF1                   ---------- ---------- ---------- ---------- ----------
CecidF4                   ---------- ---------- ---------- ---------- ----------
CecidR2                   ---------- ---------- ---------- ---------- ----------
CecidR3                   ---------- ---------- ---------- ---------- ----------


                          ....|....| ....|....| ....|....| ....|....| ....|....|
                                  360        370        380        390        400
Tenuiphantes tenuis       GCAGTTTTAT TATTATTATC TTTACCTGTT TTAGCAGGAG CTATTACTAT
Erigone dentipalpis       GCCGTATTGT TATTATTATC TTTACCTGTG CTTGCAGGAG CTATCACCAT
E. atra                   GCTGTTTTAC TTTTATTGTC TTTACCTGTG TTAGCTGGGG CTATTACTAT
Sitobion avenae           GCTATTTTAT TAATTTTATC TTTACCTGTT CTAGCAGGTG CTATTACAAT
Metopolosiphum dirhodum   GCTATTTTAT TAATTTTATC TTTACCAGTA TTAGCTGGTG CTATTACAAT
Rhopalosiphum padi        GCTATATTAT TAATTTTATC TTTACCTGTT TTAGCTGGTG CAATTACTAT
Isotoma anglicana         GCAATTTTAC TTTTACTATC ACTTCCTGTG CTAGCGGGGG CAATTACTAT
Campsicnemus curvipes     GCTATTTTAT TATTACTTTC ATTACCTGTT TTAGCTGGAG CTATTACTAT
Pteremis fenestralis      GCTTTATTAC TACTTTTATC TTTACCCGTA TTAGCAGGAG CTATTACAAT
Megaselia sp.             GCTCTTCTAT TATTATTATC TTTACCTGTT TTAGCTGGAG CTATTACTAT
Sitodiplosis mosellana    ACAGTTTTAC TTTTATTATC ATTACCAGTA TTAGCAGGAG CAATTACTAT
Putoniella sp.            ACAGTTTTAC TTTTATTATC ATTACCAGTA TTAGCAGGAG CAATTACTAT
Unidentified Cecidomyiidae GCAGTATTAT TGCTATTATC TTTACCTGTA TTAGCAGGAG CAATTACTAT
Bradysia difformis        GCAGTTTTAT TATTATTATC TTTACCAGTA TTAGCAGGAG CAATTACTAT
Lycoriella castanecens    GCAATTCTTC TTCTTCTATC CCTACCCGTT TTAGCCGGAG CAATTACTAT
L.castF1                  ---------- ---------- ---------- ---------- ----------
L.castF2                  ---------- ---------- ---------- ---------- ----------
L.castR1                  ---------- ---------- ---------- ---------- ----------
L.castR2                  ---------- ---------- ---------- ---------- ----------
L.castR3                  ---------- --CTTCTATC CCTACCCGTT TTA------- ----------
CecidF1                   ---------- ---------- ---------- ---------- ----------
CecidF4                   ---------- ---------- ---------- ---------- ----------
CecidR2                   ---------- ---------- ---------- ---------- ----------
CecidR3                   ---------- ---------- ---------- ---------- ----------
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Appendix 5 (part 5)

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                                 ....|....| ....|....| ....|....| ....|....| ....|....|
                                         410        420        430        440        450
Tenuiphantes tenuis              ATTATTAACT GATCGAAATT TTAATACTTC TTTTTTTGAT CCAGCAGGAG
Erigone dentipalpis              GCTTTTAACT GATCGAAATT TTAATACTTC TTTTTTTGAT CCTTCTGGAG
E. atra                          ATTATTAACT GATCGTAATT TTAATACATC ATTTTTTGAT CCATCTGGTG
Sitobion avenae                  ATTATTAACT GATCGTAATT TAAATACTTC ATTTTTTGAC CCAGCAGGGG
Metopolosiphum dirhodum          ATTATTAACT GATCGAAATT TAAATACATC ATTCTTTGAT CCAGCAGGAG
Rhopalosiphum padi               ACTTCTTACT GATCGTAATT TAAATACATC ATTCTTTGAC CCTGCAGGAG
Isotoma anglicana                ATTGTTGACC GATCGAAACT TAAATACATC ATTTTTTGAC CCGGCCGGTG
Campsicnemus curvipes            ATTATTAACT GATCGAAATC TTAATACATC ATTTTTTGAC CCAGCAGGAG
Pteremis fenestralis             ATTATTAACA GATCGAAATT TAAATACCTC CTTTTTTGAT CCAGCCGGAG
Megaselia sp.                    ATTATTAACA GATCGAAATT TTAATACTTC TTTCTTCGAC CCAGCTGGAG
Sitodiplosis mosellana           ATTACTAACA GATCGAAATC TTAATACATC ATTTTTTGAT CCTATAGGAG
Putoniella sp.                   ATTATTAACA GATCGAAATC TTAATACATC ATTTTTTGAT CCTATAGGAG
Unidentified Cecidomyiidae       ATTATTAACA GATCGAAATT TAAATACTTC TTTTTTTGAC CCA-------
Bradysia difformis               ATTATTAACT GACCGAAATT TAAATACTTC ATTTTTTGAT CCAGCAGGAG
Lycoriella castanecens           ATTATTAACA GACCGAAACC TTAATACCTC ATTTTTTGAT CCCGCGGGTG
L.castF1                         ---------- ---------- ---------- ---------- ----------
L.castF2                         ---------- ---------- ---------- ---------- ----------
L.castR1                         ---------- ---------- ---------- ---------- ----------
L.castR2                         ---------- ---------- ---------- ---------- ----------
L.castR3                         ---------- ---------- ---------- ---------- ----------
CecidF1                          ---------- ---------- ---------- ---------- ----------
CecidF4                          ---------- ---------- ---------- ---------- ----------
CecidR2                          ---------- ---------- ---------- ---------- CCTATAGGAG
CecidR3                          ---------- ---------- ---------- ---------- CCTATAGGAG


                                 ....|....| ....|....| ....|....| ....|....| ....|....|
                                         460        470        480        490        500
Tenuiphantes tenuis              GAGGGGATCC TGTTTTATTT CAACATTTAT TTTGAT-TTT TTGGG-----
Erigone dentipalpis              GGGGTGATCC TGTGTTATTT CAACATTTAT TTTGATCTTT TTGGACACCC
E. atra                          GAGGGGACCC TGTTTTATTT CAACATTTGT TTTGAT-TCT TTGGGCATCC
Sitobion avenae                  GAGGTGACCC AATCTTGTAT CAACATTTAT TTTGAT-TTT TTGGTCATCC
Metopolosiphum dirhodum          GAGGAGATCC TATTCTATAT CAACATTTAT TTTGAT-TCT TTGGACATCC
Rhopalosiphum padi               GAGGAGATCC AATTCTTTAT CAACATTTAT TTTGAT-TTT TTGGACATCC
Isotoma anglicana                GTGGGGATCC TATCTTATAC CAACACTTAT TTTGAT-TTT TTGGGCACCC
Campsicnemus curvipes            GAGGAGACCC TATTTTATAC CAACATTTAT TTTGAT-TTT TTGGGCATCC
Pteremis fenestralis             GAGGAGACCC AATTTTATAT CAACATTTAT TTTGAT-TTT TTGGTCATCC
Megaselia sp.                    GAGGAGATCC TATTTTATAC CAACATTTAT TCTGAT-TTT TTGGGCATCC
Sitodiplosis mosellana           GAGGAGATCC AGTTCTTTAT CAACATTTAT TTTGAT-TTT TTGGACATCC
Putoniella sp.                   GAGGAGATCC AGTTCTTTAT CAACATTTAT TTTGAT-TCT TTGGACATCC
Unidentified Cecidomyiidae       ---------- ---------- ---------- ---------- ----------
Bradysia difformis               GGGGAGACCC AATTTTATAT CAACATTTAT TTTGAT-TTT TTGGACACCC
Lycoriella castanecens           GTGGAGACCC AATCCTATAT CAACACTTAT TTTGAT-TTT TTGGGCACCC
L.castF1                         ---------- ---------- ---------- ---------- ----------
L.castF2                         ---------- ---------- ---------- ---------- ----------
L.castR1                         ---------- ---------- ---------- ---------- ----------
L.castR2                         ---------- ---------- ---------- ---------- ----------
L.castR3                         ---------- ---------- ---------- ---------- ----------
CecidF1                          ---------- ---------- ---------- ---------- ----------
CecidF4                          ---------- ---------- ---------- ---------- ----------
CecidR2                          GAGGAGATCC ---------- ---------- ---------- ----------
CecidR3                          GAGGAGAT-- ---------- ---------- ---------- ----------
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Appendix 5 (part 6)

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                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                        510        520        530        540        550
Tenuiphantes tenuis             ---------- ---------- ---------- ---------- ----------
Erigone dentipalpis             CAGAATTT-- ---------- ---------- ---------- ----------
E. atra                         ATTTGTTTTG ATTCTTTGGG CATCCAGAAG TTTATATTTT AATTTTACCC
Sitobion avenae                 TGAAGTTTAT ATTTTAATTT TACCGGG--- ---------- ----------
Metopolosiphum dirhodum         TGAAGTTTAT ATTTTAATTT TACCGGGA-- ---------- ----------
Rhopalosiphum padi              AGAAGTTTAT ATTTTAATTT TACCGGGA-- ---------- ----------
Isotoma anglicana               C--------- ---------- ---------- ---------- ----------
Campsicnemus curvipes           AGAAGTTTAT ATTTTAATTT AACCGGGA-- ---------- ----------
Pteremis fenestralis            TGAAGTTTAT ATTTTAATTT TACCGGG--- ---------- ----------
Megaselia sp.                   AGAAGTTTAT ATTTTAATTT TACCC----- ---------- ----------
Sitodiplosis mosellana          AGAAGTTTAT ATTTTAATTT TACCGGGA-- ---------- ----------
Putoniella sp.                  AGAAGTTTAT ATTTTAATTT TACCGGGA-- ---------- ----------
Unidentified Cecidomyiidae      ---------- ---------- ---------- ---------- ----------
Bradysia difformis              TGAAGTTTAT ATTTTAATTT ---------- ---------- ----------
Lycoriella castanecens          AGAAGTTTAT ATTTTAATTT A--------- ---------- ----------
L.castF1                        ---------- ---------- ---------- ---------- ----------
L.castF2                        ---------- ---------- ---------- ---------- ----------
L.castR1                        ---------- ---------- ---------- ---------- ----------
L.castR2                        ---------- ---------- ---------- ---------- ----------
L.castR3                        ---------- ---------- ---------- ---------- ----------
CecidF1                         ---------- ---------- ---------- ---------- ----------
CecidF4                         ---------- ---------- ---------- ---------- ----------
CecidR2                         ---------- ---------- ---------- ---------- ----------
CecidR3                         ---------- ---------- ---------- ---------- ----------


                                ..

Tenuiphantes tenuis             --
Erigone dentipalpis             --
E. atra                         GG
Sitobion avenae                 --
Metopolosiphum dirhodum         --
Rhopalosiphum padi              --
Isotoma anglicana               --
Campsicnemus curvipes           --
Pteremis fenestralis            --
Megaselia sp.                   --
Sitodiplosis mosellana          --
Putoniella sp.                  --
Unidentified Cecidomyiidae      --
Bradysia difformis              --
Lycoriella castanecens          --
L.castF1                        --
L.castF2                        --
L.castR1                        --
L.castR2                        --
L.castR3                        --
CecidF1                         --
CecidF4                         --
CecidR2                         --
CecidR3                         --
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