Molecular Tracking of Arthropod Predator-Prey Interactions

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Cover: *Pardosa* spider, bird cherry-oat aphids and springtails in the field. Illustration by Hernán Guzmán, UppsalAnimation ©

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Abstract

Local generalist predators can be exploited in conservation biological control of agricultural pests. To conserve and promote the most important predators, it is critical to have detailed knowledge about their prey range under natural conditions. I have studied predator-prey interactions between lycosid spiders from the genus *Pardosa* and a major aphid pest in spring-sown cereals*; Rhopalosiphum padi*. I have also studied the link between *Pardosa* and springtails; a potential source of alternative food that may sustain generalist predators when pest species are absent. To reveal what the spiders had been feeding on, I collected them in farmers´ fields and searched for prey- specific DNA remains in their stomach contents. In the same fields, I also assessed abundances of aphids and alternative prey, including springtails. For detection of *R. padi* remains I used available PCR primers, but to be able to study predation on springtails, I had to develop novel group-specific springtail primers. This thesis also evaluates how fast *R.padi* and springtail DNA is digested in laboratory-fed spiders and how temperature influences detection of prey remains in a model predator; the two-spotted lady beetle, *Adalia bipunctata*. Such knowledge is critical when applying PCR-based gut-content analysis to field-caught predators. By combining the consumption data with measurements of prey availability I was able to demonstrate that *Pardosa* feed on *R. padi* when it is most critical for successful biological control, i.e. when aphid densities are low. Spiders testing positive had consumed the pest shortly before capture, a conclusion based on results from the feeding experiment, which revealed that *R.padi* was digested rapidly in laboratory-fed spiders. I also found that springtails are an important source of alternative food for *Pardosa* and conclude that springtail occurrence may contribute to spider population maintenance and, indirectly, to enhanced predation pressure on co-occurring pests. However, I also found evidence that high abundances of alternative prey, temporarily, might divert spiders away from feeding on *R. padi*. Altogether, this work has generated new knowledge about the feeding habits of *Pardosa* spiders that will improve our understanding of the role of generalist predators as natural pest suppressors in agroecosystems.

Keywords: biological control, generalist predators, gut-content analysis, PCR, *Pardosa*, *Rhopalosiphum padi*, springtails, group-specific primers

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To my husband, Hernán.

Hoy es el dia, mañana es tarde...
Los Nocheros

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I McMillan, S., Kuusk, A-K., Cassel-Lundhagen, A. & Ekbom, B. (2007) The influence of time and temperature on molecular gut content analysis: *Adalia bipunctata* fed with *Rhopalosiphum padi*. *Insect Science*, 14, 353-358.

II Kuusk, A-K., Cassel-Lundhagen, A., Kvarnheden, A. & Ekbom, B. (2008)Tracking aphid predation by lycosid spiders in spring-sown cereals using PCR-based gut-content analysis. *Basic and Applied Ecology*, 9, 718-725.

III Kuusk, A-K. & Agustí, N. (2008) Group-specific primers for DNA-based detection of springtails (Hexapoda: Collembola) within predator gut contents. *Molecular Ecology Resources,* 8, 678-681.

IV Kuusk, A-K. & Ekbom, B. Lycosid spiders and alternative food: feeding behavior and influence on biological pest control (submitted manuscript).

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Abbreviations

BLAST	Basic local alignment search tool
bp	Base pairs
COI	Cytochrome c oxidase subunit I
COII	Cytochrome c oxidase subunit II
ELISA	Enzyme-linked immunosorbent assay
IGP	Intraguild predation
MAbs	Monoclonal antibodies
PCR	Polymerase chain reaction
qPCR	Quantitative PCR
rDNA	Ribosomal DNA

1 Introduction

1.1 Arthropod predation and biological control

Changes in numbers of animal populations are to a large extent driven by mortality caused by predators. In agricultural habitats, a wide range of arthropod predatory taxa, for example spiders, lady beetles, lacewings, hoverflies and ground beetles, are present (Van Driesche *et al.,* 2008). Many of these predators feed on major agricultural pests such as aphids (Sunderland *et al.,* 1987), caterpillars (Ma *et al.,* 2005), planthoppers (Fournier *et al.,* 2008) and slugs (Dodd *et al.,* 2003). Consequently, arthropod predators are a valuable component of the natural enemy group that can contribute to natural pest suppression.

When natural enemies are exploited to suppress densities of pest populations it is called biological control. This plant protection strategy is one of the cornerstones in integrated pest management and provides an important ecosystem service to the agricultural sector. There are three principal biological control methods: introduction, augmentation and conservation (Van Driesche & Bellows, 1996). Introduction, which also is called classical biological control, is used against invasive pests that have been accidentally introduced to a new geographic area without associated natural enemies. By tracking the immigrant species to its native country, it is often possible to find efficient natural enemies that, after a rigorous quarantine process, can be released into the new environment and hopefully provide successful control. The second type, augmentation, is used when natural enemies are absent, arrive too late or are too few to provide control. It can be used against both native and exotic pests and involves releases of reared natural enemies. The third type, conservation biological control, is the exploitation of local natural enemies and the aim is to increase their numbers

and to enhance their pest suppression efficacy. Several human activities, for example application of broad spectrum chemical pesticides, mechanical crop treatments, and removal of overwintering sites, have negative impacts on naturally occurring biological control agents. To allow local natural enemies to flourish, these forces have to be identified and altered. Apart from careful timing and selective use of agrochemicals, local natural enemies can be conserved through provision of refuge areas and supplementation of food as well as by moderating physical conditions through the use of ground covers (Riechert & Bishop, 1990; Thorbeck & Bilde, 2004). Of the three major forms of biological control, conservation has received the least amount of attention (Landis *et al.,* 2000)

One of the first proposals that predators should be used to combat agricultural pests was made by Carl Linnaeus in 1752. According to Hörstadius (1974), Linnaeus explicitly stated: "Since people noted the damage done by insects, thought has been given to ways of getting rid of them, but so far nobody has thought of getting rid of insects with insects. Every insect has its predator which follows and destroys it. Such predatory insects should be caught and used for disinfesting crop-plants". Around a century later, in 1873, the first classical biological control program involving a predacious invertebrate was initiated. In a project led by the American entomologist Charles Valentine Riley, the mite *Tyroglyphus phylloxerae* was sent to France to combat the exotic grape pest phylloxera (*Daktulosphaira vitifolii*) (Van Driesche & Bellows, 1996). The predator established successfully but had no significant effect on the devastating pest. Later in the 1880s, C.V Riley led another classical biological control program that ended in great success; the introduction of the Australian lady beetle *Rodolia cardinalis* for suppression of the exotic cottonycushion scale, *Icerya purchasi*, in Californian citrus groves (Caltagirone & Doutt, 1989). However, the action of arthropod predators and their potential as pest suppressors had been observed by primitive agriculturalists long before the days of Carl Linnaeus and C.V Riley; in ancient China, farmers utilized nests of an ant to control caterpillars and large boring beetles in citrus trees (Debach, 1974). To facilitate dispersal of the ants, bamboo branches were used as bridges between the trees.

1.2 Generalist predators and alternative prey

Certain arthropod predators feed on a rather restricted number of taxonomically related prey species while others have a broader diet that includes prey from various taxa. The lady beetles *Rodolia cardinalis* and

Hyperaspis pantherina, which feed on scale insects, are two examples of specialized predators (Caltagirone & Doutt, 1989; Folwer, 2004). Predators with a wider diet are called generalists. Spiders, carabid and staphylinid beetles, and earwigs are typical examples of generalist natural enemies (Edwards *et al.,* 1979; Symondson *et al.,* 2002).

Which type of predator, generalist or specialist that is most effective against a certain pest depends on the objective of the biological control program. If the purpose is to suppress an already epidemic pest population, this can be achieved through mass releases of reared predators or, if the pest is invasive, introductions of classical agents (Wiedenmann & Smith, 1997). It is obvious that a specialized natural enemy is a more appropriate choice than a generalist in such situations. However, if the aim is to prevent a native pest from building up high densities, rather than reducing an already escalating pest population, conservation of local generalist predators is generally considered as a more effective strategy than mass-releases or introductions of specialist enemies, particularly in temporary agroecosystems (Riechert & Lockley, 1984; Wiedenmann & Smith, 1997; Symondson *et al.,* 2002). One of the important factors is the broad diet of the generalists. When pest species are absent, these predators can, in contrast to specialists, be sustained in the field or in nearby habitats by utilizing alternative sources of nutrition (Wiedenmann & Smith, 1997; Chen & Wise, 1999; Settle *et al.,* 1996). Thus, when the cropping season starts, generalist predators have the potential to start feeding immediately on colonizing pests. Ideally, pest populations are maintained at low, non damaging levels. In contrast, specialist predators may, particularly in temperate regions, arrive to annual crop fields too late when the size of the pest population already has passed the action/damaging threshold (Symondson *et al.,* 2002).

Apart from being present in the crop early in the season, it should be emphasized that it is very important that the generalist predator quickly finds and consumes the scarce pest. Both modeling and experimental exclusion of predators in the field have shown that early season predation by assemblages of ground-living generalist predators is critical for maintaining pest populations at low levels (Edwards *et al.,* 1979; Ekbom *et al.,* 1992; Chiverton, 1986; Östman *et al.,* 2001). However, in exclusion experiments it is not revealed which individual predatory taxa are indeed feeding on the scarce pest. Or in other words, it is not known which predatory species are actually contributing to biological control.

Although there may, from a biological control perspective, be positive effects attributed to the broad diet of generalist predators, there may also be negative aspects. Good availability of high-quality alternative prey may

distract generalist predators from feeding on the target pest. This has been observed in studies in which densities of generalist predators and/or prey have been manipulated (Musser & Shelton 2003; Koss & Snyder 2005; Prasad & Snyder 2006; Birkhofer *et al.,* 2008a). However, the extent to which local generalist predators feed on *natural, unmanipulated* populations of alternative prey has only been evaluated in a few studies (Agusti *et al.,* 2003a; Juen & Traugott, 2007; Harwood *et al.,* 2007ab, 2009) and extensive data for most combinations of predator and prey is lacking. Also, there is generally little information on how presence of alternative prey influences pest consumption by generalist predators in natural, undisturbed agricultural systems (but see Harwood *et al.,* 2004).

1.3 Revealing predator-prey interactions

Although there are many examples of successful biological control programs, the exploitation of natural enemies in plant protection is generally considered as a rather uncertain method. To make biological control more predictable and reliable, and to increase confidence in biological control by the agricultural sector, new knowledge, about all three types of biological control, is required. When it comes to implementation of effective and sustainable biological control based on the action of local generalist predators, it is critical to obtain more information about their prey range under natural conditions; which predator species are exploiting low-density pest populations, and which alternative prey are included in their diet.

Predator-prey interactions occurring in natural habitats can be studied by various approaches (reviewed by Sunderland, 1988; Harwood & Obrycki, 2005). The choice of method depends on the characteristics of the study organisms (size, mobility, biology, behavior etc.) and the question to which the scientist seeks an answer. When studying large mammal predators, for example lions, it may not be too difficult to visually observe how often these animals feed and what kind of prey they consume. It is, however, generally much more complicated and time consuming to visually observe interactions between arthropod predators and their prey directly in the field (Symondson, 2002). Both the predator and the prey are generally very small and live beneath dense vegetation, in leaf litter or below the soil surface. In addition, many arthropods are very mobile and some species hunt only at night. Any attempt to facilitate observations of the study organisms (for example by cutting down vegetation, or by using lamps during night time surveys) will cause disturbance to the system and invalidate the results (Symondson, 2002). By excluding predators from field plots, using physical

barriers, and comparing the population development of the target pest inside and outside the barriers, it is possible to reveal the biological control potential of assemblages of natural enemies (Edwards *et al.,* 1979; Chiverton; 1986; Östman *et al.,* 2001). However, with this method it is difficult to identify the contribution of individual predatory species, genera, or families. An alternative approach is to remove all natural enemies from a plot and then enclose the study predator together with the target prey (Birkhofer *et al.,* 2008b). Either way, the physical barriers cause disturbance to the system and thigmotactic responses to boundaries is always a problem when enclosing mobile organisms (Symondson, 2002). Also, caging of generalist predators may alter abundances of alternative prey. Consequently, the measured predation rates will probably not mirror natural rates (Sunderland, 1988).

One way to obtain very specific consumption data at the species level is to collect predators in their natural environment, preserve them and search for remains of ingested prey in their gut-contents (Sunderland, 1988; Symondson, 2002). Also, this approach generates data on predator-prey interactions that have occurred under minimal experimental disturbance. Today, the two most popular gut-content methods are: detection of prey-specific proteins using monoclonal antibodies (MAbs) and detection of prey-specific DNA using polymerase chain reaction (PCR) primers (Symondson, 2002; Sheppard & Harwood 2005; Harwood & Obrycki, 2005; Fournier *et al.,* 2008).

2 Aims of the thesis

Detailed knowledge about arthropod predator-prey interactions in agricultural ecosystems is an important step towards establishing sustainable and efficient pest management strategies based on biological control. The aim of this thesis was to optimize, develop and use PCR-technology to gather specific knowledge about the feeding habits of common generalist spider predators from the genus *Pardosa* (Araneae: Lycosidae). In the field, at several locations, we investigated if the spiders consumed an economically important aphid pest, *Rhopalosiphum padi* (Homoptera: Aphididae). In parallel, we examined to what extent the spiders fed on springtails (Arthropoda: Collembola), which are ubiquitous arthropods that may be an alternative source of food and sustain generalist predators in agricultural habitats when pest species are absent. Alternative prey may also divert predators away from feeding on pests and therefore we investigated how presence of such food influenced spider consumption of the target aphid. The studied arthropods are all small (< 1cm) and live beneath dense vegetation. In addition, spiders and springtails are highly mobile. For these reasons it is very difficult to observe interactions between them by direct visual observation in the field. In order to realise our research goals we considered that a molecular gut-content analysis was a suitable approach.
The more specific aims of the different studies were to:

- Study the effect of ambient temperature on PCR-based prey detection (*Paper I*)
- Develop group-specific springtail PCR primers (*Paper III*)
- Investigate the detection success of *R. padi* and springtail DNA in laboratory-fed *Pardosa* spiders (*Paper II, IV*)
- Investigate if *Pardosa* spiders feed on *R. padi* in spring-sown cereals when pest densities are low (*Paper II*)

- Obtain information about the importance of springtails as alternative food for *Pardosa* spiders in two habitats; spring-sown cereals and leys (*Paper IV*)
- Investigate how availability of alternative prey affects predation rates of *R. padi* and the spiders' value as biocontrol agents (*Paper IV*)

3 The study system

3.1 The bird cherry-oat aphid (*Rhopalosiphum padi*)

The target pest, the bird cherry-oat aphid (*R. padi*) (Fig. 1) has a virtually world-wide distribution and it is considered as one of the most important pests in cereal crops in Europe (Blackman & Eastop, 2000; Leather *et al.,* 1989). In Scandinavia, *R. padi* is host alternating between the bird-cherry tree (*Prunus padus*) and numerous grasses including cultivated cereals (Wiktelius & Chiverton, 1985). The aphids overwinter as eggs on *P. padus*, the eggs hatch at bud burst and aphids usually start migrating to the summer hosts around the end of May to mid June (Wiktelius, 1982, 1984). Spring migration and colonization of cereal fields during periods of dry warm weather can result in an exponential population growth and severe outbreaks. In the first half of July, the population usually crashes and the aphids migrate to grasslands (Wiktelius & Ekbom, 1985). In the autumn, winged sexual aphids appear and egg laying on *P. padus* begins.

Figure 1. The bird cherry-oat aphid, *Rhopalosiohum padi*, on the stem base of a barley plant. The aphids are small, approximately 1-2 mm, and damage the cereal plants both directly through feeding of phloem sap and indirectly by the transmission of plant viruses. The photo is taken during an outbreak season. If generalist predators feed on *R. padi* when pest densities are still low, outbreaks can be prevented. Photo by Hernán Guzmán.

Immigrating aphids have higher survival rates on young plants compared to older. As a consequence, the importance of *R. padi* as a direct pest is greatest in countries where the cereal production is dominated by spring-sown cultivars, which are at the seedling stage when aphids colonize the fields (Leather *et al.*, 1989). In Sweden, large areas of spring-sown cereals, mainly barley followed by oats and wheat, are cultivated. In 2008, 62 % of the total cereal producing area was sown with one of these cereals (Jordbruksstatistisk årsbok 2009). In our country, *Rhopalosiphum padi* may cause yield losses up to 600 kg/ha (~15 %) (Hallqvist, 1991) and outbreaks occur approximately every 2-3 year. During such seasons, it is possible to find as many as 50 - 100 aphids per shoot (personal observation) (Fig. 1). Early in the season, over 75 % of the *R. padi* population occurs on the bases of plants, at or even slightly below the soil level (Wiktelius, 1987). Consequently, the aphids are highly exposed to attack by ground-dwelling predators.

3.2 *Pardosa* spiders

When trying to elucidate which predatory taxa contribute to biological control the value of a gut-content analysis becomes apparent. Indeed, over 100 studies have utilized some kind of gut-content analysis to assess aphid predation (reviewed by Harwood & Obrycki, 2005). In more than 70 cases, carabid beetles were studied, clearly demonstrating a need for more data on other taxa. In general, spiders have received little attention despite their high abundance in cereals fields in Europe (reviewed by Sunderland, 1987). Wolf spiders (Araneae: Lycosidae) form a major part of the generalist predatory fauna in temperate agroecosystems (Öberg *et al.,* 2007; Samu & Szinetár, 2002; Schmidt *et al.,* 2005) and in this thesis we focus on members of the diurnal, ground-living genus *Pardosa* (Fig. 2). *Pardosa* spiders do not build webs; instead they hunt for food by running around, or by lying in ambush waiting motionless until a prey comes close enough to be captured in one rapid movement (Edgar, 1969; Foelix, 1996).

Recent work by Öberg & Ekbom (2006) and Öberg *et al*. (2007) has revealed that the most common *Pardosa* species in agroecosystems around Uppsala in central Sweden are *P. agrestis*, *P. palustris* and *P. prativaga*. As a group, lycosid spiders seems to prefer the field margin over the inner field habitat, but activity densities of the most dominating species, *P. agrestis*, do not differ between habitat types (Öberg *et al.,* 2007). It has also been shown that *P. agrestis* is unaffected by sowing, and that this species is uniformly distributed in spring-sown cereals early in the season (Öberg & Ekbom, 2006). The temporal and spatial synchronization with *R. padi,* together with high activity densities, suggest a high conservation biological control potential for *P. agrestis*. Therefore, we decided to pay special attention to the diet of this species and other members of the genus *Pardosa*.

Figure 2. A *Pardosa* spider searching for food in a spring barley field. Members of this genus have long legs, a body covered with dense brownish hair and measure approximately 5-9 mm. Most species reproduce during spring-summer and overwinter as juveniles. Photo by Hernán Guzmán.

3.3 Springtails

In addition to pest herbivores, the diet of spiders includes alternative, non-pest prey. One potential source of alternative food for ground-living generalist predators in agroecosystems are springtails (Arthropoda: Collembola); small, wingless, organisms that generally feed on fungal hyphae or decaying plant material (Fig. 3). Springtails have a very wide global distribution and usually occur in very high numbers in soils and leaf litter (Hopkin, 1997). Most surface-dwelling springtails are equipped with a furca, which enables them to instantaneously shoot into the air, a very effective escape mechanism from attacking predators. In species which live in the soil, the furca is greatly reduced or even absent.

The taxonomic composition of springtails in spring-sown cereals and grass leys in Sweden is relatively diverse with more than 20 identified species representing various families from both springtail suborders, Arthropleona (elongated springtails) and Symphypleona (globular springtails) (Lagerlöf & Andren, 1991; Curry, 1986). Laboratory studies have shown that some springtails are high quality food for wolf spiders (Toft & Wise, 1999;

Oelbermann & Scheu, 2002) and inputs of detrital subsidies have caused a simultaneous increase in springtail and wolf spider abundances (indicating that the spiders are exploiting springtails) (Chen & Wise, 1999; Halaj & Wise, 2002; Oelbermann *et al.,* 2008). It has also been observed that wolf spiders carry springtails in their jaws (Edgar, 1970; Nyffeler & Benz, 1988). Despite such evidence, doubts have been raised as to whether or not this alternative prey forms a significant part of the diet of large, non-web based spiders such as *Pardosa* (Harwood *et al.,* 2005; Halaj & Wise, 2002). We wished to obtain more knowledge about predator-prey interactions between *Pardosa* spiders and springtails.

Figure 3. Springtails are small arthropods (most species measure only a few mm) that are abundant in soil and leaf litter. In agroecosystems, springtails are a potential source of non-pest, alternative prey for generalist predators. From a biological control perspective, presence of alternative prey is associated with trade offs; good availability of alternative prey may lead to increased spider condition, elevated predator populations, and an increased predation pressure on co-occurring pests. On the other hand, high abundances of profitable alternative prey may divert predator away from feeding on the target pest. The species in the photo is *Isotoma anglicana*. Photo by Arne Fjellberg.

4 Background to predator gut-content analysis

In the following section I will describe gut-content analysis of arthropod predators in some more detail. Emphasis is placed on DNA-based approaches as this was the method I used.

4.1 Gut-dissection

If a predator ingests solid prey parts that are easy to count and identify, gut dissection can be a suitable and easily applied approach for studies of predator-prey interactions (Symondson, 2002; Harwood & Obrycki, 2005; Sheppard & Harwood, 2005). Certain predatory taxa sometimes do so, but the majority of arthropod predators (for example all spiders and hemipteran bugs) feed by liquid ingestion and solid prey parts are never found in their guts. For this reason, and also because identification of prey remains in predator gut-samples generally requires great taxonomical skills, a wide range of tools that enables identification of prey at the molecular level has been developed (Symondson, 2002; Harwood & Obrycki, 2005). Today, the most widely applied approaches are detection of prey-specific proteins using ELISA and detection of prey-specific DNA remains using techniques based on PCR.

4.2 Detection of prey proteins using antibodies

In ELISA, prey proteins are detected with either poly- or monoclonal antibodies. Polyclonal antisera, which are raised in and harvested from mammals that have been injected with target prey proteins, contain a mixture of antibodies that can bind to various epitopes (the antibody binding sites on a protein). Therefore, the potential for cross reactivity with

a protein from a nontarget organism is rather high (Dodd *et al.,* 2003; Symondson, 2002). Another limitation of polyclonal antisera is that when a stock is used, a new antiserum needs to be characterized and because two antisera are never identical, results are not reproducible (Dodd *et al.,* 2003). In contrast, monoclonal antibodes (MAbs) can be produced in limitless supply from cell cultures (Köhler & Milstein, 1975; Dodd *et al.,* 2003). They are all identical and therefore, ELISA with MAbs is more specific compared to ELISA with polyclonal antisera. MAbs may be specific to almost any taxonomic level, for example an insect order, family, genus or species (Symondson, 2002; Harwood, 2007a). MAbs can even be stage- or instar specific, which enables studies of arthropod cannibalism (Sigsgaard *et al.,* 2002), a phenomenon that can not be revealed by DNA-based approaches.

Although MAbs are powerful tools in studies of arthropod predator-prey interactions (see for example Harwood *et al.,* 2004; 2007a; Hagler & Naranjo, 2005) relatively few research groups use them. The main reason is that MAbs are expensive and time consuming to develop (Sheppard & Harwood, 2005). This might not be a major problem if studying specialist predators, but if the intention is to reveal the diet of generalist predators, it may be impossible to develop a sufficient number of MAbs (Sheppard & Harwood, 2005).

4.3 Detection of prey DNA using PCR

Although MAbs are still utilized, there is a clear trend that DNA-based methods are becoming more and more important in studies of arthropod predator-prey interactions (King *et al.,* 2008). In contrast to MAbs, development and application of these techniques require skills and equipment that are common in many labs (Greenstone & Shufran, 2003; Sheppard & Harwood 2005). As an ingested prey generally is much smaller than the predator, and because the prey DNA is broken down into smaller and smaller pieces during digestion, it is necessary to use a method that is capable of amplifying extremely small amounts of semi-digested prey DNA to amounts that are possible to visualize. This demand is fulfilled by the PCR-technique (Saiki *et al.,* 1985, 1988).

Basically, PCR copies a predefined short fragment of DNA in a test-tube. The region of DNA to be copied is defined by two sequence-specific oligonucleotide primers that bind to their complementary sequence on the DNA strand according to normal base pairing rules (McPherson & Møller, 2006). As the name implies, PCR is a chain reaction driven by the enzyme DNA-polymerase. The reaction is repeated several times, and after each

cycle the target fragment is duplicated. At the end of the assay, millions of copies of the predefined target fragment have been produced, which enables visualization on, for example, agarose gels. Figure 4 illustrates the work procedure that I used when trying to reveal if an arthropod predator had consumed a certain prey.

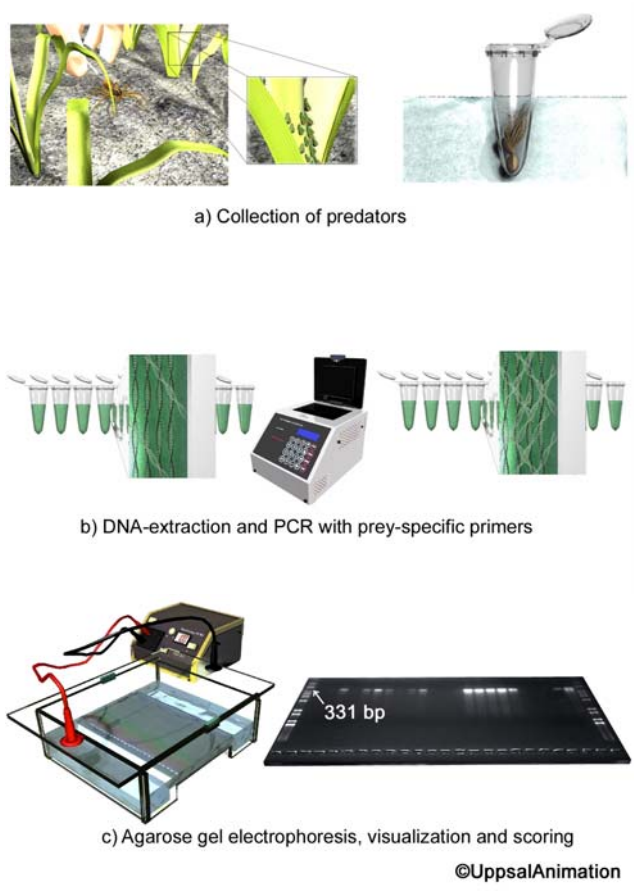

Figure 4. Schematic overview of PCR-based gut-content analysis of arthropod predators**. a)** The bird cherry-oat aphid, *Rhopalosiphum padi*, is a major pest in spring-sown cereals. Wolf spiders are common generalist predators in these crops, but are they really feeding on the aphids? To find out, predators were collected in their natural environment. To minimize degradation of DNA in their stomach contents, predators were frozen immediately after capture. **b)** DNA was extracted from whole predators and a specific *R. padi* DNA fragment of a predefined length, in this case 331 bp, was amplified by PCR. **c)** The PCR products were separated by agarose gel electrophoresis and the gels were stained with a dye that binds to DNA and illuminates under UV-light. If the visualized PCR products (which appear as bright bands) were of expected length (331 bp) the samples were scored as positive and it was concluded that those predators had consumed *R. padi*. If no bands appeared on the 331 bp region of the gel, samples were scored as negative.

4.3.1 PCR primers

Dietary samples are usually complex DNA sources containing a mixture of genomes from the predator itself, various consumed prey, gut-parasites and intestinal symbionts (Jarman *et al.,* 2004). The key to amplify DNA only from the target prey lies in the design of the primers. PCR primers can be designed to complement regions of DNA that are conserved in as wide a range of species as possible (Jarman *et al.,* 2002). Such sets of primers are usually termed "universal" and have been utilized in studies of predator-prey interactions (Malmqvist *et al.,* 2004; Kasper *et al.,* 2004: Harper *et al.,* 2006). PCR primers can also be group-specific, which means that they are designed to amplify a DNA region from only a certain range of species in a particular higher taxon (Jarman *et al.,* 2004), for example an insect order. The most common approach in studies of arthropod predator-prey interactions has been to develop species-specific primers that target only one specific type of prey (King *et al.,* 2008).

Genes that contain regions of DNA with a high degree of variability are suitable targets for development of species-specific primers. The protein coding mitochondrial genes, COI and COII, contain such variable DNA regions. These genes have been utilized in a number of studies for development of species-specific primers for various pest species such as the pollen beetle *Meligethes aeneus* (Cassel-Lundhagen *et al.,* 2009), various cereal aphids (Chen *et al.,* 2000), the pear psylla *Cacopsylla pyricola* (Agustí *et al.,* 2003b), the glassy-winged sharpshooter *Homalodisca coagulata* (de León *et al.,* 2006), the Colorado potato beetle *Leptiontarsa decemlineata* (Greenstone *et al.*, 2007) and root-feeding coleopteran pests (Juen & Traugott, 2005, 2006, 2007).

When working in species rich habitats, where the diversity of a potential prey group is high and knowledge about the links between a predator and different species within the prey group is sparse, group-specific PCR primers can be a powerful tool (Jarman *et al.,* 2004; Admassu *et al.,* 2006). To be able to design group-specific primers it is necessary to identify DNA regions that are conserved within all members of the target group but unique with respect to nontarget organisms. Several ribosomal genes, both mitochondrial (12S and 16S) and nuclear (18S and 28S), contain conserved regions that are suitable targets for design of group-specific primers (King *et al.,* 2008). During this study, group-specific springtail primers from 18S rDNA were developed (*Paper III*). Group-specific primers intended for arthropod gut-content analysis have also been designed from conserved regions in the COI- and COII genes (Chen *et al.,* 2000; Admassu *et al.,* 2006).

Apart from containing variable and conserved regions that allow differentiation between species at various taxonomic levels, there is another important reason as to why mitochondrial and ribosomal genes have been so frequently utilized in studies of DNA-based detection of arthropod predation. Both types of genes are, namely, present in hundreds or thousand of copies in each cell (Hoy, 1994), which greatly increases the sensitivity of the PCR assay and the probability that the target prey will be successfully amplified (King *et al.,* 2008).

4.3.2 Evaluation of the PCR assay

Cross-reactivity tests on nontarget organisms: When developing a PCR assay intended for gut-content analysis of field-caught predators, various aspects have to be considered (King *et al.,* 2008). Perhaps the most obvious is to empirically test the specificity of the assay to ensure that PCR products of the same size as the target prey is not produced from the predator itself, or from other organisms that might be consumed by the predator.

Success of prey DNA detection in laboratory-fed predators: Before analyzing field-caught predators it is also necessary to ensure that the prey DNA really can be detected within predators that have consumed the prey. This can be achieved by investigating the detection success of the prey DNA in laboratory-fed predators. In short, such tests are carried out by feeding starved predators with the target prey and then freezing fed predators at various time-points after consumption. Subsequently, DNA is extracted from the predators, PCRs with prey-specific primers are run, and the number of predators testing positive at each time point is expressed as a percentage or proportion of the tested predators. By estimating for how long after a meal that it is possible to detect the prey in the predator (i.e. the detection period), it is possible to determine that a predation event must have occurred within a specific time frame (Greenstone & Hunt, 1993; Greenstone *et al.,* 2007). Knowledge about detection periods is also critical when comparing the biological control potential of different predatory taxa. If unknown, a species in which the prey is detectable for very long intervals could incorrectly be considered as a more important predator than a species in which the prey is digested relatively faster (Chen *et al.,* 2000; Greenstone & Shufran, 2003).

Numerous studies have demonstrated the possibility of detecting semi-digested, multi-copy arthropod prey DNA in laboratory-fed predators (reviewed by Sheppard & Harwood, 2005; King *et al.,* 2008). A general conclusion is that the detection success of prey DNA can be enhanced by targeting short DNA fragments, no longer than approximately 100 - 500 bp

(Zaidi *et al.,* 1999; Agustí *et al.,* 1999, 2000; Hoogendoorn & Heimpel, 2001). The underlying reason is that during digestion, prey DNA molecules are broken down into smaller and smaller pieces. Consequently, shorter target fragments generally stay intact in the gut-contents for longer time periods. Another general conclusion is that the detection success of prey DNA is negatively influenced by time since feeding. Usually, the time point for when the prey DNA can be detected in 50 % of the fed predators is calculated. This value has been found to vary considerably, from a few hours (Chen *et al.,* 2000; Read *et al.,* 2006) up to several days (Sheppard *et al.,* 2005; Ma *et al.,* 2005), depending on the predator-prey system under study. Intrinsic factors such as gender, predator size/weight and developmental stage have generally not been found to have any significant effects on the success of prey DNA detection (Hoogendoorn & Heimpel, 2001; Sheppard *et al.,* 2005; Foltan *et al.,* 2005; Hosseini *et al.,* 2008). In some cases, ingestion of larger meals has prolonged the detection time of prey DNA (Ma *et al.,* 2005; Foltan *et al.,* 2005), in some cases not (Zaidi *et al.,* 1999). Because the body temperature and basic metabolism of insects vary with the temperature of the surrounding environment it is reasonable to assume that the rate of digestion and hence the detectability of prey remains may be temperature dependent. Previous studies have shown that serological detection of prey proteins in predator gut-contents can be significantly affected by ambient temperature (for example Hagler & Cohen, 1990; Hagler & Naranjo, 1997). There are also a handful of studies that demonstrate that temperature can influence the detection success of prey DNA in arthropod predators (Hoogendoorn & Heimpel, 2001; Read, 2007; Hosseini *et al.,* 2008; von Berg *et al.,* 2008a).

We have investigated the influence of time since feeding on detection success of *R. padi* and springtail DNA in *Pardosa* spiders and evaluated how temperature affects the detectability of *R. padi* remains in a model predator; larvae of the two-spotted lady beetle, *Adalia bipunctata*.

4.3.3 Examples of applied studies in agroecosystems

Initially, most DNA-based studies of arthropod predator-prey interactions presented results solely from laboratory evaluations. However, an increasing number of papers now present PCR-data obtained by screening of field-caught predators. Here, I give an overview of the results from some of these studies. Agustí *et al.* (2003a) was first to apply DNA-based gut-content analysis to field-caught predators and demonstrated that linyphiid spiders in winter wheat were exploiting three different species of springtails and that the predators were exercising prey choice. Molecular tracking of prey DNA

remains has also enabled studies of cryptic predator-prey interactions in below-ground food webs; Read *et al.* (2006) identified three soil-dwelling micro-arthropod taxa as important predators of an insect pathogenic nematode and Juen & Traugott (2007) tracked significant trophic links between myriapod predators and root feeding scarab larvae in alpine grasslands. DNA based-gut content analysis has also revealed that a hemipteran bug (*Orius insidious*) is an important natural enemy of an invasive aphid pest (*Aphis glycines*) in soybeans in the US (Harwood *et al.,* 2007b). In the same system, it has also been demonstrated that immature bugs exploit *A. glycines* to a greater extent than adult specimens (Harwood *et al.,* 2009). Fournier *et al.* (2008) made an extensive DNA-based survey to identify the arthropod predator guild of the glassy-winged sharpshooter (*Homalodisca coagulata),* an important pest in vineyards in California. This thesis presents new knowledge about the feeding habits of *Pardosa* spiders obtained by PCR-based gut-content analysis of predators collected in farmers´ fields at several locations. The obtained results are one addition to the growing body of information on the interpretation of data obtained by molecular gut-content analysis of arthropod predators.

5 Methods

5.1 Collection of predators and assessment of natural prey abundances

In 2004 and 2005, adult *Pardosa* spiders of both genders were collected at random from fields of spring-sown cereals at organic farms near Uppsala, Sweden (*Papers II*, *IV*). During 2005, we also collected spiders in leys (*Paper IV*). To reduce the breakdown of the gut-contents to a minimum, captured predators were frozen immediately in the field in individual tubes. In leys, all spiders were collected by hand. In cereals, most spiders were hand-collected as well, but when the vegetation cover was very dense, we also used dry pit-fall traps. To prevent captured arthropods from feeding on each other the traps contained moist paper, which allow smaller animals to find refuges from larger predators. In addition, the traps were open for at most two hours and emptied immediately thereafter.

In the cereal fields, we counted the numbers of *R. padi* on randomly selected shoots (*Papers II*, *IV*) and to assess the availability of springtails and other potential alternative prey we used mini-sticky traps that were randomly anchored to the soil surface (*Paper IV*). The captured alternative prey was identified to the following taxa: springtails (Collembola), Aphidoidea, Diptera, Cicadoidea, Thysanoptera, Hymenoptera, Coleoptera, Araneae and others.

5.2 Laboratory feeding experiments

To ensure that it was possible to detect *R. padi* and springtails in spiders that had consumed either prey, two laboratory feeding experiments were carried out (*Papers II, IV*). The purpose of these experiments was also to investigate the influence of time since feeding on *R. padi* and springtail DNA detection success.

To study the effect of ambient temperature on *R. padi* DNA detection success we used purchased two-spotted lady beetle (*Adalia bipunctata*) as a model predator (*Paper I*). Starved larvae were fed with one live *R. padi* at either 21°C or 14 °C. After ingestion, fed larvae were allowed to digest the prey for various time periods, ranging from 0 up to 24 hours. The behavior of the predators; the time until attack and the feeding time was observed and recorded to within one minute at each temperature.

5.3 DNA extractions

Prior to DNA extraction, all field-collected and laboratory fed-spiders were stored at -70 °C. Once during storage, all predators were briefly handled to determine species and gender. DNA from all arthropods tested in this study was extracted using a commercial kit (*Papers I, II, III, IV*). DNA concentration was assessed on a random selection of samples from each extraction setup.

5.4 PCR assays

When testing spiders for *R. padi* consumption we used a pair of primers that amplify a 331 bp fragment from the mitochondrial COII-gene (Chen *et al.,* 2000) (*Paper II*). The same pair of primers was also used to screen *A. bipunctata* larvae for *R. padi* DNA remains (*Paper I*). To be able to track springtail consumption we developed novel group-specific springtail primers (*Paper III*). Positive and negative controls were included in all PCR runs as a control for amplification failure and contamination among samples.

5.4.1 Development of group-specific springtail primers (*Paper III*)

To find a suitable DNA region for the development of group-specific springtail primers, we screened a number of candidate multi-copy genes in the public database GenBank. Apart from sequences of springtails representing various families and both springtail suborders, we searched for closely related insects and other arthropods that are common in agricultural fields, and thus potential prey for spiders. Multiple sequence alignments

indicated that the 18S rDNA gene contained regions that were well conserved within all selected springtails. When aligning these regions with sequences from nontarget species, potential primer binding sites that would exclude amplification of nontargets were discovered. Two forward and one reverse primer were designed and the two resulting combinations (Col3F/Col5R and Col4F/Col5R) amplified springtail fragments of 272 and 177 bp, respectively. Although the primer binding DNA-sequences were well conserved in the springtail species, a few positions differed among them. Therefore, both forward primers were designed as degenerate, which means that a primer stock contains a mix of different primers that covers all nucleotide variation between the target species. The primers were tested on DNA from 17 springtail species representing 14 genera, eight families and both springtail suborders. The tested springtails had been extracted from soil cores taken in the same fields as used for the collection of spiders (see above). The extracted springtails were identified to at least genus following Fjellberg (1982, 1998).

5.4.2 Cross-reactivity tests on nontarget organisms

To avoid false positive results when screening field-collected spiders, the specificity of the aphid and springtail PCR assays were evaluated by running PCR on DNA extracts from nontarget taxa (*Papers II, III*). All extracts from nontarget species had initially been tested with universal invertebrate primers (LR-J-12887 and LR-N-13398) (Simon *et al.,* 1994; Kasper *et al.,* 2004) to ascertain that they contained amplifiable DNA.

5.4.3 Visualization and scoring of PCR products

All PCR products were separated by agarose gel electrophoresis. The gels were stained with ethidium bromide and the PCR products subsequently visualized under UV light. A sample was scored as positive when a band of approximately 331 bp (aphid amplicon) - or 272 bp (springtail amplicon) appeared on the gel. All negative samples from the first PCR run were tested a second time.

5.5 Sequencing of PCR products

To verify that amplified products from field-collected spiders originated from either *R. padi* COII DNA or springtail 18S rDNA, a selection of PCR products were purified and sequenced by Uppsala Genome Center (*Papers II, IV*). Obtained sequences were compared with sequences in GenBank using the Basic Local Alignment Search Tool (BLAST). In addition to direct

sequencing, some of the purified aphid PCR products were also cloned and subsequently sequenced on both strands (*Paper II*).

6 Results and discussion

6.1 Development of group-specific springtail primers (*Paper III*)

The novel sets of group-specific springtail primers designed from 18S rDNA successfully amplified all 17 tested springtail species. Both combinations yielded strong bands of expected size, i.e. 177 and 272 bp, respectively whereas no cross-reactivity was observed with nontarget taxa (Fig. 5).

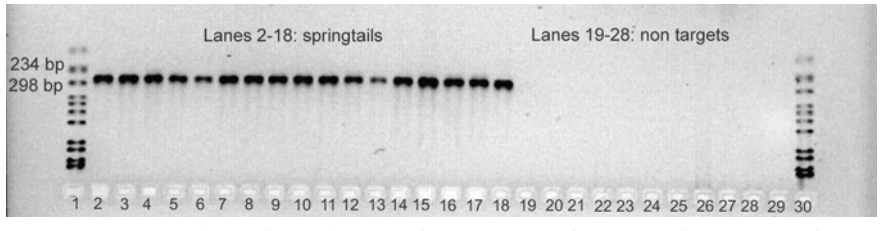

Figure 5. PCR products obtained using the group-specific springtail primers Col3F and Col5R (272 bp). Lanes 2-18 show 17 different springtail species; (2) *Ceratophysella* sp., (3) *Neanura muscorum*, (4) *Protaphorura* sp., (5) *Stenaphorura* sp., (6) *Desoria intermedia*, (7) *D. olivacea*, (8) *Folsomia fimetaria*, (9) *Folsomia* sp., (10) *Isotoma viridis*, (11) *Parisotoma notabilis*, (12) *Entomobrya lanuginosa*, (13) *E. nicoleti*, (14) *Lepidocyrtus* sp., (15) *Bourletiella hortensis*, (16) *Deuterosminthurus sulphureus*, (17) *Sminthurinus sp*., (18) *Sminthurus* sp. Lanes 19-28 show a selection of nontarget species (19) Diplura, (20) Protura, (21) Thysanoptera, (22) Coleoptera, (23) Homoptera, (24) Heteroptera, (25) Araneae, (26) Opiliones, (27) Myriapoda, (28) Annelida. Lane 29, negative control (H_20); Lane 1 and 30 DNA bp size marker.

To optimize detection of semidigested prey DNA, it is necessary to target genes with multiple copies per cell and primers should preferentially amplify fragments no longer than 300 bp (reviewed by King *et al.,* 2008). The primers developed here fulfill both these requirements. Several of the tested springtails are cosmopolitan and our expectation is that they will facilitate

studies of trophic links between springtails and generalist predators world wide. Also, it is probable that the primers amplify additional springtail species, which would extend their application even further.

6.2 Cross-reactivity tests on nontarget organisms

To be a useful tool in studies of predator-prey interactions, it is critical to ascertain that a PCR assay intended for gut-content analysis do not cross-react with DNA from nontarget species. The group-specific springtail assay developed during this thesis did not amplify DNA from any of the 41 selected nontarget invertebrates, which is encouraging (*Paper III*). However, given the myriad of species that can be encountered in the field, we emphasize that the cross-reactivity test list must be tailored for each particular system in which the primers will be applied.

Following the original *R. padi* PCR assay described in Chen *et al.* (2000), products of the same size as the target *R. padi* fragment were amplified from other aphid species (*Paper II*). Consequently, the PCR conditions were altered resulting in a modified protocol. Despite these modifications, amplification of the rose-grain aphid (*Metopolophium dirhodum*) DNA could not be avoided. This was, however, not considered to be a problem because PCR with *M. dirhodum* extracts resulted in a distinct triple band. None of the other nontarget species produced any amplicon of a size similar to the target *R. padi* fragment (*Paper II*).

6.3 Laboratory feeding experiments

6.3.1 Detection success of *R. padi* and springtail DNA in *Pardosa* spiders

To interpret PCR data obtained from field-collected predators it is essential to estimate the length of time after a feeding event that it is possible to detect the target prey in the predator of interest. The laboratory feeding experiment revealed that the detection success of *R. padi* DNA in *Pardosa* spiders was negatively influenced by time since feeding and that ingested aphids rapidly became undetectable (Fig.1 in *Paper II*). After approximately four hours we could detect aphid remains in 50 % of the fed predators and after 8 hours, only 10 % tested positive. The detection period for one springtail in *Pardosa* spiders was less than 24 hours (*Paper III*). Prey DNA remains were detected in 11 out of 12 spiders (92 %) that were frozen immediately after prey ingestion. When the springtail had been digested for 24 hours it was not possible to detect the remains in any of the fed spiders.

For us, when screening field-collected predators, the above presented results meant that positive PCRs would most likely be the result of predation events occurring shortly before capture. A predator that had consumed an aphid or a springtail more than 24 hours before being captured would not give a positive result. Similar results for detection periods of aphid COII DNA were obtained by Chen *et al.* (2000) when testing chrysopid and lady beetle larvae. However, compared to feeding experiments with other lycosid spiders, targeting fragments of approximately the same length, prey DNA in our study became undetectable much more quickly. Hosseini *et al.* (2008) and Ma *et al.* (2005) were able to detect prey DNA in 50 - 80 % of the lycosid spiders fed one 4th instar *Plutella xylostella* (Lepidoptera: Plutellidae) as long as 49 - 72 hours after ingestion. It is possible that meal size, and/or the size ratio between the predator and the ingested prey explain the observed differences in detection periods between this study and the above cited papers. The lycosid spiders tested by Hosseini *et al.* (2008) and Ma *et al.* (2005) both consumed a 4th instar *P. xylostella* larvae which is considerably more food than one *R. padi*.

Juen & Traugott (2006) demonstrated that inhibitory substances present in DNA extracts from soil-dwelling predators can preclude amplification of prey DNA and lead to false negative results. In the cited study, the detection success of prey DNA in predators frozen immediately after consumption was only 56 %. By adding a PCR enhancer (bovine serum albumin) to the reaction mix, inhibition was overcome. As the detection success of *R. padi* DNA in *Pardosa* spiders at time zero was 100 % we feel confident that the short detection time was not caused by false negatives.

Hagler & Naranjo (1997) pointed out that relatively short detection periods of prey proteins may be favorable compared to longer ones (> 24 hours) when analyzing predators feeding under natural conditions. I believe that a short detection interval of prey DNA shares the same advantages. Compared to a short detection period, a long one will less clearly define the period within which predation occurred and may confound several predation events (Dodd *et al.,* 2003). Shorter prey detection intervals may also allow a better understanding of diel feeding cycles (Chen *et al.,* 2000).

6.3.2 The effect of temperature on detection success of *R. padi* DNA in *Adalia bipunctata* (*Paper I*)

Temperature (14 vs. 21°C) clearly influenced *A. bipunctata* feeding behavior; at the lower temperature, the larvae were slower to attack the aphids, and once they did, they took a longer time to complete feeding. Despite these effects**,** no influence of temperature on PCR-based detection of *R. padi*

COII DNA in *A. bipunctata* gut-contents was observed. We therefore pooled the data from both temperature treatments, which revealed a negative relationship between proportion-positive larvae and time since feeding (Fig. 2 in *Paper I*). The time point when *R. padi* DNA could be amplified from 50 % of the fed larvae was equal to approximately five hours.

The lack of significant impact of temperature in our study could be due to the relatively narrow interval studied. It is possible that higher temperatures than 21°C may influence detection success of *R. padi* DNA in *A. bipunctata*. Hagler & Naranjo (1997) observed that digestion at 15, 20 or 25 °C had little or no effect on detection of prey proteins in the predatory bug *Orius insidiosus*. At post meal temperatures ≥ 30 °C there was, on the other hand, a clear decline in the proportion of individuals testing positive. That temperature starts to influence detection success of prey DNA at a certain "threshold" value has also been observed for the springtail *Folsomia candida* (Read, 2007), and the carabid beetle *Pterostichus melanarius* (von Berg *et al.,* 2008a).

Two other studies have investigated the influence of temperature on detection success of prey DNA in lady beetles. In agreement with our results, Hoogendoorn & Heimpel (2001) found no effect of temperature (20°C vs. 27°C) on detection of lepidopteran eggs in larvae of the coccinellid *Coleomegilla maculata*. They did, however, observe an effect of temperature in the adults; digestion at 27 °C significantly decreased the number of detectable prey fragments compared to digestion at 20 °C. Hosseini *et al*. (2008) fed adult *Hippodamia variegata* with *P. xylostella* larvae and subsequently held the predators at 15, 20, 25 or 30 °C for various time periods. The experiment was also carried out with a wolf spider; adult *Venator spenceri*. Interestingly, there was a negative effect of temperature on detection success of *P. xylostella* in the lady beetle but not in the wolf spider, which suggests that that prey digestion in the spider was less affected by increased temperatures. Sopp & Sunderland (1989) similarly demonstrated that digestion of prey proteins is less affected by temperature in linyphiid spiders than in carabid and staphylinid beetles.

If a defined digestion temperature range has proven not to influence PCR-based prey detection, data obtained from predators collected within that particular range can be interpreted in the same way. We investigated a temperature range that is reasonable for field conditions in Sweden, but although *R. padi* DNA was equally detectable in *A. bipunctata* larvae, held at either 14 or 21 °C, we can't be sure that the same is true for *Pardosa*, our study predator in the field. Therefore, we can not rule out that differences in ambient temperature between sites (and between different micro habitats

within sites) may have influenced our results. We believe, though, that the effect is of minor importance. One reason is that prey detection in spiders seems to be more resistant to high temperatures compared to other arthropod predators (Sopp & Sunderland, 1989; Hosseini *et al.,* 2008). Furthermore, all spiders in our study were collected during the same time period of the day during the same part of the growing season in both 2004 and 2005. Also, the plant canopy in both leys and cereal crops was rather dense, which should dampen temperature fluctuations. Von Berg *et al.* (2008a) argued that it is probably more important to consider temperature effects when analyzing predators that have been exposed to high temperature fluctuations than predators living in soils, or epigeic species that hunt beneath dense vegetation. Hosseini *et al.* (2008) pointed out that temperature is important to consider if predators are collected during different parts of the year. To date, no field based study presents temperature data collected during sampling.

6.4 Tracking *R. padi* predation by *Pardosa* in spring-sown cereals (*Paper II*)

A total number of 372 spiders were captured in five fields of spring-sown cereals and analyzed by PCR. Aphid densities were low with means between 0 and 0.28 aphids per shoot and did not differ between sampling occasions (Table 1). Despite low prey availability and the short detection period of the target aphid fragment, many spiders (26 % in 2004 and 19 % in 2005) tested positive for *R. padi* DNA. The percentage of spiders that tested positive varied considerably between sampling occasions ranging from 0 – 58 % in 2004, and 2 – 46 % in 2005 (Table 1). Location (farm) and year affected the probability of detecting aphid DNA in field-collected spiders whereas gender, *Pardosa* species and collection method were not significant (Table 2 in *Paper II*). Cloning and sequencing demonstrated that randomly selected PCR products indeed originated from *R. padi* COII DNA.

Previous studies in this system have shown that *Pardosa* spiders are one of the most abundant groups of natural enemies during aphid establishment (Öberg & Ekbom, 2006). The dietary evaluation carried out in this thesis demonstrates that *Pardosa* spiders exploit scarce pests on a regular basis. Together, these results strongly indicate that *Pardosa* spiders, under certain conditions, are important natural enemies of *R. padi*. Therefore, we argue that for conservation biological control of cereal aphids, specific attention to *Pardosa* is warranted.

Average predation rates of approximately 20 %, as found in the present study, are within the range of results that have been reported from two other molecular studies of predator-prey interactions between natural populations of lycosid spiders and cereal aphids (Sunderland *et al.,* 1987; Chiverton, 1987). Predation on *R. padi* by lycosid spiders in spring-sown cereals has been demonstrated previously with serological techniques (Chiverton, 1987). Spiders collected over five years showed approximately 12 % positive reactions to a polyclonal *R. padi* antiserum. However, no *R. padi* antigen detection time in lycosid spiders was estimated, precluding interpretation about the timing of predation.

One major methodological limitation that is associated with PCR-based gut-content analysis is that the technique can not discriminate between consumption of live prey and dead prey, i.e. scavenging (Juen & Traugott, 2005; Foltan *et al.,* 2005). When evaluating the biological control potential of a particular predator this is clearly a potential source of error. The reason is that predators consuming carrion prey are not contributing to biological control. Such individuals would, however, be scored as positive resulting in an overestimation of the predator's pest suppression capacity. Natural mortality rates of cereal aphids have, however, been proven very low until plants start to mature (Watt, 1979). Because crops had not reached this stage when predators in our study were collected, we do not believe that *R. padi* predation frequencies are overestimated due to scavenging. Later in the season, when aphids begin leaving plants, this might be a problem. Abiotic factors, such as wind and rain, may dislodge aphid from plants and cause aphid mortality (von Berg *et al.,* 2008b). As aphid densities were low, and since most of them occurred near the bases of plants, this mortality factor was likely low in our study.

When using PCR-based gut-content analysis there is also a risk that predation rates are overestimated due to secondary predation, i.e. the study predator eats another predator which in turn has ingested the target prey. Sheppard *et al.* (2005) evaluated the potential error caused by detection of prey DNA following secondary predation in an aphid-spider-carabid model system. The aphid (*Sitobion avenae*) could be detected in 50 % of carabids and spiders as long as 30 and 60 hours after ingestion, respectively. However, only one beetle out of 25 tested positive for aphid DNA eight hours after consuming a spider that had just eaten the aphid. Given that the detection success of the target pest in our study was less than 50 % after only four hours, such a large *relative* decrease in prey DNA detection success suggests that our data can only marginally be influenced by secondary predation.

6.5 Springtails as alternative food for *Pardosa* (*Paper IV*)

Spiders collected in spring-sown cereals 2005 were not only screened for aphid consumption. We also tested them for presence of springtail DNA together with spiders collected in leys the same year. The availability of springtails, Dipterans and other potential prey as monitored by mini-sticky traps varied between sampling occasions (Table 1).

Of all tested spiders (n = 469), 21 % were found to have preyed upon springtails. The percentage of springtail eating spiders per sampling occasion varied between 13 - 30 % (Table 1). There was no difference between the proportion of spiders testing positive for springtail consumption in leys and spring-sown cereals. Therefore, PCR-data from all sampling occasions were pooled and subsequently analyzed together.

Pardosa spiders did not feed on springtails in relation to availability and neither was springtail consumption affected by availability of Diptera or the pooled number of other potential prey. Direct sequencing and BLAST searches demonstrated that selected PCR products, in every case showed highest sequence identity to springtail 18S rDNA. The probability of detecting springtail DNA in field-collected spiders was not affected by sampling occasion, *Pardosa* species, sexual category or collection method.

Some authors have expressed doubts that springtails constitute a significant part of the diet of larger non-web building spiders because these prey items are relatively small and difficult to capture (Harwood *et al.*, 2005; Halaj & Wise, 2002). Here we show that *Pardosa* spiders in our system are quite capable of catching springtails and that these prey items are regularly included in their diet. We suggest that nutrient specific foraging is one explanation as to why springtail predation was so similar at all sampling occasions. There is evidence that arthropod predators, including *Pardosa* spiders, sometimes feed selectively to obtain a diet with an optimal composition of nutrients rather than just forage at random to optimize prey capture rates and the intake of calories (Greenstone, 1979; Waldbauer & Friedman, 1991; Maynts *et al.,* 2005). Our results suggest that the nutritional value of one or several species of springtails is of such importance that a more or less fixed proportion of spiders regularly feed on springtails, even when they are scarce. As the proportion of springtail eating spiders did not increase at relatively higher prey densities it seems, though, that *Pardosa* have to complement springtails with other prey to fulfill their nutritional requirements. One possible explanation as to why spiders are capable of performing nutrient-specific foraging is that these arthropods have very low resting metabolic rates and therefore are extremely well adapted to cope with periods of food shortages (Anderson, 1970; Greenstone & Bennet,

1980). Consequently, spiders may not be "forced" to eat every prey they encounter, rather they can wait until food of suitable quality become available.

In experimental studies, inputs of detrital subsidies have caused a simultaneous increase in springtail and wolf spider abundances (Chen & Wise, 1999; Halaj & Wise, 2002; Oelbermann *et al.,* 2008). It has also been demonstrated that removal of spiders from enclosed areas results in increased springtail populations (Clark & Grant, 1968). Although both situations indicate that spiders are exploiting springtails, experimental studies provide no direct evidence that consumption has occurred. Apart from this work, there is only one previous molecular study that has investigated predation by generalist predators on natural springtail populations. Agusti *et al.,* (2003a) utilized species-specific primers for three different springtails and revealed predation rates between 20 - 38 % by linyphiid spiders collected in a winter wheat field. Furthermore, it was demonstrated that the predators were exercising prey choice; they preferred to feed on the springtail that was least common.

Altogether, the results indicate that springtails are an important source of alternative food for *Pardosa* spiders in Swedish agroecosystems. Thus, presence of springtails may contribute to spider population maintenance and, indirectly, to enhanced predation pressure on low density, co-occurring pests. To support a reproductive numerical response it is, however, necessary that ingested springtails are of high nutritional quality (Marcussen *et al.,* 1999). Group-specific PCR opens possibilities for interesting follow-up studies concerning prey quality; predator testing positive can be re-screened with sets of species-specific springtail primers. By comparing the proportions of springtail species in the predator gut with relative abundances in the field, it can be investigated if the spiders are exercising prey choice. Alternatively, if the aim is to obtain a more complete picture of which prey species that are utilized by the predator, group-specific PCR products can, under certain conditions, be further analyzed by cloning and sequencing (Jarman *et al.,* 2004).

Table 1. *Sampling scheme, mean numbers of available prey, sample sizes and detection of R.padi and springtail DNA in Pardosa* spiders by *PCR-based gut-content analysis (Papers II and IV).*

Habitat and year	Location	Sampling occasion	*R.padi*⋆	Springtails⋆⋆	Diptera⋆⋆	Other potential alternative prey⋆⋆	No of analyzed specimens	% *Pardosa* tested *R.padi* positive	% *Pardosa* tested springtail positive
Leys 2005	Farm A	June 16	-	22.3	3.4	2.0	47	-	13
	Farm B	June 21	-	12.8	23.2	4.5	49	-	27
	Farm C	June 23	-	41.8	8.7	1.3	50	-	16
	Farm D	June 28	-	68.9	19.2	0.9	50	-	20
Cereals 2005	Farm A	June 16	0.05	20.6	21.7	2.1	54	46	30
	¨	June 30	0.09	6.2	18.6	2.0	60	17	17
	¨	July 7	0.3	1.2	15.2	2.4	50	26	28
	Farm B	June 21	0.01	6.7	30.7	1.9	47	4	19
	Farm C	June 23	0	111.1	3.6	3.3	62	2	19
Cereals 2004	Farm A	June 22	0	-	-	-	32	12	-
	¨	June 29	0	-	-	-	22	0	-
	Farm D	June 21	0	-	-	-	24	58	-
	¨	June 28	0.3	-	-	-	21	38	-

⋆ *R. padi* densities are the average number of aphids per shoot. ⋆⋆ Densities of alternative prey were monitored by mini-sticky traps.

6.6 Effects of alternative prey on biological control of aphids (*Paper IV*)

In spring-sown cereals, availability of all alternative prey, springtails, and Diptera differed between sampling occasions. As already presented, aphid predation varied considerably between farms (Table 1). Because aphid densities on plants did not differ between sites, we hypothesized that the variation depended on the availability of alternative prey. By combining the aphid consumption PCR-data with the population monitoring of alternative prey, we found evidence supporting our hypothesis.

There was a negative relationship between the probability of detecting aphid DNA in a *Pardosa* spider and the availability of all captured alternative prey. Subsequent analyses with densities of specific prey groups showed that springtails contributed significantly to the negative effect. Thus, in our system there seems to be a risk that *Pardosa* spiders switch from feeding on aphids when alternative prey are present at high densities and hence that aphid biological control might be disrupted. Apart from our study, the only data on negative relationships between pest consumption and availability of *natural* populations of alternative prey was presented by Harwood *et al.,* (2004). Data from four fields of winter wheat in Great Britain showed that there was a negative relationship between availability of springtails and aphid predation by linyphiid spiders. Our data, however, includes relatively few sampling points (n = five sampling occasions in spring-sown cereals). There was a significant negative relationship between aphid predation and densities of all alternative prey and springtails, but both logistic regression models had low fits. To improve the models more sampling points at intermediate and high alternative prey abundances are needed.

The negative association between aphid predation and springtail availability is puzzling when considering that springtail *consumption* did not increase at sites where springtails were more abundant. *Pardosa* spiders are generally considered as active foragers but there is also evidence that these natural enemies hunt with a sit-and-wait strategy, i.e. the spiders wait motionless for the prey to come within reach, thereafter grabbing it in one rapid movement (Edgar, 1969; Foelix, 1996). We suggest that when densities of alternative prey, which almost exclusively consisted of mobile species (springtails, Diptera and Hymenopera), increased, *Pardosa* spiders became more sedentary and hunted with a sit-and-wait strategy. This would lead to reduced encounter rates with *R. padi* which is sedentary on the plant stem base, at least when aphid densities are low (Wiktelius, 1987), which was the case on all sampling occasions in our study. In contrast, at sites where availability of mobile prey was relatively low, we argue that *Pardosa*

foraged more actively leading to increased encounter rates with *R. padi* and consequently more spiders tested positive for aphids at such sites. Computer simulations carried out by Rosenheim & Corbett (2003) are supporting this explanation; widely foraging predators are predicted to be much more effective in suppressing a sedentary prey than an ambush hunter. This assumption has also received support in experimental studies (Rosenheim *et al.,* 2004; Gavish-Regev *et al.,* 2009).

7 Conclusions

The main conclusions from the results presented in this thesis are:

- *Pardosa* spiders exploit an economically important aphid pest, *Rhopalosiphum padi*, in spring-sown cereals when it is most critical for successful biological control, i.e. at low pest densities.

- Spiders testing positive for *R. padi* DNA most likely consumed the aphid shortly before capture.

- Under certain conditions, *Pardosa* spiders may be important in suppressing *R. padi* populations below economic thresholds. Therefore, *Pardosa* spiders deserve special attention in conservation biological control of *R. padi*.

- The development of the group-specific springtail primers was successful and made it possible to track predator-prey interactions between *Pardosa* spiders and springtails in the field.

- *Pardosa* spiders utilize springtails as an alternative source of food in both annual and perennial agricultural habitats on a regular basis.

- *Pardosa* spiders did not feed on springtails in relation to availability.

- The fact that a high proportion of *Pardosa* spiders regularly feed on springtails in both an annual and a perennial habitat suggests that springtail occurrence may contribute to spider population maintenance and, indirectly, to enhanced predation pressure on recently colonized, low-density pests such as *R. padi*.

- It will be cheap and easy to apply the group-specific springtail primers in other systems and my expectation is that they will facilitate studies of trophic links between springtails and arthropod predators worldwide.

- We found evidence that high abundances of alternative prey, including springtails, may temporarily disrupt biological control of *R. padi* in spring-sown cereals.

- Temperature did not have a significant effect of *R. padi* DNA detection success in laboratory-fed model predators.

8 Future research

The long term goals of our work are 1) to determine the optimum composition and density of generalist predators necessary to achieve durable control of insect pests and 2) to develop guidelines on how to achieve optimal predator populations. Using these goals as a starting point, I present some ideas about future research that will help us realise successful conservation biological control.

Apart from *Pardosa* spiders, other groups of generalist predators, such as linyphiid spiders and carabid beetles, are common in spring-sown cereals during *R. padi* establishment. I suggest that DNA-based gut-content analysis of *R. padi* remains should be carried out for these taxa as well, as it would allow comparisons of the biological control potential of the different predators. To make correct comparisons, it would be necessary to determine the detection period of the aphid COII fragment in linyphiids and carabids.

This thesis is based on observational studies and reports about the magnitude of the proportions of *Pardosa* spiders that exploit *R. padi*. In individual fields as many as half of the collected spiders had consumed the aphid. But what does this mean in terms of aphid control? To answer this question I suggest that molecular gut-content analysis could be combined with experimental exclusion of predators from *R. padi* infested plots. With a careful design it would be possible to estimate how large the proportions of predator populations consuming the aphid need to be in order to keep the pest populations below economic thresholds. We did not assess predator abundance in the present studies, but it will be necessary to consider this factor in future work.

I have conducted single-plex PCRs, which means that DNA from only one type of prey has been amplified in each run. In future work, I suggest that the potential for performing a multiplex PCR assay, in which DNA remains from several prey types can be amplified simultaneously, should be

examined. Apart from tracking predation of both pest and alternative prey in the same run, it would be meaningful to include primers that reveal if generalist predators are feeding on each other. Do for example, *Pardosa* spiders consume small carabid beetles or linyphiid spiders during the aphid establishment phase or more specialized enemies, such as lady beetles, lacewings and syrphid larvae, later in the season? When predators feed on each other it is called intraguild predation (IGP) and this phenomenon is important to consider as it may interfere with biological control and lower the predators' status as pest suppressors. Primers for predators need to be developed and the detection periods of the intraguild prey have to be determined. It is reasonable to believe that IGP is more common when predator densities are high. It is also possible that generalist predators feed more on each other if there is a lack of alternative prey in the habitat. Therefore, when IGP is studied it will be important to assess predator densities in addition to availability of alternative prey.

This thesis demonstrates that springtails are utilized by adult *Pardosa* spiders during the period when *R. padi* is present in the field. To get a more complete picture of the importance of springtails as alternative food for *Pardosa,* I suggest that future research should test the hypothesis that springtail consumption in the autumn is important for winter survival. If an initial screening with group-specific primers reveals that juvenile spiders utilize springtails after harvest, it would be meaningful to elucidate if they prefer some springtails over others. One way to approach this question is to determine the availability of individual springtail species in the field, design species-specific primers for abundant as well as less common species, and then re-screen spiders that tested positive with the group-specific primer set. By comparing the ratios of the springtail species in the field with the number of spiders that tested positive for each of those species, it would be possible to examine if the spiders prefer some springtails over others. Subsequently, spiders could be fed preferred species under laboratory conditions and then be kept outside in individual cages during winter. Survival of these spiders should be compared with spiders subjected to starvation or other diets.

Further, I suggest that the hypothesis that the presence of springtails attracts generalist predators into cereal crops early in the season should be tested. Our results suggest that higher numbers of alternative prey may lead to lower per capita rates of *R. padi* consumption. However, if densities of generalist predators increase, then the net effect on pest control may still be positive. One way to approach this question is by computer simulations/modeling. In the field, predator activity densities could be

compared between large, open cereal plots with enhanced availability of springtails and unmanipulated control areas. Aphid and springtail consumption by generalist predators in the different treatments should be compared and the effect on aphid densities and crop yields could ultimately be assessed.

We suggested that *Pardosa* spiders may change their foraging behavior when proportions of sedentary and mobile prey change. This hypothesis is important to test as it will influence the outcome of conservation biological control. Possible methods would be to vary availability of different types of prey in cage experiments.

Using conventional PCR, it is generally not possible to obtain a quantitative measure of how much prey a predator has consumed. The results indicate if prey DNA is present or not, and together with the detection period one can conclude that a predation event must have occurred within a specific time frame. Recently, attempts have been made to apply quantitative PCR (qPCR), in studies of arthropod predation (Zhang *et al.,* 2007; Schmidt *et al.,* 2009; Weber *et al.,* 2009). Given that an extensive and careful calibration of the assay has been carried out, qPCR can, ideally, provide an estimate of the amount of undigested prey in the predator gut-contents at the moment of analysis, but it does not reveal how large amounts, or how many prey items, the predator consumed in the field (King *et al.,* 2008).

In general, I believe that DNA-based gut-content analysis will be a valuable complementary tool in many future projects concerning conservation biological and help us to generate knowledge that would have been impossible to obtain using "non molecular" approaches.

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Översikt på svenska

DNA-teknik spårar interaktioner mellan vargspindlar, havrebladlöss och hoppstjärtar

Jordbruksgrödor angrips ofta av skadeinsekter vilket kan orsaka stora skördeförluster. I odlingslandskapet finns naturligt förekommande predatorer som äter skadeinsekter. Dessa så kallade naturliga fiender bör bevaras och gynnas, eftersom de kan begränsa skadeinsekternas populationstillväxt och på så sätt indirekt minska skadorna på växten. För att gynna de predatorer som är mest effektiva behövs detaljerad kunskap om deras födointag. Att med blotta ögat se vad ett leddjur äter är emellertid en utmaning; predatorn och bytet är ofta små, rörliga och/eller lever bland tät vegetation. Genom att fånga in predatorer och analysera vad de har i magen kan man dock övervinna dessa problem.

Havrebladlusen (*Rhopalosiphum padi*) kan orsaka stora skördeförluster i vårsådd spannmål. För att naturliga fiender ska kunna begränsa havrebladlusens populationstillväxt måste de hitta och äta upp lössen tidigt på säsongen, d.v.s. när skadeinsekterna fortfarande är få till antalet. I denna avhandling har jag undersökt i vilken mån marklevande vargspindlar från det vanliga släktet *Pardosa* äter havrebladlöss i vårsådd spannmål.

Vargspindlar äter emellertid inte enbart bladlöss, de är allätare och kan konsumera många olika slags byten. En potentiell källa till så kallad alternativ mat för *Pardosa* är hoppstjärtar; små leddjur som ofta förekommer i stort antal i jordbruksmark. Att *Pardosa* äter andra byten än havrebladlöss kan, från en lantbrukares synvinkel, innebära både för och nackdelar. Alternativ mat kan å ena sidan förse spindlarna med näring, förbättra deras kondition och på så sätt bidra till att upprätthålla en stor spindelpopulation på gården. Men om den alternativa maten, t.ex. hoppstjärtar, är mer åtråvärd än havrebladlusen kan god tillgång till alternativ mat innebära att predationen på skadeinsekten

minskar. I denna avhandling har jag undersökt betydelsen av hoppstjärtar som alternativ mat för *Pardosa* i vårsådd spannmål och i vallar. Jag har också studerat hur förekomst av hoppstjärtar och andra alternativa byten påverkar spindlarnas intag av havrebladlusen.

Samtliga studieorganismer är små (< 1 cm) och lever bland tät vegetation. Vargspindlar och hoppstjärtar är dessutom mycket rörliga. Därför är det svårt att studera interaktioner mellan *Pardosa*, havrebladlöss och hoppstjärtar direkt i fält. För att kunna ta reda på vad vargspindlarna äter fick jag istället samla in dem och söka efter DNA-rester av havrebladlöss och hoppstjärtar i deras maginnehåll med hjälp av så kallad PCR-teknik. I de fält där spindlarna samlades in uppskattade jag också förekomsten av de båda bytesdjuren.

För att kunna detektera rester av uppätna byten i en predator med PCR måste man ha tillgång till så kallade primers. Primers är korta artificiellt tillverkade DNA-sekvenser som under själva analysen specifikt skiljer ur och parar ihop sig med unika delar av bytets DNA. Primers för havrebladlusen fanns att tillgå men för att kunna studera interaktioner mellan *Pardosa* och hoppstjärtar utvecklade jag nya primers som en viktig del av projektet. Denna avhandling innehåller vidare resultat från laboratorieförsök där vi studerade hur lång tid efter en måltid som det gick att detektera DNA-rester av havrebladlöss och hoppstjärtar i magen på *Pardosa*. Utan sådan kunskap är det omöjligt att veta om en positiv PCR-reaktion är resultatet av en måltid för flera dagar sedan eller strax före infångandet. Resultaten visade att lusens och hoppstjärtens DNA bröts ned snabbt i spindlarna. I praktiken betydde det att vi enbart skulle kunna detektera predation som ägt rum några få timmar innan en spindel fångades in. Om spindeln däremot hade ätit en lus eller en hoppstjärt t.ex. ett dygn tidigare så skulle vi inte kunna spåra det. Vi har också studerat hur nedbrytningsprocessen av havrebladlusens DNA påverkas av omgivningstemperaturen. Detta experiment utfördes med en modellpredator; den tvåprickiga nyckelpigan *Adalia bipunctata*.

Det fanns få havrebladlöss i samtliga insamlingsfält men trots det hade många vargspindlar (26 % 2004 och 19 % 2005) ätit skadegöraren. I vissa fält hade cirka hälften av alla analyserade spindlar ätit havrebladlöss. Att så många spindlar testades positiva pekar på att *Pardosa*, under vissa förhållanden, kan vara betydelsefulla predatorer med potential att begränsa havrebladlusens populationstillväxt. Resultaten från fältstudierna visade också att *Pardosa* regelbundet konsumerar hoppstjärtar både i spannmålsgrödor (22 % positiva) och i vallar (19 % positiva). Detta indikerar att förekomst av hoppstjärtar kan bidra till att upprätthålla stora spindelpopulationer. Om spindlarna blir fler kan det i sin tur innebära att predationstrycket på havrebladlusen ökar. Resultaten pekar emellertid också på att det, temporärt, finns en risk att färre

Pardosa äter havrebladlöss i fält där hoppstjärtar och andra alternativa byten är vanligt förekommande.

Sammanfattningsvis så har mitt arbete genererat ny kunskap om vargspindlars födovanor och det visar att DNA-baserad maganalys är ett användbart verktyg när man vill spåra specifika interaktioner mellan leddjur i naturliga system. Resultaten bidrar till att öka vår förståelse kring betydelsen av naturliga fiender som skadedjursbekämpare i jordbruksgrödor.

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