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DIETS OF LADYBIRD BEETLES (COLEOPTERA: COCCINELLIDAE)

IN UTAH ALFALFA FIELDS

by

L. Nicole Davidson

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Biology
(Entomology)

Approved:

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UTAH STATE UNIVERSITY
Logan, Utah

2008

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ABSTRACT

Diets of Lady Bird Beetles (Coleoptera: Coccinellidae)
in Utah Alfalfa Fields

by

L. Nicole Davidson, Master of Science

Utah State University, 2008

Major Professor: Dr. Edward W. Evans
Department: Biology

Aphidophagous lady beetles rely on multiple sources of food in their environment. Alfalfa fields provide both aphids and many alternate foods, such as other arthropod prey, pollen, and fungi. Alfalfa fields (*Medicago sativa* L.) in Utah have low aphid densities, which may require lady beetles to consume alternative sources of food. Many methods can be used to determine these diets; frass analysis is used here to compare the diets of the introduced species *Coccinella septempunctata* L. with two native species, *C. transversoguttata richardsoni* Brown and *Hippodamia convergens* Guérin-Ménéville, that occur in the Utah alfalfa habitat.

In initial laboratory experiments to examine the feasibility of frass analysis, 48 hours at 20°C was sufficient time for adult lady beetles to pass prey cuticle through their guts. When consumed by these adults, pea aphids (*Acyrtosiphon pisum* [Harris]), alfalfa weevil larvae (*Hypera postica* [Gyllenhall]), and *C. septempunctata* larvae produced distinctive fragments in the frass. Such fragments could also be distinguished in frass

collected in a field experiment in which aphid densities in plots of alfalfa were manipulated. Furthermore, additional consumed foods could be distinguished in the field experiment, including pollen, fungi, and other types of arthropods.

Frass analysis demonstrated higher use of aphid prey by *C. septempunctata* adults collected from high versus low aphid density plots during the field experiment. Use of other types of prey, such as alfalfa weevil larvae, other arthropods, pollen and fungi, was similar between plots with high and low aphid densities.

A field census was performed over two years to track the diets of the three species of lady beetles during the first crop of alfalfa, when two sources of prey in particular were present, aphids and alfalfa weevil larvae. Comparisons of diets revealed that the three species utilized different types of prey to similar degree during both years. In general, however, higher percentages of *C. septempunctata* adults were found to have consumed aphids and weevils during both years. Also, *C. septempunctata* was found to produce more frass and consume larger quantities of prey than either native species during the second year.

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L. Nicole Davidson

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CHAPTER 1

INTRODUCTION

In this thesis, I use frass analysis (dissection and examination of insect frass) to examine the diets of aphidophagous lady beetles in alfalfa fields (*Medicago sativa* L.) of northern Utah. Here I first review the biology and feeding habits of these predators, and the techniques and methods (including frass analysis) that have been developed to study the natural diets of lady beetles and other insect predators.

Aphidophagous Lady Beetles

Aphidophagous Coccinellini (Coleoptera: Coccinellidae, subfamily Coccinellinae) are common and important members of predatory guilds in a variety of agricultural and natural habitats. Despite their preference for aphid prey, these lady beetles will readily consume a wide range of food items, including other types of arthropod prey and foods such as pollen, nectar, and fungal spores (Hodek 1973, 1996). Because of their polyphagous nature, these lady beetles are a critical element in the biological control of pest species in agricultural environments. Their ability to persist in the environment prior to the arrival of aphid pests, or during periods of low pest densities, may enhance their suppressive impact on aphid pests more than specialist predators could exert (Elliot et al. 1996, Harwood and Obrycki 2005).

The many types of food consumed by these aphidophagous lady beetles are placed into two categories: essential and alternative prey (Hodek 1962, 1996; also termed nursery and food prey by Dixon 2000). Essential prey is characterized as that which will support the growth and development of larvae to adulthood, as well as the

development of ovarioles by females and ultimate production of another generation through egg-laying. Aphids are readily recognized as being essential or nursery prey for these lady beetles, though different species of aphids vary in their suitability for particular lady beetle species (Hodek 1973). Aphid populations have a tendency to be ephemeral, with patchy distributions and unstable population dynamics (Agarwala et al. 1998). Many aphid species build to high numbers slowly after lady beetles colonize aphid habitats following winter hibernation. Additionally, the migration of some aphids between plant species can produce local extinctions of aphid prey sources for lady beetles (Hagen 1962). Because of these constraints on aphidophagous lady beetles, alternative foods can fill an important dietary void. The use of such food resources can allow lady beetles to continue to survive by filling their basic metabolic needs. Non-aphid foods are useful in maintaining female lady beetles in a state of reproductive readiness (Hodek 1996, Dixon 2000) by limiting their use of fat body reserves or re-adsorption of eggs for survival. This means that when sufficient aphid prey is once again available, they will be able to resume reproductive activities quickly (Evans et al. 2004, Evans and Gunther 2005).

Lady beetle species may differ in how readily they use the variety of alternative foods available to them in the environment. While many laboratory experiments have demonstrated that lady beetles will consume a broad suite of arthropods as prey, these experiments are generally conducted under periods of starvation, and may not reflect the lady beetle's diet in their natural environment (Putnam 1957, Triltsch 1999). Much work has been done in laboratory and field studies to describe the dietary breadth of aphidophagous lady beetles. Lady beetles have been documented to feed on a variety of

arthropods, including Thysanoptera, Acari, the larvae and eggs of multiple orders (including Diptera, Coleoptera, and Lepidoptera), as well as Coccoidea, and other members of Sternorrhynca (Putnam 1957, 1964; Hodek 1973, 1996; Triltsch 1997, 1999; Triltsch and Freier 1998; Kalaskar and Evans 2001; Ricci and Ponti 2005; Ricci et al. 2005). In addition, they have been found to rely extensively on non-arthropod foods such as plant pollen, floral and extra-floral nectaries, fungal spores, and conidiospores of common plant pathogens, all of which are readily available in their environment (Putnam 1964; Hodek 1973, 1996; Hemptinne and Desprets 1986; Triltsch 1997, 1999; Triltsch and Freier 1998; Lundgren et al. 2004, 2005; Ricci and Ponti 2005; Ricci et al. 2005).

While a seemingly broad list of arthropods is potentially utilized as prey by these lady beetles, such a list cannot adequately reflect what is being consumed at any given time in the field, or in a given environment. Adult lady beetles must balance their diets between “alternative foods” that will allow them to survive and “essential foods” that will allow them to reproduce (Hodek 1962, 1996; Dixon 2000). Interest in understanding the interplay between predatory species and their available prey has increased of late. By better understanding the diets of predaceous insects, an enhanced understanding of ecological links and complex interactions that drive some species to dominate their guild could be attained. Many methods are available to study the diets of invertebrate predators in general, and can be applied specifically to aphidophagous lady beetles.

Methods of Characterizing Invertebrate Diets

Many techniques (reviewed by Sunderland 1987, Powell et al. 1996, Symondson 2002, Harwood and Obrycki 2005) have been employed to determine the foods consumed by generalist predators. These can be broadly characterized as techniques that are performed on live subjects or through post-mortem analysis after feeding events. These techniques can range from those that are simple and low-cost to those requiring technical skill and high cost inputs. Most of the methods dealing with live subjects will be discussed first, followed by methods dealing with post-mortem analysis. An additional method utilized on live subjects to determine predation after feeding events take place (frass analysis) will be discussed last as it is applied to this research project.

One of the most basic techniques utilized on live subjects is direct observation in the field. This method can be performed via observers stationed in fields, or with predation events captured with video surveillance. Direct observation is hampered by the time required to observe individuals eating, and the inability to collect large amounts of data. It also has the potential to overemphasize prey that may ultimately be rejected by the predator (Harwood and Obrycki 2005). This technique is especially suitable for sit-and-wait or sedentary predators and prey (Sunderland 1987). Direct observation can provide useful information, however, such as the handling time required by the predator for particular types of prey, as well as a range of potential prey the predator may use in the field (Sunderland 1987).

Another technique often utilized on live subjects is the use of laboratory feeding experiments wherein the suitability of typical prey available in the field is contrasted with the predators' ability to survive, or complete development and reproduction while eating

the prey. These types of feeding experiments can also determine whether a predator is capable of capturing and subduing the prey in order to consume them (Harwood and Obrycki 2005). Laboratory experiments are hampered by their artificial nature, but do provide insight into whether or not a particular type of prey is a suitable food for a generalist predator.

Cage experiments in the field utilizing live subjects are another alternative to study the interactions between particular predators and prey. These types of experiments, while they can be used to test specific hypothesis about predator/ prey interactions, can be hampered by their artificial nature. By limiting the types of prey available, cage experiments can artificially increase predation on target prey as the full suite of alternatives are not present. If predator densities are too high, interference between predators may complicate their use of the prey available, and in turn complicate conclusions drawn from results (Sunderland 1987).

Many post-mortem techniques have been developed that can overcome the obstacles presented by the above types of studies. One such method is gut dissection. In this technique the crop, proventriculus, mid and hind gut are removed and inspected for diagnostic fragments of different types of prey, as well as an assessment of feeding based on how full the gut is with liquid or solid food (Powell et al. 1996). This method has the benefit of describing multiple types of prey within the predator's gut, but is only viable to determine the dietary range based on solid, indigestible remains of its prey. Additionally, care must be taken during collection and preservation of the predator so that expulsion of food from the gut through regurgitation or voiding frass does not take place (Powell et al. 1996). This method can make interpretation of results complex, as the ability of the

person performing the technique to identify the contents of the gut can vary, and is not generally quantified (Sunderland 1987). Also it can be difficult to quantify the amount of biomass consumed of the different types of prey. Nonetheless, gut dissection has proven to be a valuable asset in determining dietary breadth of many arthropods, including Coccinellidae (Hemptinne and Desprets 1986; Ricci 1986; Triltsch 1997, 1999; Triltsch and Freier 1998; Hoogendoorn and Heimpel 2004; Lundgren et al. 2004, 2005; Ricci and Ponti 2005; Ricci et al. 2005) as well as other predators belonging to Carabidae, Staphylinidae, Opilione, and Dermaptera (Harwood and Obrycki 2005). This technique is also low-cost, requiring little in the way of laboratory equipment and supplies.

Several chemical, serological, and molecular techniques have been employed post-mortem on predatory arthropods to discover their diets. Electrophoresis is one example, and has been used on Anthocoridae, Notonectidae, and Staphylinidae, as well as Acari (Powell et al. 1996, Harwood and Obrycki 2005). This technique is less expensive than other molecular and chemical methods, but necessitates careful sampling of predators so that cross contamination with prey does not occur. Electrophoresis uses an electric current to separate proteins based on their size and shape (Powell et al. 1996). The ability to determine what prey proteins are present in the predator homogenate depends entirely on whether the prey consumed will produce specific bands of proteins that do not overlap with proteins of alternative prey (Sunderland 1987, Harwood and Obrycki 2005). This technique can give an idea of quantities consumed based on the intensity of the stain used to visualize the protein bands, if an assay has been performed previously to correlate stain intensity (based on protein concentration) to specific amounts of prey consumed (Harwood and Obrycki 2005). Also, this technique allows

the determination of prey consumed by biting/ chewing and fluid-feeding predators. A potential deterrent to use of this technique is the nature of the chemicals used, many of which are known carcinogens, or highly toxic (Powell et al. 1996).

Another, but lesser used, chemical technique is to measure the isotopic carbon (C) and nitrogen (N) ratios of predators and compare these to prey available from different C and N sources. This technique was used to determine how particular species of Coccinellidae are utilizing habitat, specifically as foraging sites in consumption of plant pollen (Ostrom et al. 1997) and aphids (Prasifka et al. 2004). Broad generalizations can be made about the source of the predators' diets (i.e., prey or plant material from one agricultural crop versus another, based on C and N signatures), but this method cannot provide information about the specific prey consumed, or the quantities of prey consumed (Harwood and Obrycki 2005).

Serological techniques utilize polyclonal or monoclonal antibodies and some type of immunoassay to determine presence of the prey antigen in the predator (Powell et al. 1996, Harwood and Obrycki 2005). The most common immunoassays performed have been enzyme-linked immunosorbent assay (ELISA), with many different styles used to varying success. Polyclonal antibodies are developed by injecting prey extracts into vertebrates and allowing an immune response to develop wherein antibodies are formed and subsequently extracted for use. This results in a mix of multiple antibodies that must be tested for cross-reactivity against proteins common throughout insects, including target predators and non-target prey. Because the mix of antibodies present in the anti-sera can be difficult to characterize, and the potential for individual vertebrates to produce different antibodies to injected prey extracts, polyclonal antibodies are limited in

their reproducibility (Powell et al. 1996, Harwood and Obrycki 2005). Additionally, because of the lack of specificity that they may offer, polyclonal antibodies provide only a crude representation of predation (Harwood and Obrycki 2005). Monoclonal antibodies provide a more sensitive test, as they are comprised of a single clone of one type of antibody to specific proteins present in the prey. These are considerably more complex to derive and may require an entire year to produce a clone, with no guarantee of success (Chen et al. 2000). Monoclonal antibodies are grown in vitro in hybridoma cell lines and as such require cell culturing facilities to produce them (Symondson 2002, Harwood and Obrycki 2005). Because of this extensive characterization, monoclonal antibodies are reproducible and can target any taxonomic level including species or the specific stage of a species (Symondson 2002). ELISA allows for quantification of time elapsed since a predation event and/ or of the amount consumed by the resulting color intensity achieved in the test (Symondson 2002, Harwood and Obrycki 2005). Considerable effort has gone into modeling this relationship, and polyclonal or monoclonal antibodies have been used on many types of predators including Carabidae, Staphylinidae, Aranaea, Coccinellidae, Geocoridae, Anthocoridae, and Miridae, as well as many other arthropods to determine predation on a wide variety of prey (Sunderland and Sutton 1980; Turner 1984; Sunderland et al. 1987; Sopp and Sunderland 1989; Symondson and Liddell 1993; Hagler and Naranjo 1997, 2004, 2005; Agusti et al. 1999; Harwood et al. 2001; Naranjo and Hagler 2001). Serological techniques can be used on predators regardless of their manner of feeding, but antibodies can be difficult and expensive to produce. Also, many researchers have found that each species of predator requires characterization, as antigen

decay rate varies widely between even closely related species (Sopp and Sunderland 1989, Symondson and Liddell 1993, Symondson 2002).

An emerging molecular technique is the use of deoxyribonucleic acid (DNA) polymerase chain reaction (PCR) to determine predators' diets. This generally targets multiple copy sequences of differing lengths of prey DNA in homogenized extracts of the predator, and can target specific species of prey rather than general groups of prey (Zaidi et al. 1999). An advantage of PCR is that the DNA is amplified, and so the test is sensitive to even very small amounts of prey consumed (Symondson 2002, Juen and Traugott 2005). While start-up costs can be prohibitive, they can be much less than for other techniques such as ELISA, and in general, most research institutions have PCR facilities and many PCR kits are available for specific insect species' primers (Chen et al. 2000, Symondson 2002). PCR requires knowledge of potential prey species so that specific DNA primers can be selected. There is also the option to identify unknown DNA in the guts of predators by cloning and sequencing the DNA present and matching it with a basic local alignment search tool or "BLAST" search of databases of nucleotide sequences, such as with the National Institute of Health's (NIH) open access database GenBank (Sheppard and Harwood 2005). Many of the PCR tests of predator diets have focused on single species or types of prey, which does not account for alternative prey sources, or dietary breadth of generalist predators. Another option available is multi-plex PCR which simultaneously amplifies the DNA of several target species at one time. While this overcomes the problems of overemphasizing one target prey, multi-plex PCR can be hard to implement in large-scale studies because of monetary costs and time required (Sheppard and Harwood 2005).

A problem, in general, with all PCR techniques has been estimating the amount of prey consumed as well as the time elapsed since consumption (Hoogendoorn and Heimpel 2001, Harwood and Obrycki 2005, Sheppard and Harwood 2005). Because even very small amounts of DNA are amplified, PCR may produce a historical perspective of what the predators are consuming rather than insight into recent predation events (Sheppard and Harwood 2005). To compare prey consumption among multiple species of predators, consumption rates must be characterized because each predator can have strikingly different digestion rates of prey DNA (Sheppard et al. 2005). While this technique has not been applied to a wide variety of field studies, it has been tested on Carabidae (Zaidi et al. 1999, Foltan et al. 2005, Harper et al. 2005, Juen and Traugott 2005, Sheppard et al. 2005), Lycosidae and Nabidae (Ma et al. 2005), Coccinellidae (Chen et al. 2000, Hoogendoorn and Heimpel 2001), a variety of Aranea (Agusti et al. 2003, Greenstone and Shufron 2003), Miridae (Agusti et al. 2000), and Chrysopidae (Chen et al. 2000). PCR techniques are appropriate to assess the diets of biting/ chewing and fluid-feeding predators.

An additional technique is the use of fecal (i.e., frass) analysis carried out on live subjects after feeding events. Frass analysis has been used in two different ways to assess predation. In one technique, the quantity (weight) of frass pellets produced was used to estimate the amount of predation on aphids by a variety of species of Coccinellidae (Honěk 1986). This technique did not document actual consumption of aphids in the field, and also could not assess the impact of sources of alternative prey on the lady beetles' diet, or the impact of these prey sources on frass production (Honěk 1986). A similar technique was employed with harvestmen (Opilione) (Phillipson 1960), but again

the types of prey consumed were not identified; only the weights of fecal material produced provided an assessment of predation. When frass analysis is used in this manner, the speed of digestion of different types of prey could make interpretation of results difficult, particularly if only the weight of frass produced is assessed (Sunderland 1987).

Another method of frass analysis is similar to gut dissection; save that the analysis is on prey remains contained in the frass pellet, rather than the gut. An important distinction between frass analysis and the other molecular and dissection techniques is that the predator does not need to be sacrificed, and potentially larger sample sizes can be collected without disturbing the natural populations of potentially beneficial predators. As with gut dissection, this method is only possible if the organism consumes indigestible pieces of its prey. In the past this technique has been used on a variety of slug species (with predominantly herbivorous diets, though some arthropod fragments were found; Pallant 1969), odonate nymphs (Lawton 1970, Thompson 1978), and coccinellids (Putnam 1964) of a variety of species. Some drawbacks to this technique, in addition to the inability to use it on fluid-feeders, are that quantitative data on prey consumed can be difficult to collect (Harwood and Obrycki 2005). When the technique was used for odonates, prey remains were sufficiently whole to allow separation into categories based on length and width of prey. Biomass of each prey size category could be assessed (Lawton 1970, Thompson 1978). This assumed that the odonates were consuming their prey whole and intact. With Coccinellidae, while some very small prey can be found intact (such as mites and small aphids), it was found to be more feasible to quantify approximate numbers consumed by counting the number of cheliceral plates from mites,

or rostra and tarsi of aphids contained in the frass as an index of predation rate (Putnam 1964).

Ultimately the choice of methods used to determine the diet of generalist predators should consider the study organism, the types of prey expected to be included in the diet, the need for qualitative and quantitative data, and the time and financial constraints of the project (Sunderland 1987, Powell et al. 1996, Symondson 2002, Harwood and Obrycki 2005). Many of the chemical, serological, and molecular methods are best suited to systems comparing use of single types of prey by multiple predators because of the difficulty of performing multiple tests on one sample. Multi-plex PCR does provide an alternative if multiple prey types are to be assessed. Nonetheless, multi-plex PCR could prove difficult and costly if primers are not readily available for all prey to be assessed. Additionally, if quantitative data are necessary, PCR could require extensive experimentation to develop methods to determine quantities of prey consumed and time elapsed since consumption to calibrate data collected from the field. Frass analysis and gut dissection provide realistic alternatives for studies where access to equipment needed for chemical, serological, and molecular methods is limited. Additionally, these methods require little specialized equipment other than dissecting or compound microscopes, and as such, have a much lower cost (Sunderland 1987, Powell et al. 1996). For any method chosen, if care is taken there is the potential to develop solutions to quantify amounts of prey consumed. All methods can provide qualitative data on the types of prey incorporated into a predator's diet.

Development and Use of Frass Analysis to Compare Diets of Lady Beetles in Alfalfa

Several species of lady beetles utilize the alfalfa habitat to forage for food and carry out reproduction (Evans 2004). In recent years, pea aphid (*Acyrtosiphon pisum* [Harris]) densities have been very low in northern Utah alfalfa fields, likely requiring the extensive use of alternative food sources within the fields by lady beetles. A wide variety of alternative food is available, including both arthropod prey and non-arthropod foods such as pollen and fungi. Alfalfa fields may also provide large populations of one particular type of arthropod prey to these lady beetles, the larvae of the alfalfa weevil (*Hypera postica* [Gyllenhal]) (Richards and Evans 1998, Evans and Gunther 2005), which these lady beetles have been known to readily incorporate into their diets (Evans and England 1996, Evans 2004; Kalaskar and Evans 2001 see also references within).

As this complex of lady beetles must share the alfalfa habitat, species could interact with one another in ways that affect the diets of individual species. Intra- and interspecific consumption (i.e., cannibalism and intraguild predation) of eggs and larvae could occur because of the spatial and temporal overlap in the alfalfa habitat (Burgio et al. 2002, Schellhorn and Andow 1999), particularly if the preferred aphid prey occurs in low densities. Additionally, competition between the species present for resources may result in differences among the many lady beetles species in their use of aphid, alfalfa weevil, and other food resources in the field.

In recent years, the introduced *Coccinella septempunctata* L. has become abundant in alfalfa while native species have declined in abundance (Evans 2004; see also Elliot et al. 1996, Obrycki et al. 1998, Turnock et al. 2003, Hoogendoorn and

Heimpel 2004). By documenting the use of prey by lady beetles, comparisons could be drawn that might provide insight into the rise in dominance of *C. septempunctata* relative to its native counterparts, particularly in the alfalfa fields of northern Utah.

For the thesis research presented here, frass analysis was chosen as a suitable technique to determine diets of lady beetles in alfalfa. This choice was made for a number of reasons. Frass analysis has been used less often than other methods, but provides a simpler alternative to gut dissection. Additionally, lady beetles used during experiments would not have to be sacrificed and could be either returned to the field or utilized for other experiments. Given that lady beetles are a beneficial insect, being able to return them to the field once experiments are completed is an asset in favor of the frass analysis method. Lady beetles are well suited to the technique as well, because they are biting/ chewing predators which do ingest fragments of their prey. The technique has been used in the past with success at differentiating types of prey consumed by lady beetles in peach orchards (Putnam 1964).

Although testing at the outset of this research project would need to be undertaken for the specific prey present in alfalfa, the overlap of similar types of prey between alfalfa and peach orchards (aphids, Collembola, Thysanoptera; Putnam 1964) suggested that frass analysis would likely be a suitable method to determine the use of prey by lady beetles in the alfalfa habitat. Chapter 2 presents the results of that initial testing, and Chapter 3 presents the results of applying frass analysis to examining the diets of adult lady beetles in alfalfa fields. In particular the objectives of this study were to test the utility of frass analysis in the alfalfa habitat with lady beetle predators. Additionally the method would be used to compare the field diets of the introduced *C. septempunctata*

with native lady beetle species. As demonstrated in these chapters, development of the technique of frass analysis for particular field settings allows for comparison of the dietary breadth of common lady beetle species with relatively low monetary input, and it enables conclusions to be drawn concerning the similarities and differences among the diets of individual species.

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CHAPTER 2

THE UTILITY OF FRASS ANALYSIS TO DECIPHER

THE DIETS OF APHIDOPHAGOUS

LADY BEETLES

Abstract

Many methods have been used to evaluate the diets of insect predators in the field. One promising method for predators that ingest pieces of their prey is frass analysis. Aphidophagous lady beetles are candidates for this technique, as they are biting/ chewing predators that eat a variety of foods, especially when their preferred aphid prey is scarce. The aim of this study was to test the utility of frass analysis for determining the diet of *Coccinella septempunctata* L., an aphidophagous lady beetle that also consumes alfalfa weevil larvae (*Hypera postica* [Gyllenhall]) in alfalfa fields (*Medicago sativa* L.). Laboratory experiments at 20°C revealed that within 48 hours after consumption of prey, almost all cuticular fragments had been voided. Feeding experiments demonstrated that diagnostic cuticular fragments could be readily distinguished for specific types of prey (aphids, weevil larvae, and conspecific larvae). A field experiment was conducted to compare consumption rates of aphids and weevils by *C. septempunctata* in areas of high and low aphid densities. Pea aphids (*Acyrtosiphon pisum* [Harris]) reared in a greenhouse were added to half of the experimental plots. All plots were sprayed with sugar-water, to attract larger sample sizes of lady beetles. Frass analysis of adult lady beetles showed that consumption of alfalfa weevil larvae, pollen, and fungi was similar between aphid treatments and sexes of *C. septempunctata*. The

percentage of individuals utilizing aphids as prey was higher in plots with increased aphid densities; however, the highest densities were insufficient for beetle satiation. Consumption of weevils (detected frequently) did not differ significantly in plots with high versus low aphid density. These laboratory and field results demonstrate the utility of frass analysis as a technique to assess field diets of aphidophagous lady beetles.

Introduction

Numerous methods have been used to decipher the diets of predatory insects in the field. These methods include, for example, laboratory and field observational studies, gut dissection, serological tests (such as enzyme-linked immunosorbent assay or ELISA), and electrophoretic tests. Excellent reviews provide assessments of the relative merits and drawbacks of each of these methods, including their usefulness in providing qualitative and quantitative field data on predator-prey relations (Sunderland 1987, Powell et al. 1996, Symondson 2002, Harwood and Obrycki 2005).

Frass analysis is one method of assessing predator diets, but it has been seldom used. In one approach to frass analysis, the mass of fecal material produced is used as an index of predation rate (Phillipson 1960, Honěk 1986). A second approach uses the contents of the frass to identify types of prey consumed by a predator. This technique has been used for a few invertebrate predators, including species of Odonata (Lawton 1970, Thompson 1978) and Coleoptera (Coccinellidae; Putnam 1964). Although not widely used to determine prey composition in a predator's diet, frass analysis holds promise for a variety of reasons. Study insects need not be sacrificed, but instead can be returned to the field, or used for additional laboratory experiments as well. The technique is inexpensive

and simple, and requires little specialized equipment. Frass analysis also provides results quickly, and frass samples may be stored for long periods of time (Powell et al. 1996).

The study presented here builds on the work of Putnam (1964) to explore further the utility of frass analysis for determining diets of aphidophagous lady beetles (Coleoptera: Coccinellidae, subfamily Coccinellinae, tribe Coccinellini). These insects are common and important predators in a variety of habitats. They readily attack and appear to prefer aphid prey, but given that aphid populations are ephemeral, they often must also consume other types of arthropods and non-arthropod food to sustain themselves (Hodek 1962, 1996; Dixon 2000). Various species have been documented feeding on a variety of arthropods, including Thysanoptera, mites, the larvae and eggs of multiple orders (including Diptera, Coleoptera, and Lepidoptera), as well as coccids, and other members of Sternorrhynca (Putnam 1957, Triltsch 1999, Ricci et al. 2005). In addition, they have been found to rely extensively on non-arthropod food such as plant pollen and fungal spores, which are readily available in their environment (Hemptinne and Desprets 1986, Triltsch and Freier 1998, Lundgren et al. 2004, Ricci et al. 2005).

This broad list of possible dietary components does not adequately reflect what is consumed in any given field situation, however. Adult lady beetles must balance their diets between “alternative foods” that will allow them to survive and “essential foods” that will allow them to reproduce (Hodek 1996, Dixon 2000). Individual species in this large group of lady beetles differ in which prey types are essential, but all are polyphagous to some extent and will readily incorporate alternative prey into their diet.

The present study is focused on species of lady beetles in the intermountain west

of North America that frequent alfalfa fields (*Medicago sativa* L.), both to forage for food and to reproduce. In such alfalfa fields, these species feed especially on the essential prey, pea aphids, *Acyrtosiphon pisum* (Harris) (Evans 2004). However, pea aphid populations are often low (e.g., they build slowly in the spring), necessitating the use of alternative prey by lady beetles at these times. A wide variety of alternative prey is generally available, including both arthropod and non-arthropod food. Among such arthropod prey available each spring are the highly abundant larvae of the alfalfa weevil, *Hypera postica* (Gyllenhal) (Richards and Evans 1998, Evans and Gunther 2005). *Coccinella septempunctata* L. in particular and other lady beetles in general, are known to feed on larvae of the alfalfa weevil in both the old and new worlds when in an alfalfa crop (Evans and England 1996, Kalaskar and Evans 2001, and references therein). Faced with low numbers of aphids, *C. septempunctata* and co-occurring lady beetle species may also supplement their diets by engaging in cannibalism and intraguild predation of eggs and larvae (Schellhorn and Andow 1999, Burgio et al. 2002).

The objective of this study was to develop the use of frass analysis in alfalfa field situations with the specific goal of determining the diets of common lady beetle species. Thus, laboratory studies were carried out first to determine whether frass analysis could be used to detect inclusion of aphids, alfalfa weevil larvae, and lady beetle larvae in the diets of adult lady beetles. An important goal of these laboratory studies was to determine the time course over which frass pellets with diagnostic prey fragments were produced following a lady beetle's consumption of the prey. A field experiment was then conducted as proof of the concept that frass analysis could provide valuable insight on diets of natural populations.

Materials and Methods

Insects

Lady beetles used in the following laboratory experiments were collected during the spring and summer of 2003 and 2006 in a variety of sites in Cache County, Utah. For the most part, lady beetles were collected in alfalfa fields, though some were also gathered in weedy pastures and wooded edges. Once collected, and during each of the experiments, the lady beetles were maintained at 20°C and 16L: 8D in an incubator. They were maintained in groups of a given sex and species in large (14 cm diameter) Petri dishes, and fed an excess of pea aphids (*A. pisum*). Aphids were reared in a greenhouse on fava beans (*Vicia faba* L.), and collected the same day as used for food. Alfalfa weevil larvae (*H. postica*) used in the experiments were collected by sweep netting in alfalfa fields. Weevils were maintained in Rubbermaid® Servin Saver 2.8 liter canisters (6 cm × 6 cm × 7 cm) with mesh lids and fresh alfalfa clippings in an incubator at 12°C and 16L: 8D. Lady beetle larvae were reared in the laboratory from eggs laid by field-collected adults. They were maintained under the same conditions as described above for lady beetle adults.

Gut-clearing Experiments

Two similar experiments were performed. In the first experiment, 20 field-collected adults of each sex of *C. septempunctata* were provided aphids as prey. Because of mortality, ultimate sample sizes were 19 females and 17 males. Adults of each sex were held in large (14 cm diameter) Petri dishes (i.e., one dish for each sex), and were fed an excess of pea aphids (such that ample prey was leftover each day) for 48 hours. Sugar

water (15% solution) was provided, in 12 x 75 mm borosilicate glass tubes plugged with cotton. Freshly collected aphids were added to Petri dishes each morning. If needed, additional aphids were added in the afternoon to ensure that there was ample prey in the dish. Because the lady beetles were provided as a group with aphids, it was not possible to determine whether all lady beetles indeed fed on the aphids.

After being held for 48 hours with aphids, lady beetles were placed in individual, small (5.5 cm diameter) Petri dishes with a drop of sugar water (included to prevent mortality). Individuals were transferred to clean dishes with fresh sugar water every 24 hours for a period of 72 hours. For each individual, the number, appearance, and color of frass pellets was noted for each of the three 24-hour periods.

All frass pellets were dissected. When dissections could not be completed immediately, frass pellets were stored in the Petri dishes they were produced in. Pellets were placed in the well of a depression slide and softened with a 20% solution of sodium hydroxide for approximately 5 minutes, until it was possible to break apart the peritrophic membrane with tweezers and dissecting needles. The slide was then inspected under the 10× objective of a compound microscope to note the presence or absence of aphid cuticular fragments. Identification of aphid fragments was aided by initial dissections of frass from additional (i.e., non-experimental) lady beetles that had fed on aphids, and by illustrations from Triltsch (1999).

In the second experiment, lady beetle females of the five most abundant species that occur in local alfalfa fields were studied: *C. septempunctata*, *C. transversoguttata richardsoni* Brown, *Hippodamia convergens* Guérin-Ménéville, *H. quinquesignata* (Kirby), and *Harmonia axyridis* (Pallas). Individual females of each species were

provided with either pea aphids (*A. pisum*) or alfalfa weevil larvae (*Hypera postica*) as prey, and the frass that they produced was dissected for prey remains.

For 48 hours prior to the start of the experiment, females were held in large, communal (14 cm diameter) Petri dishes with only tubes of sugar water (and no prey) to allow them to clear their guts and to stimulate their hunger. At the start of the experiment, females were transferred to individual, small (5.5 cm diameter) Petri dishes and were randomly assigned to receive aphids or weevils as prey. All females received a drop of sugar water each day in addition to the prey provided. Females fed aphids received an excess supply of mixed instars. Females fed weevils received six weevil larvae (late 2nd to late 3rd instars).

Individuals were given fresh prey every day for 3 days. If an individual did not consume prey on any of the three days she was removed from the experiment. Final sample sizes varied among species and diets (treatments) because some individuals did not eat the prey provided and species were collected in different numbers due to their availability (Table 2.1).

At the outset of the experiment, 33-43 individuals of each species were provided with weevils. Some of these individuals (but none of those provided with aphids) failed to feed on the prey. The numbers of individuals failing to feed on weevils varied among species, and included 5 of 40 *C. septempunctata*, 6 of 41 *C. transversoguttata*, 10 of 43 *Hippodamia convergens*, 14 of 40 *H. quinquesignata*, and three of 33 *Harmonia axyridis*.

After three days during which the predators were allowed to feed on aphids or weevils, the prey were removed and the lady beetles were transferred to clean, small (5.5 cm diameter) Petri dishes with a drop of sugar water. At 6, 12, 24, and 48 hours after

this initial transfer, lady beetles were again transferred to new Petri dishes (also without prey, and with only a drop of sugar water). The lady beetles were removed from the final Petri dishes 72 hours after removal from prey. The frass that was produced in the series of Petri dishes enabled the extent of gut clearing to be quantified at 6, 12, 24, 48, and 72 hours after removal from prey. At each of these times, the number, color, and appearance of frass pellets produced by each female was noted, and all frass pellets produced by individuals were dissected and inspected (methods for scoring pellets and pellet contents were the same as those of the first experiment). If dissections were not completed immediately, pellets were held in the Petri dishes they were produced in. Data were analyzed with SAS (SAS Institute 2003) using chi-square tests of independence and repeated measures ANOVA. All data met assumptions of normality.

Prey Indicator Experiment

In the laboratory, 80 males and 80 females of *C. septempunctata* were chosen at random from field-collected populations to examine their frass for diagnostic cuticular fragments from different kinds of prey. Initially, each adult was held individually for 48 hours in a small (5.5 cm diameter) Petri dish. During this time, food was withheld and water was offered in vials plugged with cotton. Individuals cleared their guts of their prior aphid diet, and grew hungry in the absence of prey.

Thereafter, 20 individuals of each sex were randomly assigned to each of four diets, provided daily: (1) six young larvae (2nd – early 3rd instars) of the alfalfa weevil, (2) four older alfalfa weevil larvae (late 3rd instars), (3) four *C. septempunctata* larvae (2nd – early 3rd instars), or (4) an excess number of pea aphids. Individuals were maintained on these diets for 5 days, and then were held for a sixth day with no food. As

the objective of this experiment was to discover the types of prey fragments produced in frass pellets from different types of prey, it was considered sufficient to hold individuals for only 24 hours past their last feeding. If individuals did not consume any prey over the 5 day feeding period, they were removed from the experiment. All individuals provided with conspecific larvae or pea aphids consumed some of the prey offered. For individuals provided with early or late instars of alfalfa weevil larvae, a small number of both males and females failed to feed on the prey provided: three males and three females failed to consume early instars (reducing sample sizes to 17 for each sex), and three males and two females failed to consume late instars (reducing sample sizes to 17 and 18, respectively).

Frass was collected daily from each dish. A single pellet (the largest produced over the 6 day period) was examined for each individual as described above. Structures seen under the 10× objective of a compound microscope were categorized based upon where they came from on the prey's body, and sketches and photographs (Appendix A) were made of the most representative samples for future use (e.g., as in examining the frass of field-caught lady beetles in the field experiment described below). In addition, pellets were categorized as to how many cuticular fragments they contained. Dissected pellets were scored as having few (20 or fewer) pieces of cuticular fragments, or as having many (more than 20) fragments. Data were analyzed with chi-square tests of independence with SAS (SAS Institute 2003).

Field Experiment

A field experiment was conducted to collect frass samples from natural (versus laboratory) populations of *C. septempunctata* to test whether frass analysis could be used to assess diet composition in free-living adults of this species (adults of species other than *C. septempunctata* were collected in too few numbers to examine population patterns). Eight plots of 1.5 m x 3 m were marked in an alfalfa field (USU Animal Science Farm, Wellsville, Cache County, Utah), with individual plots placed 5-10 m apart. Plots were placed where alfalfa grew in especially lush patches, in hopes of attracting large numbers of lady beetles. Plot dimensions were set such that beetles could be hand picked from all sides of the plot, without having to physically enter the plot. All eight plots were sprayed at the start of the experiment with 1.25 liters of 15% sugar-water solution. Lady beetles aggregate in response to aphid honeydew as well as to the aphids themselves, and sugar-water can act as a substitute for aphid honeydew in eliciting lady beetle aggregation (Evans and England 1996). It was hoped that large numbers of lady beetles would accumulate in the plots in response to the sugar water, thereby yielding large sample sizes.

To compare the adult diet of *C. septempunctata* (and the tendency to consume alfalfa weevil larvae in particular) in the presence of high versus low numbers of aphids, half of the plots were randomly selected to be seeded with aphids (reared on fava beans in a greenhouse). Immediately following spraying of plots with sugar, aphids were added to each of the four “high aphid density” plots, while no aphids were added to the remaining four “low aphid density” plots (which reflected natural field densities; estimated from stem samples as 0.25 aphids per stem). An estimated 22,500 pea aphids were added to

each plot from fava beans, thereby raising the estimated numbers of aphids in “high aphid density” plots to 2.25 aphids per stem. Natural populations of lady beetles typically are semi-starved (Honěk 1986), and lady beetle adults collected over several years from similar alfalfa fields in Cache County, Utah, were found to weigh significantly less than adults reared in the laboratory under conditions of satiation (Y. Kajita, personal communication). While the two treatments allowed for differential responses by the resident lady beetles, neither treatment would provide sufficient aphids to completely satiate the lady beetles.

Beginning on the day after plot manipulations, the plots were visited to collect as many adult lady beetles as possible. Immediately upon collection, individuals were held in small (5.5 cm diameter) Petri dishes with a drop of sugar water for 48 hours (i.e., a sufficient time to them to void their gut contents). The plots were visited again to collect adults on the next three days (i.e., the second and fourth days) with individuals being treated in the same way on each collecting occasion.

Collected beetles were identified as to species and sex, and were held for 48 hours (they were incorporated thereafter into laboratory populations). All five species studied previously were collected, but sufficient sample sizes were obtained only for *C. septempunctata*. Frass was collected, with the largest pellet being reserved for dissection. Pellets were dissected as described above. Contents were scored as to whether aphid, weevil, or lady beetle larval remains could be found. Non-identifiable pieces were noted, with either sketches or photos made (Appendix A). Additional categories of other arthropods (not including the above types) and pollen and fungi were also considered during dissections. A fragment was designated as derived from “other arthropods” if it

could not readily be identified as derived from aphids or weevils, but nonetheless appeared to be a fragment of invertebrate cuticle. Pollen and fungi were readily distinguished from other particles by their “regular” shape. Drawings from Triltsch (1999) were used to aid in identification of particles as pollen or fungi, as were pictures and diagrams from Moore et al. (1991). Fungi were identified as belonging to the genus *Alternaria* with the aid of Triltsch (1999) and as verified by consulting Agrios (2005) and Ricci et al. (2005).

In addition, pellets were scored as to how “full” of cuticular fragments they were. Frass pellets were designated as having no prey or food fragments, small amounts (i.e., 20 pieces or less) or large amounts of prey or food fragments (i.e., more than 20 pieces). Data were analyzed with SAS (SAS Institute 2003) using chi-square tests of independence.

Results

Gut-clearing Experiments

In the first gut-clearing experiment, all individuals (females and males of *C. septempunctata*) produced frass when held without prey after feeding on aphids. Most of the frass pellets were produced within 24 hours following removal from prey (Fig 2.1). Production of frass pellets dropped to low numbers during the next 24 hours. There were no significant differences between the sexes during the first and second 24 hours in the production of frass, but there was a significant effect of time (one-way repeated measures ANOVA, effect of sex: $F_{1,34} = 0.27$, $p = 0.60$; effect of time: $F_{1,34} = 97.78$, $p < 0.0001$;

interaction of sex \times time: $F_{1,34} = 0.23$, $p = 0.64$; Appendix B). Males did not produce frass thereafter, and females produced little additional frass (Fig 2.1).

Not all frass pellets produced during the 72-hour experimental period contained fragments of aphid cuticle. However, all but 3 of 19 females (i.e., 84%) and 2 of 17 males (i.e., 88%) produced one or more frass pellets that were found upon dissection to contain fragments of aphid cuticle (because beetles were not held individually when feeding on aphids, it is not possible to determine whether adults that did not produce frass with aphid fragments had indeed fed on aphids prior to their removal from the prey). Females and males did not differ in the percentages of individuals that produced at least one frass pellet containing aphid fragments during the first 24 hours ($\chi^2 = 0.0008$, $p = 0.98$; Fig 1b), or the next 24 hours ($\chi^2 = 0.085$, $p = 0.77$; Fig 2.1).

Among female lady beetles of the five species (*C. septempunctata*, *C. transversoguttata*, *H. convergens*, *H. quinquesignata*, *Harmonia axyridis*) that fed on either aphids or weevils in the second gut clearing experiment, individuals produced most frass pellets within the first two days from removal from prey, regardless of the prey type provided (Fig 2.2). During this experiment, frass was collected and analyzed for three time periods during the first day to determine when frass pellets were voided. Individuals fed aphids generally produced the greatest number of frass pellets within the first six hours after being removed from prey. Lady beetles fed weevils, however, did not exhibit such a marked pattern of frass production during the first 24 hours (Fig. 2.2).

The amount of frass produced over the 3 days differed among lady beetle species, prey species, and days, although frass production declined similarly with time among lady beetle species (Fig. 2.2; 2-way repeated measures ANOVA with Huynh-Feldt

Epsilon correction for sphericity: effect of lady beetle species: $F_{4,301} = 3.83$, $p = 0.005$, effect of prey: $F_{1,301} = 19.56$, $p < 0.0001$, interaction of lady beetle and prey species: $F_{4,301} = 4.04$, $p = 0.003$; effect of time: $F_{2,602} = 279.01$, $p < 0.0001$, time \times lady beetle species: $F_{8,602} = 2.87$, $p = 0.01$, time \times prey species: $F_{2,602} = 12.41$, $p = 0.0001$, time \times lady beetle species \times prey species: $F_{8,602} = 4.54$, $p = 0.0004$; Appendix B). Overall, the lady beetles produced fewer frass pellets during each time period when fed weevils rather than aphids. Additionally, frass production of females fed weevils declined more rapidly with time than did frass production of females fed aphids (Fig. 2.2).

As in the first gut-clearing experiment, not all frass produced contained fragments of the prey consumed. All individuals included in the analysis of the experiment were known to have fed on the prey provided. A much higher percentage of females produced frass containing cuticular fragments among individuals that were known to have fed on aphids rather than weevils (Table 2.1; $\chi^2 = 46.48$, $p < 0.0001$ for all species combined). When aphids were consumed, between 80 and 95% of females produced one or more frass pellets containing aphid cuticle. High percentages of females of the three largest beetle species (*C. septempunctata*, *C. transversoguttata*, and *Harmonia axyridis*) produced frass with aphid cuticle, but there was no significant difference in this regard among the five species (i.e., including the smaller *Hippodamia convergens* and *H. quinquesignata* as well; $\chi^2 = 5.76$, $p = 0.22$). Greater differences occurred among species when individuals fed on weevils with percentages varying from 25 to 65% among species (Table 2.1; $\chi^2 = 15.16$, $p = 0.0044$). *C. septempunctata*, *H. quinquesignata*, and *Harmonia axyridis* did not significantly differ from one another in percentage of females producing frass with weevil fragments ($\chi^2 = 1.86$, $p = 0.39$), and *C. transversoguttata* and

Hippodamia convergens did not significantly differ from one another in percentage of females with weevil fragments ($\chi^2 = 3.28$, $p = 0.07$).

Quantitative data on sizes of frass pellets produced were not collected, but observational data were collected. Frass that contained prey cuticle was generally larger and darker than frass that did not. Also, frass pellets produced by individuals that fed on aphids tended to be larger than those produced by those that fed on weevils. Many weevil-fed individuals produced only a single pellet during the entire experiment that contained weevil fragments. In contrast, aphid-fed individuals produced multiple pellets over three days that contained aphid fragments.

The percentage of the population producing at least one frass pellet that contained cuticular fragments of their prey was highest at 6 hours after removal from prey (Fig 2.3). This was especially pronounced for individuals fed aphids ($\chi^2 = 71.25$, $p < 0.0001$, pellet production in the first versus second 6 hours after removal from prey, for all five species combined). A similar trend occurred for individuals fed weevils, but during the first day this pattern was not as distinct as for those fed aphids ($\chi^2 = 1.30$, $p = 0.25$, pellet production in the first versus second 6 hours after removal from prey, for all five species combined). Similar to the first 6 hours, the percentage of the population producing at least one pellet containing prey fragments was higher on day one than on day two. The rapid decline from day one to day two in the percentage of the population that produced frass pellets containing prey fragments was much more pronounced, particularly for those individuals fed weevils (Fig 2.3; weevils $\chi^2 = 80.52$, $p < 0.0001$, aphids $\chi^2 = 144.16$, $p < 0.0001$, pellet production in day one versus day two after removal from prey, for all five species combined). Although females continued to produce small numbers of frass

pellets on the third day (Fig. 2.2), very few of these pellets contained prey fragments.

In particular, only a few females of *Harmonia axyridis* and *C. septempunctata* that had fed on aphids produced pellets with prey fragments on the third day (Fig. 2.3).

Prey Indicator Experiment

Each type of food (weevil larvae, aphids, and conspecific larvae) yielded visually distinct cuticular fragments within the frass pellet, and no frass pellet examined contained unusual fragments (e.g., pollen, fragments from other food types, or amorphous particles). Cuticular fragments from weevil larvae were very light colored and looked like shed snakeskin. Fragments from aphids were light tan in color with distinctive patterns of setae. Fragments from conspecific lady beetle larvae were dark, almost black in color, and marked distinctively with whorls of striations.

Sections of cuticle found in the frass were counted in order to distinguish between frass pellets with large or small amounts of prey remains (i.e., many [> 20] or few [≤ 20] prey fragments). All individuals were known to have fed on the prey provided, and all produced frass that contained at least one piece of prey cuticle. A significant difference occurred, however, among prey categories in the percentage of frass pellets containing many versus few prey fragments (Fig. 2.4; $\chi^2 = 30.88$, $p < 0.0001$, for the sexes combined). When lady beetle adults were fed conspecific larvae or aphids, more than half of the frass pellets examined contained many cuticular fragments. In contrast, when adults were fed either category of weevil larvae, fewer than half of frass pellets contained many cuticular fragments. There was no significant difference for any of the four food types in the percentage of female versus male lady beetles that produced frass pellets with many versus few fragments (Fig 2.4, Table 2.2).

Frass pellets produced by lady beetles that fed on small or large alfalfa weevils contained five main categories of cuticular fragments: mandible, head capsule, portions of cuticle, cuticle with setae, and setae alone (Fig 2.5). Cuticle, cuticle with setae, and setae alone occurred especially frequently in frass pellets. Less of the carcass was left behind when lady beetles fed on small rather than large weevil larvae, and the frass contained more cuticular fragments, especially head capsules and mandibles. When large weevils were fed upon, oftentimes a dried husk of the body was left behind in the Petri dish. Females and males were similar in the regularity with which different sections of early or late weevil cuticle was included in their frass, and did not differ significantly in the percentages of their frass pellets that contained the different categories of cuticular remains (Table 2.3).

Associated with the aphid diet, six categories of cuticular fragments were found in frass pellets: mouthparts, eyes, antennae, final tarsal claws, legs and cuticle (Fig 2.5). Most commonly found were antennae, legs, and cuticle. In general lady beetles appeared to consume an aphid in its entirety, especially for small aphids. With larger sized and winged aphids, the beetle would often leave behind small bits of prey (although not as much as in the case of the weevils). As with weevil fragments, frass pellets of female and male lady beetles did not differ in the degree to which they contained different categories of aphid fragments (Table 2.3).

For the diet of conspecific lady beetle larvae, six categories of cuticular fragments were found: mandible, head capsule, legs or prolegs, cuticle, cuticle with setae, and setae alone (Fig 2.5). In general, the lady beetles tended to attack and begin consuming conspecific larvae mid-ventrally. This seemed to allow an adult to subdue and kill the

larva before it could escape. *C. septempunctata* larvae have an array of bristle-like setae on their ventral side that are quite different in morphology from the simpler setae that occur on weevils. A large proportion of detached setae, or setae still attached to cuticular fragments, were ingested by adults preying on conspecific larvae. Carcasses left behind almost always retained the head, reflecting the adult beetle's preference instead for body regions with softer tissues. In contrast to often simply taking a liquid meal of weevils, the lady beetles tended to consume considerable body tissue of conspecific larvae. With the exception of head capsules and mandibles, most categories of conspecific larval remains were found in almost all frass pellets with fragments (Fig. 2.5). Males and females did not significantly differ in how frequently their frass pellets contained different categories of cuticular remains (Table 2.3).

Field Experiment

When adults of *C. septempunctata* were collected from field plots of alfalfa with naturally low numbers of aphids, or with aphids added experimentally, a high percentage of adults produced frass pellets containing food fragments (Fig. 2.6). Almost 50% of female lady beetles, and 20-40% of males, produced frass containing large amounts of food fragments (Fig. 2.6). There was no significant difference between the treatments in percentages of females that produced frass with no, few, or many prey fragments or particles of other foods such as pollen ($\chi^2 = 1.10$, $p = 0.58$). For males, however, there was a significant difference between the two treatments ($\chi^2 = 7.93$, $p = 0.02$), as relatively more males in plots with aphids added produced pellets with large amounts of prey fragments and other food particles (Fig. 2.6). Overall, males and females did not significantly differ from one another in either plot treatment in the relative amounts of

food remains found within their frass (Fig 2.6; high aphid density $\chi^2 = 2.08$, $p = 0.35$; low aphid density $\chi^2 = 5.32$, $p = 0.07$).

Four categories of food were found in the frass pellets produced by lady beetles collected from field plots: aphid fragments, alfalfa weevil fragments, other arthropod fragments, and pollen and fungi. Collembola and Thysanoptera appeared in the frass of many individuals and were categorized as “other arthropods”. No frass pellets were found to contain fragments of lady beetle larvae. Many pellets contained amorphous particles, most likely of soil, which were not included. Pollen and fungi were readily distinguished from other particles and were found intact within the frass pellets. Several sources of pollen were available in or near the plots (e.g. alfalfa, dandelions [*Taraxacum officinale* Weber in Wiggers], and western salsify [*Tragopogon dubius* Scop.]) and the fungi appeared to be primarily from the genus *Alternaria*.

Many individuals fed on more than one category of food. More females collected from plots with high than with low aphid numbers (86% versus 65%) produced frass pellets containing more than one food category, but this difference was not significant ($\chi^2 = 2.66$, $p < 0.10$). Similarly more males collected from plots with high than with low aphid numbers (77% vs. 67%) produced frass pellets containing multiple categories of food, but again the difference was not significant ($\chi^2 = 0.0202$, $p = 0.89$).

As indicated by frass contents, a higher percentage of females consumed aphids in plots with many versus few aphids (Fig. 7; $\chi^2 = 17.25$, $p < 0.0001$). Similar percentages of females consumed alfalfa weevil larvae in the two plots (Fig. 7; $\chi^2 = 0.37$, $p = 0.54$). Overall, approximately half of the females collected from plots produced frass pellets containing weevil fragments. Similar consumption levels were observed for other

arthropods, pollen and fungi, with no significant difference between females collected from plots with many versus few aphids (Fig 2.7; other arthropod $\chi^2 = 0.027$, $p = 0.87$; pollen and fungi $\chi^2 = 0.14$, $p = 0.71$).

A higher percentage of males also consumed aphids in plots with many versus few aphids (Fig 7; $\chi^2 = 27.35$, $p < 0.0001$). Male consumption levels for alfalfa weevil larvae were similar between the two kinds of plots (Fig 2.7; $\chi^2 = 1.13$, $p = 0.29$), as were male consumption levels for pollen and fungi ($\chi^2 = 1.03$, $p = 0.31$). In contrast to females, however, males consumed significantly fewer other arthropods in plots with few aphids than in plots with many aphids ($\chi^2 = 6.27$, $p = 0.01$).

Females and males from plots with many or few aphids were similar overall in the percentages of individuals that produced frass pellets containing particular categories of food fragments (Fig. 2.7). No significant difference occurred between the sexes for any food category in either kind of plot, with the exception that a higher percentage of pellets with fragments of arthropods other than aphids and weevils were produced by females than by males in plots with few aphids (Table 2.4). Females and males also did not differ from one another in their proclivity to consume a mixed diet in either treatment (Table 2.4).

Discussion

The major purpose of this study was to further develop the underutilized method of frass analysis to characterize lady beetle diets. This technique has not been used often, though it has been used with odonate (Lawton 1970, Thompson 1978) and coccinellid predators (Putnam 1964). In the present study, frass analysis proved to be a useful

technique for determining diets of lady beetles. Each type of prey examined in the laboratory produced distinctive cuticular fragments in the frass voided by adult lady beetles. These distinctions enabled the diets of field collected adults to be determined from inspection of their frass. The presence of additional categories of food could be determined in the frass of field collected adults as well, including pollen, fungi, thrips and collembolans (results for these latter two groups were not reported individually here because they were not studied in the laboratory, and because they occurred relatively infrequently in field collected frass).

Putnam (1964) used both frass analysis and gut dissection to identify aphids, scales, thrips, and tetranychid mites as foods of several lady beetle species (including *Hippodamia convergens* and *C. transversoguttata*) in peach orchards, but also found that some insects (less than 10 percent of all insects consumed) could not be classified to their order. Triltsch (1999) used gut dissection to identify aphids, thrips, collembolans, mites, and larvae of Hymenoptera, Diptera, coccinellids and leaf beetles consumed by *C. septempunctata* in a variety of cereal crops in Europe. Future expansion on the work reported here would seem likely to enable analysis of lady beetle diets from frass to include these other types of frequently encountered arthropod prey.

As with all methods of deciphering the diets of predatory arthropods, use of frass analysis requires knowledge of how long diagnostic portions of prey remain in the digestive tracts of species being investigated. Overall, 48 hours at 20°C was sufficient time for individuals of each of five lady beetle species considered here to clear their guts of undigested prey cuticle. In some instances, individuals that had fed on either aphids or weevils failed to produce frass containing diagnostic prey cuticle. In particular, a large

percentage of adult lady beetles that fed on alfalfa weevil larvae did not incorporate weevil remains into their frass. This could lead to underestimates in the field of how often individuals were utilizing weevil larvae as prey. However, there is also the interesting possibility that in field settings, high costs of searching for prey may lead individuals to consume more of the prey that they capture, and hence produce frass pellets with high likelihood of containing cuticular fragments.

Frass analysis was successful in documenting a positive relationship between consumption rates of aphids by *C. septempunctata* adults and the number of aphids within alfalfa plots. Frass analysis further indicated that the use of other types of prey such as weevils, other arthropods, pollen, and fungi by females was not affected by the number of aphids co-occurring with these other potential foods. Lady beetles may be opportunistic predators, feeding on prey as they encounter it in the environment, rather than waiting to find their optimal prey, aphids. In particular, females must balance their metabolic needs with their reproductive needs. Feeding on prey that are not aphids can be used to meet their metabolic needs and allow them to divert essential nutrients from any aphids consumed directly to their reproductive needs (Evans et al. 2004). In contrast to females, males only need to consume enough food to fuel their metabolic processes. This may explain why one striking difference occurred between treatments wherein fewer males utilized other types of arthropod prey (besides aphids and weevils) in low aphid density areas than in high aphid density area

Frass analysis also demonstrated a tendency of both female and male *C. septempunctata* to feed on multiple types of prey over a short period of time, rather than focusing on consuming just one type. Both sexes consistently consumed alfalfa weevil

larvae at high rates when both many and few aphids were present, thus supporting the implication of previous field observations from both the old and new world that *C. septempunctata* feeds frequently on alfalfa weevil larvae (Kalaskar and Evans 2001 and references within). Pollen and fungi were major components of *C. septempunctata*'s field diet also, with roughly half of all individuals in all plots combined found to have consumed these foods. Given that these fields have had extremely low densities of aphids in recent years (Evans 2004), the lady beetles present must utilize other sources of prey or food in order to survive within this environment.

During the field experiment, no evidence of intraguild predation of lady beetle larvae was found. Similarly, in other field studies of lady beetles (both adult and larval stages), few incidences of intra-guild predation were found (Triltsch 1999, Hoogendoorn and Heimpel 2004). I observed females in the field actively laying eggs. Although no larvae were found within the plots, it is possible nonetheless that eggs and larval lady beetles were present and available for adults to consume. Frass analysis would not capture intraguild predation of eggs, however, as eggs do not contain indigestible, sclerotized fragments that would be voided in the frass (Triltsch 1999).

As noted above, a percentage of the adults (females) that were fed aphids and weevils in the gut clearing experiments failed to produce any frass containing aphid or weevil cuticle. This could mean that the percentages of lady beetles found to feed on aphids or weevils during the field experiment are underestimates. For example, more than half of all *C. septempunctata* females were found to have fed on weevils during the field experiment, but even more may have in fact done so, as a large percentage of *C. septempunctata* females that consumed weevils during the gut clearing experiment failed

to produce frass with diagnostic cuticle. In contrast, most *C. septempunctata* females fed aphids during the gut clearing experiment produced frass containing aphid fragments, so findings from the field experiment are more likely to be indicative of actual aphid consumption in the field by this species.

A frequent goal in determining the diets of beneficial predators is to quantify how much they consume of the different dietary components. While strict presence/ absence data alone can suggest the importance of different types of prey in a predator's diet, additional information on quantities consumed may be very useful in guiding the implementation of integrated pest management strategies (Harwood and Obrycki 2005). Distinguishing between frass pellets containing many or few prey remains (≤ 20 and < 20 fragments, respectively) was used as a means here to assess amounts of prey consumed by field collected adults of *C. septempunctata*. This may not be a readily implemented technique, however, to obtain comparative quantitative data across lady beetle species. Different species of lady beetles can vary greatly in size, and smaller lady beetles may ingest smaller pieces of their prey, or take smaller meals from individual prey. Simply counting the number of fragments in the frass may not adequately measure the quantity of prey consumed. Additionally, counting fragments does not take into account the size of each individual prey fragment, which could vary widely depending on the prey type, or on the section of the body the fragment came from. Other approaches may yield more useful data. One method is to count particular types of fragments such as final tarsal claws or mouthparts to estimate numbers of individuals of given prey types consumed (Putnam 1964, Triltsch 1999). Another possibility is to measure the total surface area of cuticular fragments for particular prey items in the frass.

An advantage of frass analysis in comparison to many other methods of determining the diet of lady beetles in the field (e.g., serological or molecular techniques) is that multiple prey types can be censused at the same time. In addition, this technique is inexpensive, and once preliminary laboratory work is completed, can be performed quickly. Frass analysis also stands out as one of very few methods (including direct observation in the field and laboratory feeding experiments) wherein the predator being studied does not need to be sacrificed. Unlike in laboratory experiments, frass analysis in field experiments can be used to directly measure dietary preferences across populations, and still provide live insects for subsequent studies or to be returned to the field. Direct observation of foraging predators can be time consuming, thus limiting the population sizes that can be sampled. Frass analysis allows one to collect large amounts of samples in a relatively short period and either process immediately, or store for dissection at a later date. The field experiment presented here characterized natural diets of *C. septempunctata* only during a very short period of time (i.e., over 4 days). Similar, long-term studies of diets of multiple species of lady beetles in alfalfa fields and other habitats (e.g., orchards) can readily be conducted as well, especially with further laboratory study to associate diagnostic fragments in lady beetle frass with additional species of prey.

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Table 2.1. Number of females of five lady beetle species provided with prey (either pea aphid or alfalfa weevil larvae) in the second gut clearing experiment, and percentages that produced frass containing portions of prey cuticle. Only those individuals that consumed the prey offered were included in the experiment.

Lady beetle species	Number that consumed aphids	Percentage with aphid cuticle in frass	Number that consumed weevils	Percentage with weevil cuticle in frass
<i>Coccinella septempunctata</i>	34	97.1	35	57.1
<i>Coccinella transversoguttata</i>	21	95.2	35	28.6
<i>Hippodamia convergens</i>	31	80.7	33	36.4
<i>Hippodamia quinquesignata</i>	40	87.5	26	65.4
<i>Harmonia axyridis</i>	22	90.9	30	73.3

Table 2.2. Results from chi-square tests of independence comparing the amount of cuticular remains (≤ 20 or > 20 fragments) produced by females and males for each category of prey offered (early and late instar weevils, pea aphid, and conspecific larvae). All tests are non-significant ($df = 1$).

Females vs. Males	Early Instar Weevil	Late Instar Weevil	Pea Aphid	Conspecific Larvae
p-value	0.73	0.067	0.74	0.077
X² value	0.12	3.37	0.11	3.14

Table 2.3. Results from chi-square tests of independence comparing the types of cuticular remains present in the frass of females and males for each category of prey offered (early and late instar weevils, pea aphid, and conspecific larvae). All tests are non-significant ($df = 1$).

Females vs. Males	Early Instar Weevil	Late Instar Weevil	Pea Aphid	Conspecific Larvae
p-value	0.36	0.15	0.049	0.58
X² value	0.85	2.087	3.89	0.3

Table 2.4. Results for chi-square tests of independence comparisons between the sexes of *C. septempunctata*, for two treatments (high and low aphid density) in the field experiment. Comparisons are for the percentages of the population found to have consumed the category of prey, as based on detection of diagnostic fragments in frass pellets (df = 1).

Females vs. Males		Aphid	Weevil	Other Arthropod	Pollen and Fungi	Mixed Diet
High Aphid Treatment	χ^2 value	0.22	0.022	4.02	0.091	3.00
	p value	0.64	0.88	0.045	0.76	0.083
Low Aphid Treatment	χ^2 value	0.0022	0.23	0.28	1.42	0.054
	p value	0.93	0.63	0.60	0.23	0.82

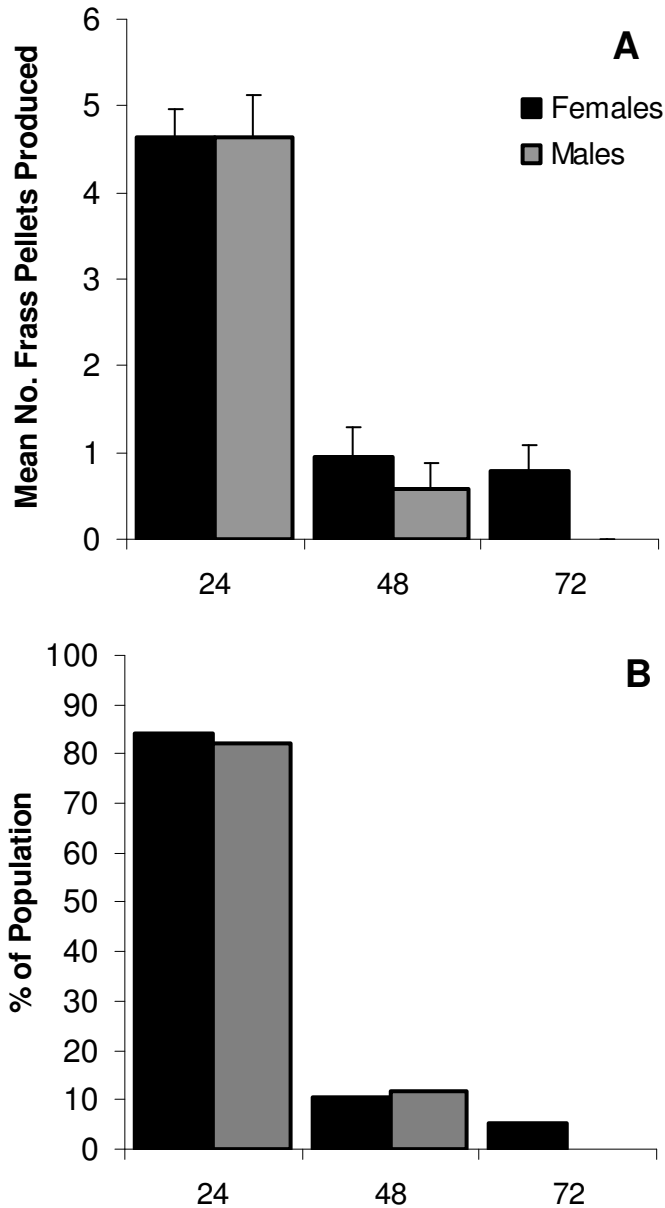


Fig. 2.1. Mean number of frass pellets produced (A) and percentage of individuals that produced at least one frass pellet containing fragments of aphid cuticle (B) by individual female and male *C. septempunctata* during three time periods (24, 48, and 72 hours) after removal from aphid prey. N = 19 females, 17 males.

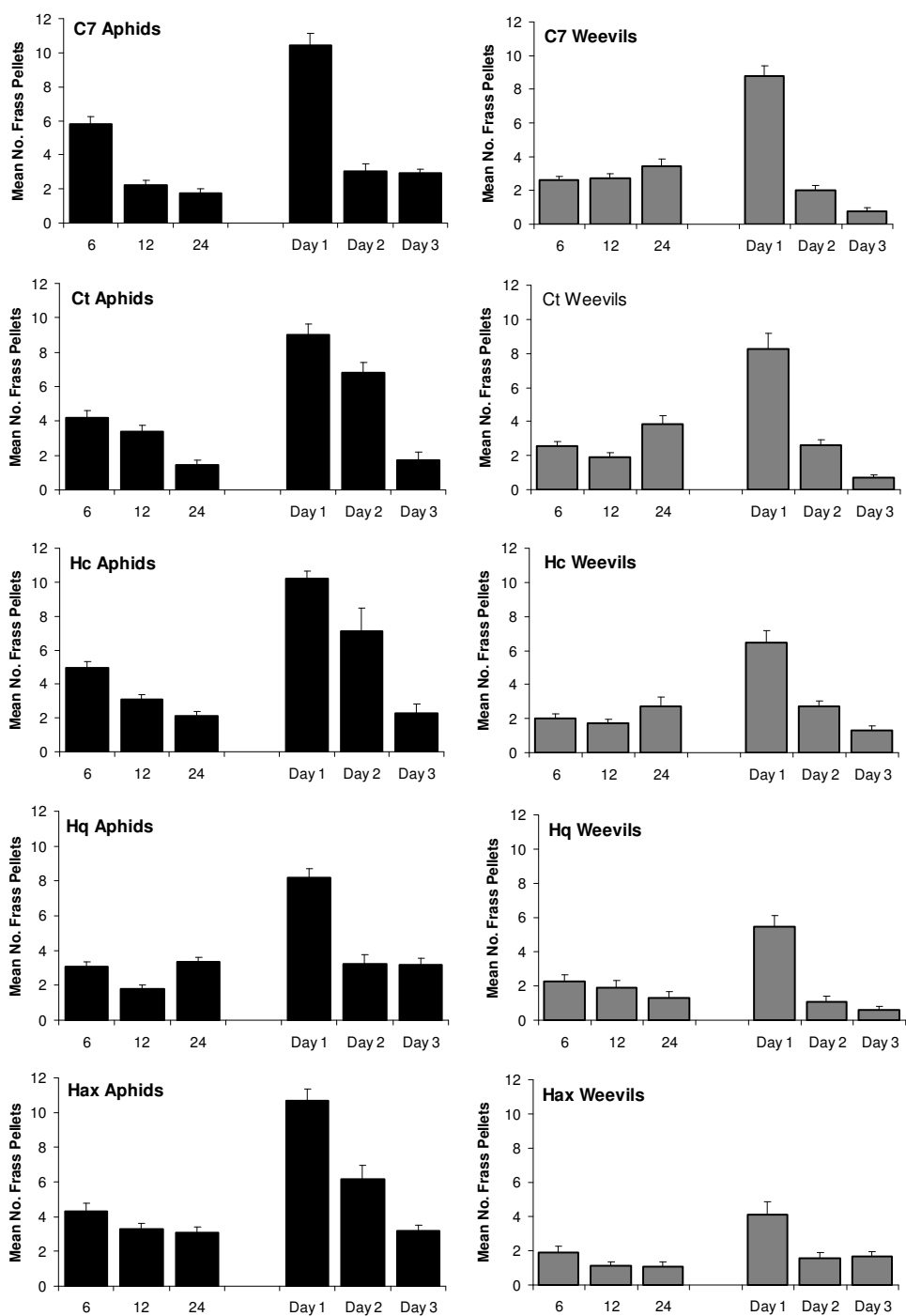


Fig. 2.2. Mean number of frass pellets produced per individual (adult female) when fed aphids or weevils for five species of lady beetles (*C. septempunctata* [C7], *C. transversoguttata* [Ct], *Hippodamia convergens* [Hc], *H. quinquesignata* [Hq], and *Harmonia axyridis* [Hax]) during different time periods. Graphs show data for 6, 12, and 24 hours and day one (first 24 hours), day two (second 24 hours), and day three (third 24 hours).

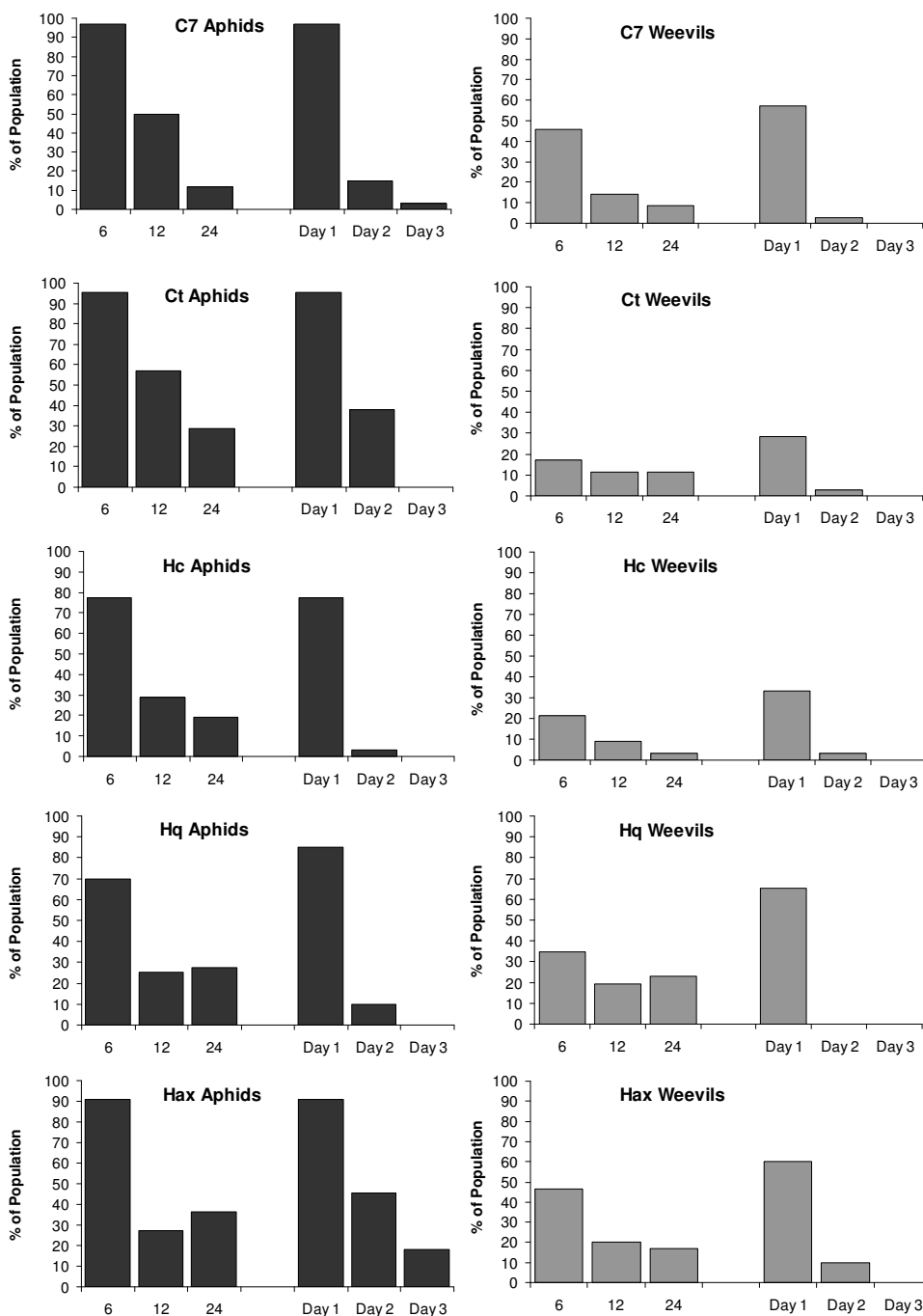


Fig. 2.3. Percentages of females of five lady beetle species (*C. septempunctata* [C7], *C. transversoguttata* [Ct], *Hippodamia convergens* [Hc], *H. quinquesignata* [Hq], and *Harmonia axyridis* [Hax]), that both consumed the prey offered (aphids or weevils) and produced at least one frass pellet which contained fragments of that prey during different time periods. Graphs show data for 6, 12, and 24 hours and day one (first 24 hours), day two (second 24 hours), and day three (third 24 hours).

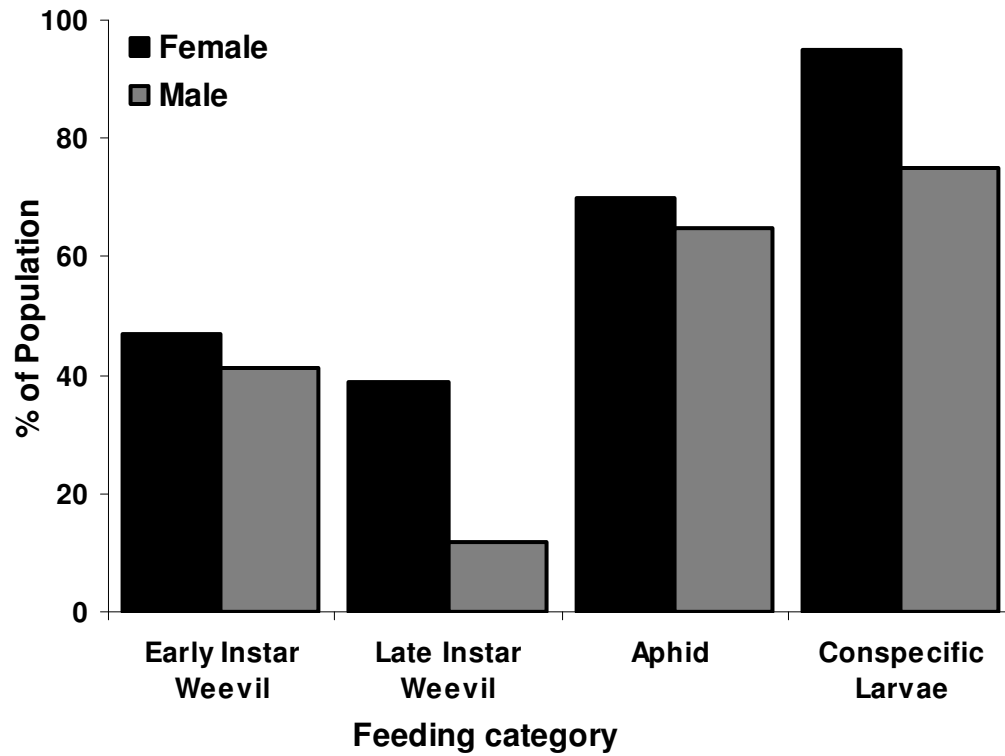


Fig. 2.4. The percentages of female and male *C. septempunctata* that contained large amounts of prey cuticle (i.e., more than 20 pieces) in their largest frass pellet. All individuals included had fed on the prey provided, and each individual produced a pellet with at least one piece of prey cuticle within it. (i.e., percentages of population with either large or small amounts would total to 100%).

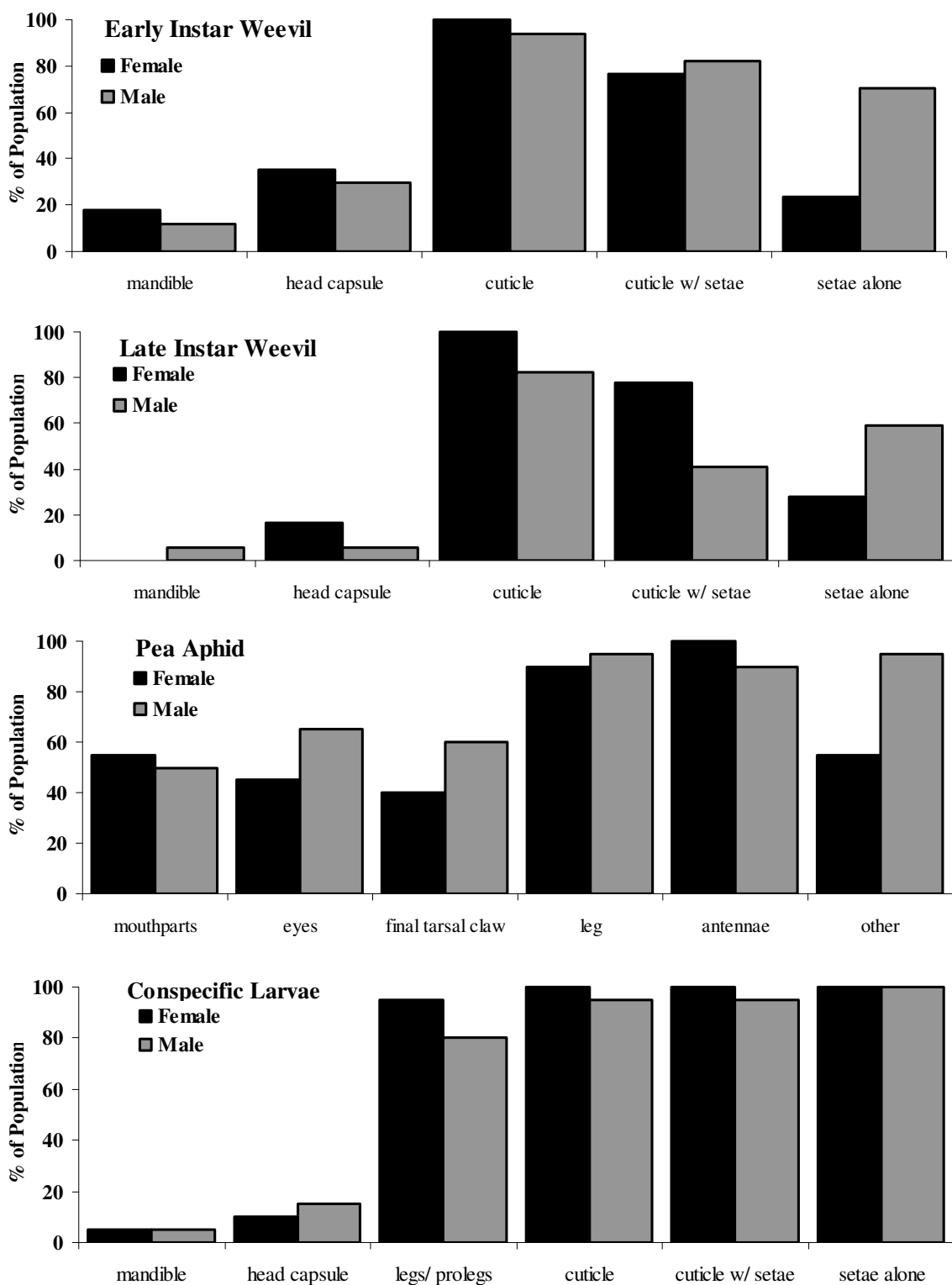


Fig. 2.5. The percentage of female and male *C. septempunctata* whose frass pellet contained noted sections of cuticle from the prey's exoskeleton given that they were fed each prey type (early or late instar weevils, pea aphids, conspecific larvae).

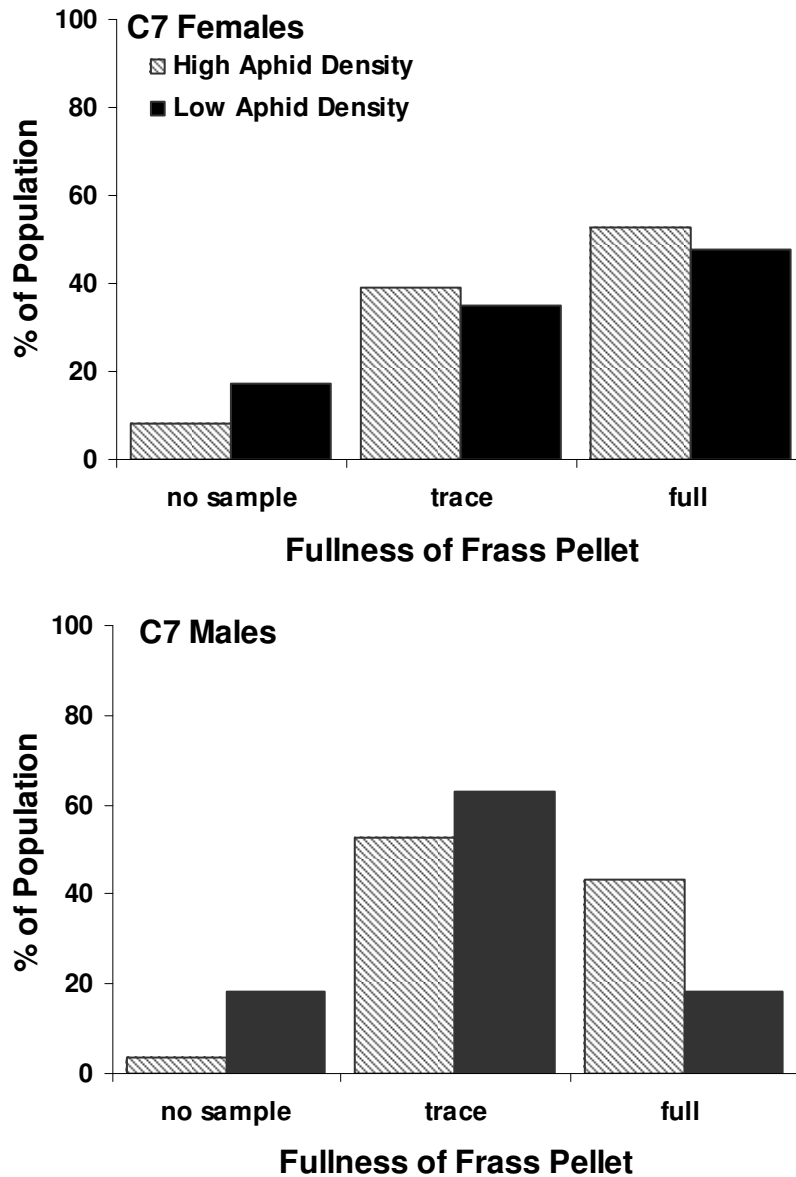


Fig. 2.6. Percentages of female and male *C. septempunctata* that produced frass pellets with no cuticular remains of prey, small amounts of prey (≤ 20 pieces), or frass pellets with large amounts of prey (> 20 pieces). Individual lady beetles were collected from plots with one of two treatments: high aphid density and low aphid density.

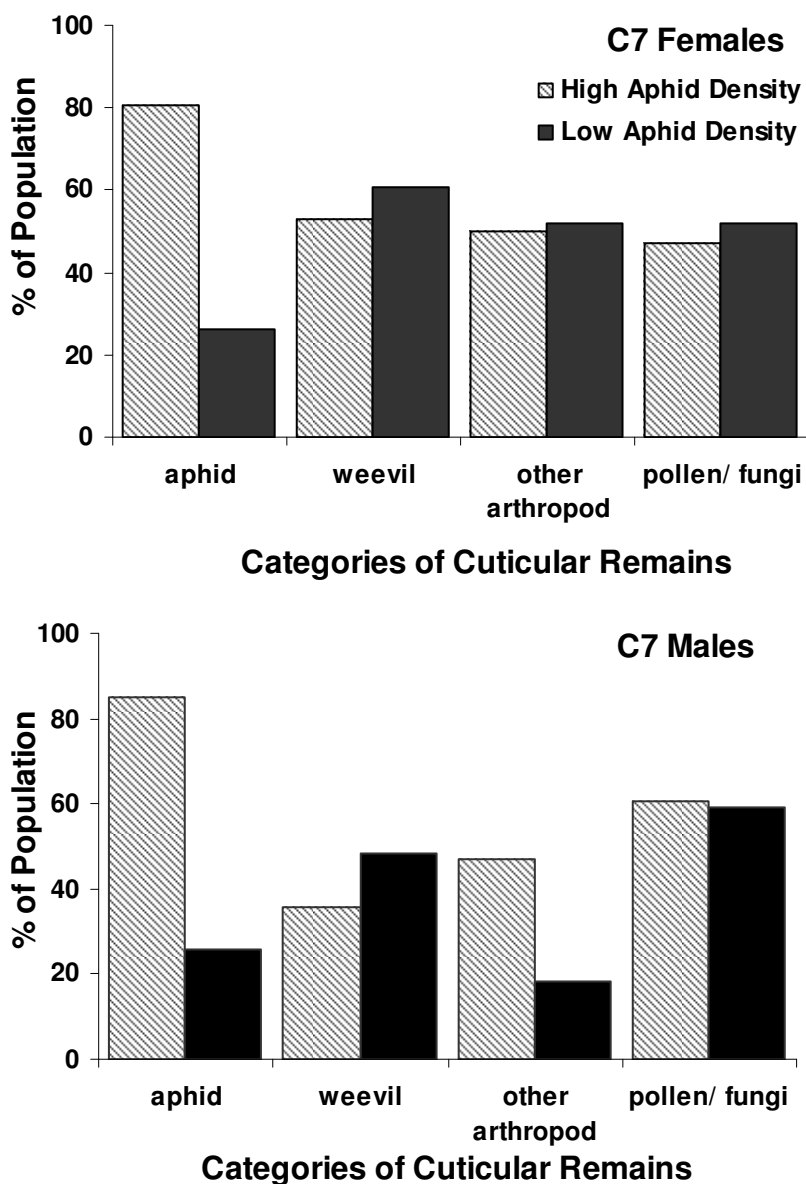


Fig. 2.7. Percentages of female and male *C. septempunctata* that contained specific categories of cuticular remains: aphid, alfalfa weevil larvae, other arthropod, and pollen and fungi. Individual lady beetles were collected from plots with one of two treatments: high aphid density and low aphid density.

CHAPTER 3

**A FIELD CENSUS TO DETERMINE THE DIETS
OF APHIDOPHAGOUS LADY BEETLES
IN UTAH ALFALFA FIELDS**

Abstract

Aphidophagous lady beetles enhance their foraging success in natural settings by consuming other types of food in addition to aphids. Alfalfa fields (*Medicago sativa* L.) in Utah provide habitat to many aphidophagous lady beetles, including the introduced *Coccinella septempunctata* L., and two native species, *C. transversoguttata richardsoni* Brown, and *Hippodamia convergens* Guérin-Ménéville. Because aphid populations in alfalfa fields are often ephemeral and fail to reach high densities, lady beetles must rely on other sources of food. These sources include other arthropods such as the abundant larvae of the alfalfa weevil (*Hypera postica* [Gyllenhal]), pollen, and fungi.

Frass analysis was used to identify and compare natural diets of female lady beetles in alfalfa fields of northern Utah. The first alfalfa crop was censused in 2004 and 2005. Overall, low densities of pea aphids (*Acyrtosiphon pisum* [Harris]) occurred in both years, although densities of aphids and alfalfa weevil larvae increased as the spring season progressed. Results showed that a high proportion of all three lady beetle species consumed arthropod and non-arthropod food. As aphid and weevil densities increased in later spring, the proportion of lady beetles feeding on these arthropods also increased. A corresponding decrease was seen in the proportion utilizing other types of arthropod prey

(such as thrips and collembolans) and non-arthropod food. In general, the diet of *C. septempunctata* was composed of arthropod prey more than that of the two native species. During 2005, all frass pellets produced by individuals were ranked by size, and the surface area of food fragments in dissected frass pellets was determined. Overall, *C. septempunctata* consumed as much or more than the native species for all food types, as indicated by the greater surface area of prey in frass pellets, and by greater total amounts of frass produced.

Introduction

Insect predators tend to be polyphagous in natural settings (Hagen et al. 1999). While they may focus on a particular type or species of prey, these predators appear to optimize their foraging by consuming many different types of prey. Because of this generalist tendency, it can often be difficult to determine the diets of lady beetles in the field. Many techniques (reviewed by Sunderland 1987, Powell et al. 1996, Symondson 2002, Harwood and Obrycki 2005) have been employed to determine what generalist predators can and will consume. These include direct observation of predation events and laboratory feeding experiments to determine suitability of prey, as well as measurements of predation events after the fact by gut dissection, and a variety of molecular techniques such as enzyme-linked immunosorbent assays (ELISA) and deoxyribonucleic acid (DNA) polymerase chain reaction (PCR).

An additional technique is the use of fecal dissection or frass analysis (Putnam 1964, Lawton 1970, Thompson 1978, Powell et al. 1996). This is similar to gut dissection, save that the analysis is on prey remains once they have been voided by the

predator. An important distinction between frass analysis and molecular and dissection techniques is that the predator does not need to be sacrificed, and hence larger sample sizes can be collected without disturbing the natural populations of potentially beneficial predators. This method is only possible, however, if the organism consumes prey containing indigestible fragments. In the past, this technique has been used with odonate nymphs (Lawton 1970, Thompson 1978) as well as coccinellids (Putnam 1964). In the study presented here, the technique of fecal analysis was used to determine the feeding habits of aphidophagous lady beetles within alfalfa fields (*Medicago sativa* L.) of northern Utah.

Aphidophagous lady beetles feed on a wide range of prey in addition to their preferred aphid prey. Aphids are recognized as essential prey for these predators, as their consumption maximally supports growth and development of larval stages as well as ovariole development and egg-laying by females (Hodek 1962, 1996). Other foods can be important in lady beetle diets because of the ephemeral nature of aphid populations (e.g., Agarwala et al. 1998). Alternative prey or plant foods, such as other arthropods, pollen and nectar do not contain the necessary nutrients to support reproduction and oviposition in lady beetles (Hodek 1996). Nonetheless, alternative foods can sustain these predators such that they can reproduce rapidly when sufficient numbers of aphids are consumed (e.g., Hemptinne and Desprets 1986, Evans and Gunther 2005).

Species of lady beetles differ in how readily they use the many types of alternative prey available in different habitats. One frequent source of food is pollen. Pollen sources include crop plants in fields of agricultural environments, plants along hedgerows, weeds, and native plants. Among lady beetles, *Coleomegilla maculata*

DeGeer is distinctive in using maize pollen as an essential food for reproduction and larval development (Lundgren et al. 2004, 2005). Other aphidophagous lady beetles regularly consume pollen as an alternative food (Putnam 1964, Hemptinne and Desprets 1986, Triltsch 1999, Lundgren et al. 2004, Ricci et al. 2005). Many field studies of aphidophagous lady beetles have recorded varied use of other arthropods besides aphids. Such prey includes thysanopterans, mites, coccids, lepidopteran eggs, and larvae of other Coleoptera, including conspecifics (Hodek 1996, Triltsch 1997, Ricci et al. 2005). Other foods consumed include spores and conidiospores of common plant pathogenic fungi such as *Alternaria* and *Puccinia* (Putnam 1957, 1964; Triltsch 1999; Ricci et al. 2005; Ricci and Ponti 2005).

Alfalfa fields provide an interesting setting to compare foods consumed by different species of aphidophagous lady beetles. These fields are visited by many such species in both North America and Europe. In addition, they offer not only essential foods (aphids), but also a myriad of potential alternative foods, including other types of arthropods, as well as pollen and fungal spores that occur both within and along the edges of the alfalfa habitat. One major alternative prey in alfalfa fields of northern Utah is the larval stage of the alfalfa weevil (*Hypera postica* [Gyllenhal]), which can be abundant during the first crop of alfalfa and is fed upon by larvae and adults of many lady beetle species (e.g., Kalaskar and Evans 2001, Evans 2004, and references within).

Comparison among lady beetle species of the foods that they consume in alfalfa may provide insight into underlying causes of the ongoing shift in community structure of aphidophagous lady beetles that is occurring in alfalfa fields of northern Utah.

Recently introduced, the European species *Coccinella septempunctata* L. has become

very abundant, while at the same time native species have declined in abundance (Evans 2004; see also Elliot et al. 1996, Obrycki et al. 1998, Turnock et al. 2003, Hoogendoorn and Heimpel 2004).

The aim of this study was to use frass analysis to identify and compare the diets of common lady beetle species within alfalfa fields in northern Utah during the first crop (i.e., during the spring and early summer) when lady beetles are especially abundant. Within Utah, approximately a dozen species of native lady beetles plus the introduced *C. septempunctata* can be found in the alfalfa habitat. Of special interest was whether patterns of consumption of aphids or alternative prey (especially the highly abundant alfalfa weevil larvae) differed between the newly dominant species in this habitat, *C. septempunctata*, and the native species that it appears to be displacing. Frass analysis was evaluated for its potential to make such comparisons.

Materials and Methods

Lady beetle adults were collected in alfalfa fields in Cache County, Utah, during the springs of 2004 and 2005. Two fields were selected for study in each year to ensure sufficiently large sample sizes for frass analysis. Due to crop rotations, the field sites changed between years. In 2004, fields were located at the Utah State University Animal Science and Caine Dairy farms near Wellsville, UT. In 2005, fields were selected at the Utah State University Wellsville North farm in Wellsville, UT and Cache Junction farm near Cache Junction, UT.

Censuses were conducted for approximately 6 weeks in each year beginning when alfalfa reached a height of 20 cm and ending at the first cutting. In 2004, lady beetles

were collected between 26 April and 31 May. During the relatively cool spring of 2005, censuses were taken between 13 May and 16 June. Pea aphids (*Acyrtosiphon pisum* [Harris]), preferred prey of lady beetles, and alfalfa weevil larvae, a secondary prey, were available during sampling periods in both years.

As the season progressed, both species of prey increased in abundance. Because the two fields in a given year were near each other (within 2-10 km), and because the general seasonal patterns of abundance of aphids, weevils, and lady beetles were similar between these fields, data collected in the two fields were combined for analysis in each year of the study. To examine possible changes with season in the lady beetles' use of foods (while still maintaining reasonably large sample sizes for individual periods), the data were separated each year into those from early spring (the first 3 weeks of the census period) and late spring (the second 3 weeks). During the early period, aphid and weevil densities were markedly low, while during the late period both prey exhibited a population spike.

During 2004, a single plot (1.5 × 3 m) was established in each field. In order to collect more lady beetles in 2005, between three and five plots (1.5 × 3 m each) were established throughout each field. Every four to 10 days in both years, plot locations were moved to help maintain similar prey densities between plots and the entire field. Plots were sprayed with 1.5 liters of a 15% sugar water solution each time they were set up, to arrest and increase lady beetle populations (Evans and Swallow 1993). Lady beetles were collected beginning 24 hours after sugar solution applications. Depending on weather conditions, lady beetles were collected for the next 2 to 4 days. Because the lady beetle species found in the alfalfa fields differed in relative abundance, sufficient

numbers of only three species could be collected to quantify and compare food habits:

C. septempunctata, *C. transversoguttata richardsoni* Brown, and *Hippodamia convergens* Guérin-Ménéville. Only female adults were examined for their food habits (of the two sexes, females of lady beetles have been well documented to consume the most prey, as they seek to maximize egg production; Hemptinne et al. 1996). Sample sizes (total numbers of females collected) for the three species from combined fields during early and late periods in both years are shown in Table 3.1.

Prey abundance was determined for aphids by randomly collecting 10 sets of 10 alfalfa stems from each field, shaking them in a bucket, and counting the number of aphids that dislodged. Weevil density was determined by randomly collecting stems from each field (25 stems in 2004 and 50 stems in 2005), freezing them, and later examining the stems in the lab. Weevil larvae were sorted to instar, as determined by head capsule size. Prey density data were collected every time new plots were set up in a field (roughly every 4 to 10 days). Relative abundance of the different lady beetle species within plots was determined by tracking how many individuals of each species were collected per person-minute of search during 2005. Pollen sources in the field were noted on each visit in both years, by identifying weedy species in flower throughout the field and at field edges using Whitson et al. (2001).

In the laboratory, beetles were sexed, and females were placed individually into small (5.5cm diameter) Petri dishes and held in an incubator at 20⁰C, 16L: 8D. A drop of 15% sugar water solution was added to each dish to prevent starvation. Beetles were held for 48 hours (as based on previous laboratory studies of digestion rates and associated

temporal patterns of frass production, presented in Chapter 2). The largest pellet produced by each individual was selected for dissection and analysis.

Pellets were placed individually in the well of a depression glass slide with a drop of 20% sodium hydroxide. When softened, the pellet was teased apart and inspected under the 10 × objective of a compound microscope. In both years, contents were scored as to whether the following fragments, associated with different types of food, were present or absent: aphid cuticle, alfalfa weevil larvae cuticle, pollen and fungi (of various species combined), and fragments of arthropods other than aphids and weevils. The distinction between cuticular fragments from alfalfa weevils, pea aphids, and other kinds of arthropods was determined in a previous study (Chapter 2). Pollen and fungi could be distinguished from arthropod prey based on their shape, and with the aid of a prior field experiment (Chapter 2). Moore et al. (1991), Triltsch (1999), Agrios (2005), and Ricci et al. (2005) were used to verify fragments as pollen or fungi. Because so many types of pollen and fungal spores could be found in frass pellets, all were combined and scored as a single category (non-arthropod foods).

Alfalfa fields harbor many types of arthropods that can serve as prey for lady beetles, including thrips, a variety of hemipterans, and other types of beetle and lepidopteran larvae. Identification to specific type or group without further diagnostic laboratory preparation was problematic. However, cuticular fragments from lady beetle larvae, thrips, and collembolans were distinctive, and could be identified and included in scoring for foods consumed. Because few individuals had distinctive chitinous arthropod remains other than from aphids and weevils, these fragments were combined into a single

“other arthropod” category for quantitative analysis, even if it was possible to categorize the cuticle more specifically.

During 2005 an additional set of data was collected. This included the number and size of all pellets produced by a female during the 48 hour holding period. In general, most lady beetles produced some frass, although there were many occurrences where frass pellets did not contain prey or food fragments. Lady beetles were likely to produce multiple frass pellets of varying sizes. Pellets were categorized into size rankings of one to five (smallest to largest) by comparison to a reference of frass samples made in 2004. As an index of the total amount of frass produced by an individual, these ranks for individual pellets were added together.

Although all pellets produced were ranked to size in 2005, only the largest pellet was selected for dissection. For each of the three species, this pellet represented about 30% of the total estimated amount of frass produced by a female in both early and late spring. Additionally in 2005, the number of fragments of each food type present was quantified upon dissection of the largest pellet. An eyepiece reticle with a 10 mm square counting grid divided into 100 (1 mm) squares (21 mm diameter Meiji Technology MA283/05) was used to determine the total number of squares containing fragments of each food type. The entire well area of the depression glass slide was observed, and squares were only counted when food fragments were present. The mean surface area of each food type was estimated for each lady beetle species.

All statistical analyses were performed using SAS version 9.1 (SAS Institute 2003). Presence versus absence data from 2004 and 2005 were analyzed with χ^2 tests of independence. Mean surface area of prey within frass from 2005 was analyzed using

two-way ANOVA (type III sums of squares) with species and time (early or late in the first crop) as main effects. Each type of prey was analyzed individually by including only those individuals that had consumed the particular prey type (individuals without any fragments of the prey in their frass were left out of the analysis to preserve normality of the data). Data for aphid fragments in the frass were square root transformed to meet assumptions of normality. For all other prey types (weevil, other arthropod, pollen, and fungi), data were log transformed to meet assumptions of normality. Prey densities in the alfalfa fields early versus late in the first crop in 2004 and 2005 were analyzed using one-way ANOVA type III sums of squares, and aphid densities were square root transformed to meet assumptions of normality.

Results

Prey and Predator Abundances in Alfalfa Fields

Prey densities exhibited similar trends in 2004 and 2005: low aphid numbers occurred in early spring, increasing to higher densities later in the first crop (Fig. 3.1). In each year, there was a significant difference in aphid densities between early and late periods (Fig 3.2.A; 2004 one-way ANOVA, effect of time: $F_{1,98} = 260.11$, $p < 0.0001$; 2005 one-way ANOVA, effect of time: $F_{1,118} = 34.56$, $p < 0.0001$; Appendix B). During 2004, aphid densities were stable throughout the entire early period, and spiked during the late period. In contrast, during 2005, aphid densities were low and stable throughout much of the season, but then spiked to a high density during the final week of the season (Fig. 3.2).

Weevil densities exhibited similar seasonal trends in 2004 and 2005 as well, with weevil populations staying relatively stable during the early period, and spiking to higher levels during the late period (Fig. 3.1). Weevil densities were significantly different between early and late periods in both 2004 and 2005 (Fig. 3.2; 2004 one-way ANOVA, effect of time: $F_{1,8} = 10.4$, $p = 0.012$; 2005 one-way ANOVA, effect of time: $F_{1,9} = 18.5$, $p = 0.002$; Appendix B). Age structure of weevil populations differed between the early and late periods during 2004 and 2005 (Fig 3.2), with the proportion of the population represented by later instars (third and fourth) increasing during the late period. During both sampling periods, a large proportion of the population was composed of early instars (first and second).

During 2005, *C. septempunctata* accounted for almost 50% of all female lady beetles collected during both time periods (Fig 3.3). The next most abundant species was *H. convergens*, accounting for an additional 25% of females captured in the early period and 30% in the late period. *C. transversoguttata* was present in much lower numbers, accounting for little more than 15% of all females collected in both periods. An assortment of other lady beetle species was collected in low numbers. These species included *Harmonia axyridis* (Pallas), *Adalia bipunctata* L., several members of the genus *Hippodamia* (*H. quinquesignata quinquesignata* [Kirby], *H. sinuata crotchii* Casey, and *H. tredecimpunctata tibialis* [Say]), and two species in the genus *Coccinella* (*C. novemnotata* Herbst, *C. difficilis* Crotch). Such low numbers were collected for these other species that they were not included in the analyses.

Prey Use in 2004 and 2005

Throughout the spring in 2004 and 2005, a high proportion of *C. septempunctata*, *C. transversoguttata*, and *H. convergens* females had fragments of arthropod prey, as well as pollen and fungal spores, in their frass (Figs. 3.4 and 3.5). Additionally, a high proportion (40-60% in 2004, and 40-70% in 2005) had consumed more than one category of prey over a short period of time, such that these multiple prey could be identified from a single frass pellet. Many females (30-85% in 2004, and 20-50% in 2005) had consumed aphids. Many females also had consumed alfalfa weevils (15-30% in 2004, and 10-50% in 2005), or other arthropods (10-35% in 2004, and 15-40 % in 2005) (Figs. 3.4 and 3.5).

For all three species combined, frass analysis revealed significant changes in diet as spring progressed (Figs. 3.4 and 3.5; Table 3.2). An increase occurred from early to late spring 2004 in the proportion of females consuming arthropod prey (all categories combined; χ^2 test, $P < 0.0001$). The proportion of individuals consuming arthropod prey was high from the outset in 2005, and hence did not increase significantly from early to late spring ($P = 0.37$). Especially in 2004, an increase from early to late spring was detected in the proportion of individuals consuming aphids (2004: $P < 0.0001$; 2005: $P < 0.01$). Also in both years, a decrease from early to late spring was detected in the proportion of individuals consuming arthropods other than aphids and weevils, and non-arthropod foods ($P < 0.0001$ for each category in each year). An increase was detected in 2005 ($P < 0.0001$), but not in 2004 ($P = 0.50$), from early to late spring in the proportion of individuals consuming weevils. No changes occurred between early and late spring, in either 2004 ($P = 0.065$) or 2005 ($P = 0.16$), in the lady beetles' proclivity to consume a

short-term mixed diet (as indicated by frass pellets containing fragments of more than one category of food).

The degree to which females fed on various foods differed among the three lady beetle species in both early and late spring (Figs. 3.4 and 3.5; Table 3.3). In most respects, the general patterns among species in the proportions of females consuming different types of food were similar in 2004 and 2005. In general, the proportion of females consuming some kind of arthropod was highest for *C. septempunctata*, intermediate for *C. transversoguttata*, and lowest for *H. convergens*. Similarly, the proportion of females consuming aphids was highest for *C. septempunctata* and lowest for *H. convergens*, with proportions for *C. transversoguttata* varying in between. The proportion of females consuming weevils did not vary significantly among the three species in either early or late spring 2004, but was higher for *C. septempunctata* than for *C. transversoguttata* and *H. convergens* in early and late spring 2005. No difference was apparent among species in the proportion of females consuming arthropods other than aphids or weevils in either early or late spring in 2004 and 2005.

In general, the proportion of females consuming non-arthropod food was highest for *C. septempunctata*, intermediate for *C. transversoguttata*, and lowest for *H. convergens* females. This pattern was more strongly expressed in late than in early spring (Figs. 3.4 and 3.5; Table 3.3). Flowering plants found within the field and on field edges most likely provided pollen for lady beetles. These sources varied from week to week, but included a wide range of plants in both years: dandelion (*Taraxacum officinale* Weber in Wiggers), bilobed speedwell (*Veronica biloba* L.), red stem filaree (*Erodium cicutarium* [L.] L'Her. ex Ait.), western salsify (*Tragopogon dubius* Scop.), a variety of

thistles (genus *Cirsium*), a variety of mustards (family Brassicaceae) including hoary cress (*Cardaria draba* [L.] Desv.), dyer's woad (*Isatis tinctoria* L.), shepherd's purse (*Capsella bursa-pastoris* [L.] Medic.), multiple types of grasses (Family Poaceae), and alfalfa (*M. sativa* L.) itself. Pollen grains from pine trees were found regularly in the frass of all lady beetle species, and likely came from trees planted by field edges, or near to fields. Fungi also factored into the lady beetle's diet, with spores of two main genera found: *Alternaria* and *Puccinia*. Other types of fungi could have been present as well, but available resources only allowed identification of these two genera.

The proportion of females exhibiting a short-term mixed diet was generally highest for *C. septempunctata*, intermediate for *C. transversoguttata*, and lowest for *H. convergens* females (Figs. 3.4 and 3.5; Table 3.3). An exception occurred in early spring 2004, when a higher proportion of females of *C. transversoguttata* than of *C. septempunctata* and *H. convergens* exhibited a mixed diet.

Quantities of Frass Produced and Prey Consumed in 2005

There were significant interactions between time period (early and late spring) and species (*C. septempunctata*, *C. transversoguttata*, and *H. convergens*) in the number of frass pellets, their average rank size, and the sum total of ranks of frass pellets produced by females when brought from the field and held for 48 hours in the lab (Table 3.4; Appendix B). In early spring, females of the three species produced similar quantities of frass (they laid similar numbers of pellets), although females of *C. transversoguttata* produced pellets of slightly larger size on average than did females of *C. septempunctata* and *H. convergens* (Fig 3.6). In contrast, later in the first crop, females of *C. septempunctata* produced the most frass: females of all three species

produced similarly sized pellets on average, but *C. septempunctata* females produced these in largest number (Fig. 3.6). Overall, the total quantity of frass produced increased from early to late spring, particularly for females of *C. septempunctata* and less so for *C. transversoguttata* and *H. convergens* (Fig 3.6). Nevertheless, the proportional amount of frass that was dissected for each individual was relatively consistent between species and time periods (Fig. 3.6).

Differences between the early and late period and lady beetle species were variable in the quantities of food consumed (as reflected by fragments present in the frass), among females that had consumed different categories of food in 2005 (Fig. 3.7; Table 3.5; Appendix B). In many cases, females of a given species consumed similar amounts of a given type of food in early and late spring. In only one case did the quantity clearly decline with season: females of *C. transversoguttata* consumed much less of non arthropod food in late versus early spring. Marked seasonal increases occurred in the quantity of all arthropods combined, and of aphids and weevils in particular, consumed by *C. transversoguttata* females. A strong increase from early to late spring occurred also in the quantity of weevils consumed by *C. septempunctata* females. No clear change from early to late spring in the amount of consumption of any food type was observed for *H. convergens* females. Overall, females of *C. septempunctata* (and of *C. transversoguttata* in late spring) consumed more of all arthropods combined and aphids than did females of *H. convergens*. Females of *C. septempunctata* also consumed the largest quantities of weevils, especially in late spring. Results for consumption of non-arthropods were noteworthy in that females of *C. transversoguttata* ate more than did females of the other two species in early spring, but less in late spring (Fig. 3.7).

Discussion

Generalist predators utilize many types of prey in their environment. Because of this broad acceptance of different types of prey, it can be difficult to determine the importance of each type to the predator's diet. Various techniques have been devised to study the diets of generalist insect predators, both to puzzle out the broad components of their diet and to determine how often they are feeding on specific pests of interest. Frass analysis has been seldom used (Putnam 1964, Lawton 1970, Thompson 1978), but was found to be a promising technique for studying the diets of lady beetles (Chapter 2). In the present study, frass analysis proved useful to characterize the diets of lady beetle species in alfalfa during spring, and to compare tendencies of three species to consume preferred aphid prey and a major alternative prey, alfalfa weevil larvae.

Frass analysis revealed a number of interesting patterns of prey use by lady beetles in spring alfalfa. Females of *C. septempunctata*, *C. transversoguttata* and *H. convergens* consumed a wide variety of foods, including pollen and fungal spores. They typically consumed more than one category of food over a short period of time, as reflected in the contents of a single frass pellet. Females fed frequently on other types of arthropods in addition to their preferred prey, aphids. In particular, cuticular fragments of alfalfa weevil larvae were found in the frass of these females between 20-50% of the time. The increase in use of aphid and weevil prey observed as spring progressed likely reflects the greater availability of these prey species in late than in early spring.

Differences between 2004 and 2005 in spring patterns of lady beetle consumption of aphids and weevils may reflect the difference in relative abundance of the two prey species in the two years. Aphid densities were low (0.1 to one aphid per stem in 2004;

0.05 to two aphids per stem in 2005) in both years, especially in 2005. Although aphid densities increased during the first crop in both 2004 and 2005, this increase did not occur in 2005 until the very end of the census period (i.e., until just before cutting). In contrast, weevil densities increased earlier in the first crop during 2005. A large proportion of alfalfa weevil larvae were early instars (first and second) throughout the first alfalfa crop in both years. This availability of smaller, more readily handled weevil prey throughout the spring season likely affected the amount of weevils consumed by the lady beetles. Thus, prey availability in 2005 reflected an extended period of low aphid densities combined with overall greater availability of weevils than in 2004. This may account for the more marked late season increase in weevil consumption, and the less marked late season increase in aphid consumption, in 2005 than in 2004.

As aphid and weevil densities increased during the first crop, and as correspondingly higher proportions of lady beetle females were found to prey upon these species, fewer females were found to include non-arthropod foods and arthropods other than weevils and aphids in their diet. Thus, it would appear that ready availability of the two primary prey species in first crop alfalfa, pea aphids and alfalfa weevil larvae, results in diminished use of other foods by lady beetle females. Similarly, *C. septempunctata* was found to vary in its diet as aphid prey became more available in both grain fields and natural environments in Europe, though aphids were consistently utilized throughout the year (Ricci et al. 2005).

Frass analysis also indicated that in general, a higher proportion of females of the dominant, introduced *C. septempunctata*, than of the two less abundant, native species (*C. transversoguttata* and *H. convergens*), produced frass containing fragments of

arthropods throughout the spring. There was also a tendency for a higher proportion of females of *C. septempunctata* versus native species to have consumed aphids. Higher proportions of *C. septempunctata* than of *H. convergens* females were found with aphid remains in their frass throughout the first crop in both 2004 and 2005. In addition, higher proportions of *C. septempunctata* versus *C. transversoguttata* females were found to have consumed aphids late in the first crop in 2004, and early in the first crop in 2005 (early in 2004 and late in 2005, however, similar proportions of *C. septempunctata* and *C. transversoguttata* females produced frass with aphid remains).

These dietary differences may be a result of *C. septempunctata* on average being a larger beetle, thus requiring it to consume more food to fuel its metabolism than its native counterparts in the same habitat. Additionally, *C. septempunctata* has been found to tolerate environments with low aphid densities (Triltsch and Freier 1998). Relatively high proportions of *C. septempunctata* have been found to consume aphid prey throughout the year, which may indicate its ability to effectively search for and consume aphid prey even when present in the environment in low densities (Triltsch 1999). Similar studies have not been undertaken with *C. transversoguttata* and *H. convergens*, however.

A previous, laboratory study found that very high and similar percentages of *C. septempunctata* and *C. transversoguttata* females (97% and 95%, respectively) that fed on aphids subsequently produced frass pellets with detectable aphid fragments (Chapter 2). These findings simplify the interpretation and comparison in the present study of proportions of field-collected *C. septempunctata* and *C. transversoguttata* females that produced frass with aphid fragments. The laboratory study also found, however, that a

lower percentage (80%) of *H. convergens* females that consumed aphids subsequently produced frass with detectable aphid fragments. Thus, analysis of frass from field-collected females of this species in particular may underestimate the frequency of aphid consumption, and accordingly the results of the present study concerning *H. convergens* females should be interpreted cautiously.

During 2005, when aphid densities were especially low throughout most of the first crop, a higher proportion of females of *C. septempunctata* than of either native species produced frass containing weevil fragments (during 2004, the three species were similar in their use of weevils in both early and late spring). During previous laboratory experiments, a large proportion of individuals of all three females that fed on weevils did not produce frass containing detectable weevil fragments (Chapter 2). This was especially the case for the two native species (71% of *C. transversoguttata* individuals, and 63% of *H. convergens* individuals), while such occurred with lower frequency (42%) among *C. septempunctata* females. If such proportions are a fair reflection of intrinsic differences in this regard among the three lady beetle species, they (rather than differences in field consumption rates) might account for patterns suggested by frass analysis for field-collected females. For example, the higher proportions of *C. septempunctata* than *C. transversoguttata* and *H. convergens* females producing frass with weevil fragments in 2005 may reflect that *C. septempunctata* females are more likely to produce such frass after feeding on weevils. Further study is needed to address this issue.

Overall, given that lady beetles that have fed on weevils often produce frass without detectable fragments, females of all three species very likely consumed alfalfa

weevils even more frequently in the alfalfa fields studied here than indicated by the observed high proportions (20-50%) of frass pellets containing weevil fragments. This supports the inference drawn previously from field observations (e.g., Kalaskar and Evans 2001, Evans 2004, and references within) that alfalfa weevil larvae are important components of lady beetle diets in spring alfalfa.

Similar types of arthropods other than aphids and weevils were consumed by the three lady beetle species, with no striking differences among the predator species. Commonly preyed upon were thrips, which have also been documented widely as alternative food for adult lady beetles (Putnam 1964, Triltsch 1997, Ricci and Ponti 2005). Cannibalism and intraguild predation on coccinellid larvae were very rare, with frass from only a few individuals containing cuticular fragments that could have been coccinellid larvae, and no tendency for this to occur more frequently in the frass of *C. septempunctata* versus native species. This concurs with a study by Triltsch (1999) where it was suggested that cannibalism occurs predominantly between larval stages, and only rarely with lady beetle adults consuming larval stages. When lady beetle adults consumed larval stages in the laboratory, a large amount of fragments were found (Chapter 2). It is therefore likely that consumption of prey in the field, if it occurred, would provide sufficient cuticular fragments to document such cannibalism taking place.

Pollen and fungal spore use was similar between years, with use by lady beetle populations declining late in the first crop when arthropod prey such as aphids and weevils were more readily available. The types of pollen found in frass were consistent across lady beetle species, suggesting that the predators exploited specific pollens simply in proportion to their availabilities (or that they had similar preferences). Pollinivory has

been recorded for many species of coccinellids. For example, adults of *Adalia bipunctata* use pollen to sustain themselves, enabling them to initiate reproduction quickly when aphids become available (Hemptinne and Desprets 1986). *H. convergens* has also been found to utilize pollen, particularly when aphids are rare, in order to build up fat reserves for overwintering (Hemptinne and Desprets 1986). *C. septempunctata* was found to utilize both pollen and fungi in agricultural and native environments in Europe, and this usage increased as aphid usage (and densities) decreased (Ricci et al. 2005). In addition, many species have been shown to consume fungi with regularity throughout adulthood (Putnam 1964, Triltsch 1999, Ricci et al. 2005). The nutritional contribution of fungal spores to lady beetle's diets is not known, but it has been suggested that use of fungal spores reflects the phylogenetic relationships with mycophagous Coccinellidae (Triltsch 1997). Given that aphid densities were extremely low in the alfalfa fields studied here, pollen and fungi likely served as important alternative foods for the lady beetles.

In summary, the results of frass analysis indicate that the diets of *C. septempunctata* and the two native species in alfalfa were similar in many respects, including in how these diets varied between 2004 and 2005. Nonetheless, it appears overall that *C. septempunctata* females may be more successful than females of *C. transversoguttata* and *H. convergens* in finding and consuming arthropod prey, particularly aphids and weevils, during the first crop of alfalfa. This is suggested both by the overall higher proportions of the *C. septempunctata* populations utilizing arthropod prey in the two years studied, and by the overall greater amounts of food consumed by *C. septempunctata* females in 2005 (as measured by frass produced, including both

quantities of frass and mean surface areas of diagnostic fragments in the pellets). In addition, *C. septempunctata* females appeared to be as successful, and sometimes more so, than native lady beetle females in finding and consuming non-arthropod food. Particularly in 2005, a higher proportion of *C. septempunctata* females were found to have consumed non-arthropod food than the native species, even though total surface area consumed was on average less than the native species.

As in the present study, Evans (2004) found low aphid densities in alfalfa fields of northern Utah throughout the spring in recent years. Adults of *C. septempunctata* appear to tolerate agricultural habitats with low aphid densities, in part by using a variety of types of arthropods as supplementary food (Triltsch and Freier 1998, Evans 2004, Evans and Toler 2007). Also consistent with patterns in previous years in northern Utah, *C. septempunctata* was the dominant lady beetle species in the alfalfa fields studied here, and accounted for almost 50% of all female lady beetles present throughout the spring. This may be due in part to their greater predilection, in comparison to native species, to tolerate habitats with low aphid densities (Evans 2004). The results of the present study suggest furthermore that such predilection may derive from the greater ability of *C. septempunctata* than native lady beetle adults to search for and find food, including aphids at low density, in these alfalfa fields.

The aim of this study was to determine the degrees to which these lady beetles utilize the many sources of food available to them in the alfalfa habitat. While the three lady beetle species were similar in their use of different prey items, there was a general trend of greater use by *C. septempunctata* of most prey types in the habitat. Additionally, for many types of prey, individual *C. septempunctata* consumed more on average than

their native counterparts (*C. transversoguttata* and *H. convergens*). Frass analysis was a useful technique in this study, as it allowed the separation of several categories of food included in the diet of these lady beetles. With further laboratory work, frass analysis could be used in the future to delve more deeply into the broad category of “other arthropod” prey and consider specific prey types such as Collembola and Thysanoptera. One problem of varying severity with frass analysis is the tendency of lady beetles in some cases to capture prey, but only consume a liquid meal from them. This can lead to underestimates of prey use (e.g., as in the case of weevil consumption), or may prevent this technique from being applied for certain prey types (e.g., arthropod eggs; Triltsch 1999). On the other hand, frass analysis can be performed quickly, and with minimal cost input, in contrast to many other methods of determining diet. Additionally, there is no need to sacrifice the insect. The results of the present study suggest that frass analysis may provide a good overview of the diets of lady beetles in a variety of environments.

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Table 3.1. Sample sizes of female lady beetle species collected in the two time periods (early and late) for the spring of 2004 and 2005.

	<i>C. septempunctata</i>	<i>C. transversoguttata</i>	<i>H. convergens</i>
2004 Early	183	67	71
2004 Late	187	58	62
2005 Early	156	115	167
2005 Late	153	135	161

Table 3.2. Results for chi-square tests of independence comparing proportions of lady beetles utilizing different types of food in early versus late spring of 2004 and 2005. Significant p-values are shown in bold type. Degrees of freedom for all tests equal 1.

Food Type	2004 Early vs. Late (All species combined)		2005 Early vs. Late (All species combined)	
	χ^2 value	p-value	χ^2 value	p-value
All Arthropods	49.85	< 0.0001	0.81	0.37
Aphids	102.49	< 0.0001	6.56	0.010
Weevil	0.46	0.50	57.20	< 0.0001
Other Arthropod	31.12	< 0.0001	2.56	< 0.0001
Non-arthropod	29.25	< 0.0001	15.92	< 0.0001
Mixed Diet	3.40	0.065	2.0	0.16

Table 3.3. Results for chi-square tests of independence comparing proportions lady beetles utilizing different types of food in early and late spring of 2004 and 2005. Significant p-values are shown in bold type. Degrees of freedom for all tests comparing species (*Coccinella septempunctata*, *C. transversoguttata*, and *Hippodamia convergens*) equal 2.

Food Type	2004 Early (Among Species)		2004 Late (Among Species)		2005 Early (Among Species)		2005 Late (Among Species)	
	χ^2 value	p-value	χ^2 value	p-value	χ^2 value	p-value	χ^2 value	p-value
All Arthropods	8.75	0.013	10.47	0.0053	13.11	0.0014	13.45	0.0012
Aphids	3.16	0.21	18.87	< 0.0001	16.93	0.0002	8.81	0.012
Weevil	1.60	0.45	3.58	0.17	8.68	0.013	16.94	0.0002
Other Arthropod	2.50	0.29	0.16	0.92	3.25	0.20	0.99	0.68
Non-arthropod	3.15	0.21	6.74	0.034	5.64	0.060	16.18	0.0003
Mixed Diet	7.43	0.024	5.85	0.054	6.65	0.036	28.43	< 0.0001

Table 3.4. Results of two-way ANOVA (time [early vs. late 2005] x species [C7, Hc, and Ct]) for attributes of frass pellets produced by individual lady beetle females, collected from alfalfa fields, including the average number of frass pellets produced, average rank size of frass pellets produced, average sum total of ranks for size of frass pellets produced, and proportion of the total amount of frass that was dissected. The proportion of the total amount of frass dissected was only determined for those individuals whose frass pellet contained some type of prey or food,. All effects are from Type III sums of squares. Significant p-values are shown in bold.

	Time x Species			Time			Species		
	df	F-value	P-value	df	F-value	P-value	df	F-value	P-value
Average Number of Pellets Produced	2, 884	14.73	<0.0001	1, 884	38.18	<0.0001	2, 884	3.26	0.039
Average Rank Size of Pellets Produced	2, 884	9.81	<0.0001	1, 884	45.86	<0.0001	2, 884	71.80	<0.0001
Average Accumulated Total Ranks of Pellets Produced	2, 884	24.52	<0.0001	1, 884	80.09	<0.0001	2, 884	32.16	<0.0001
Proportion of Total Dissected	2, 664	2.94	0.054	1, 664	4.38	0.034	2, 664	0.75	0.47

Table 3.5. Results of two-way ANOVA (time [early vs. late 2005] x species [C7, Hc, and Ct]) for mean surface area consumed of different types of prey. Prey types are as follows: all arthropod prey combined, aphids, alfalfa weevil larvae, other arthropods (not including aphids or weevils), and non-arthropod food (includes pollen and fungi). Data for each type of prey was only included if the individual had consumed that type of prey. All effects are from Type III sums of squares. Significant p-values are shown in bold.

	Time x Species			Time			Species		
	df	F-value	P-value	df	F-value	P-value	df	F-value	P-value
All Arthropods	2, 805	5.21	0.0056	1, 805	20.77	<0.0001	2, 805	21.25	<0.0001
Aphids	2, 325	5.43	0.0048	1, 325	7.09	0.0081	2, 325	8.22	0.0003
Weevils	2, 256	0.93	0.40	1, 256	8.24	0.0044	2, 256	1.93	0.15
Other Arthropod	2, 220	0.5	0.61	1, 220	0	0.95	2, 220	1.25	0.29
Non-Arthropod	2, 516	11.2	<0.0001	1, 516	28.65	<0.0001	2, 516	1.48	0.23

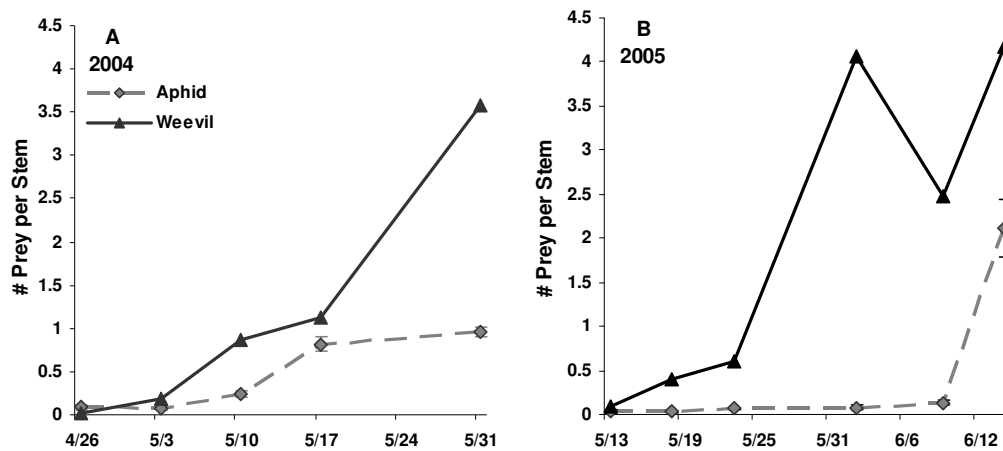


Fig. 3.1. Number of aphids and alfalfa weevil larvae per stem during early and late spring and in 2004 and 2005 (results are combined for two fields sampled in each year).

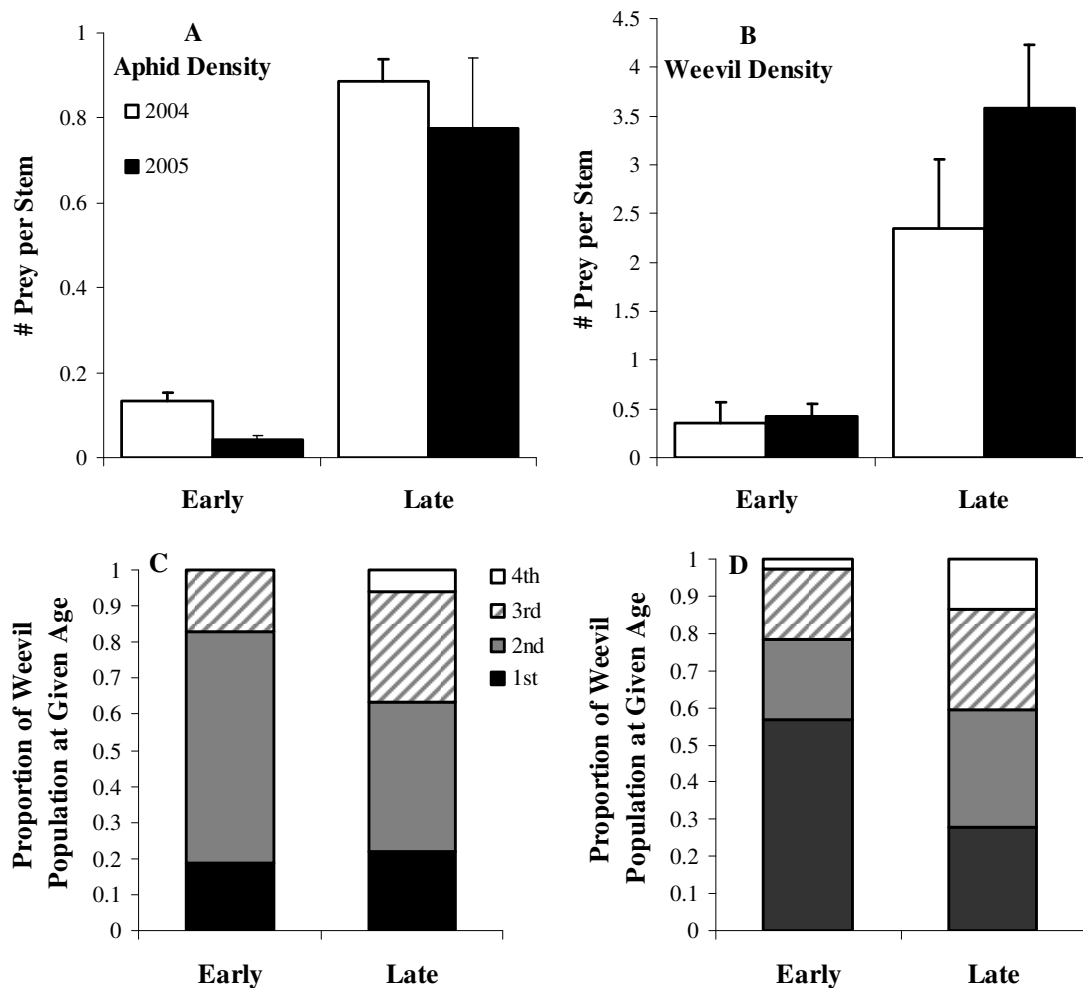


Fig. 3.2. Prey density (aphids [A] and alfalfa weevil larvae [B]) during early and late sampling periods of 2004 and 2005, and age structure of alfalfa weevil larvae during early and late sampling periods of 2004 (C) and 2005 (D).

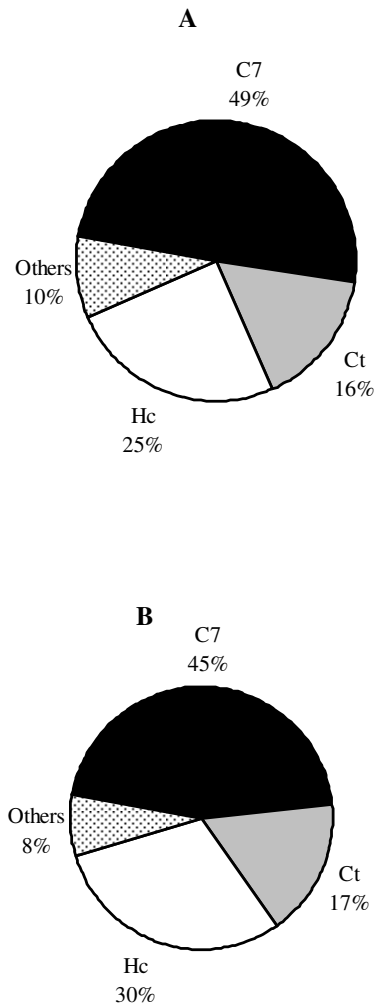


Fig. 3.3. Percentages of adult female lady beetles collected in alfalfa fields during early (A) and late (B) spring 2005. C7, Ct, and Hc stand for *Coccinella septempunctata*, *C. transversoguttata*, and *Hippodamia convergens*, respectively. The category “others” includes all other lady beetle species combined.

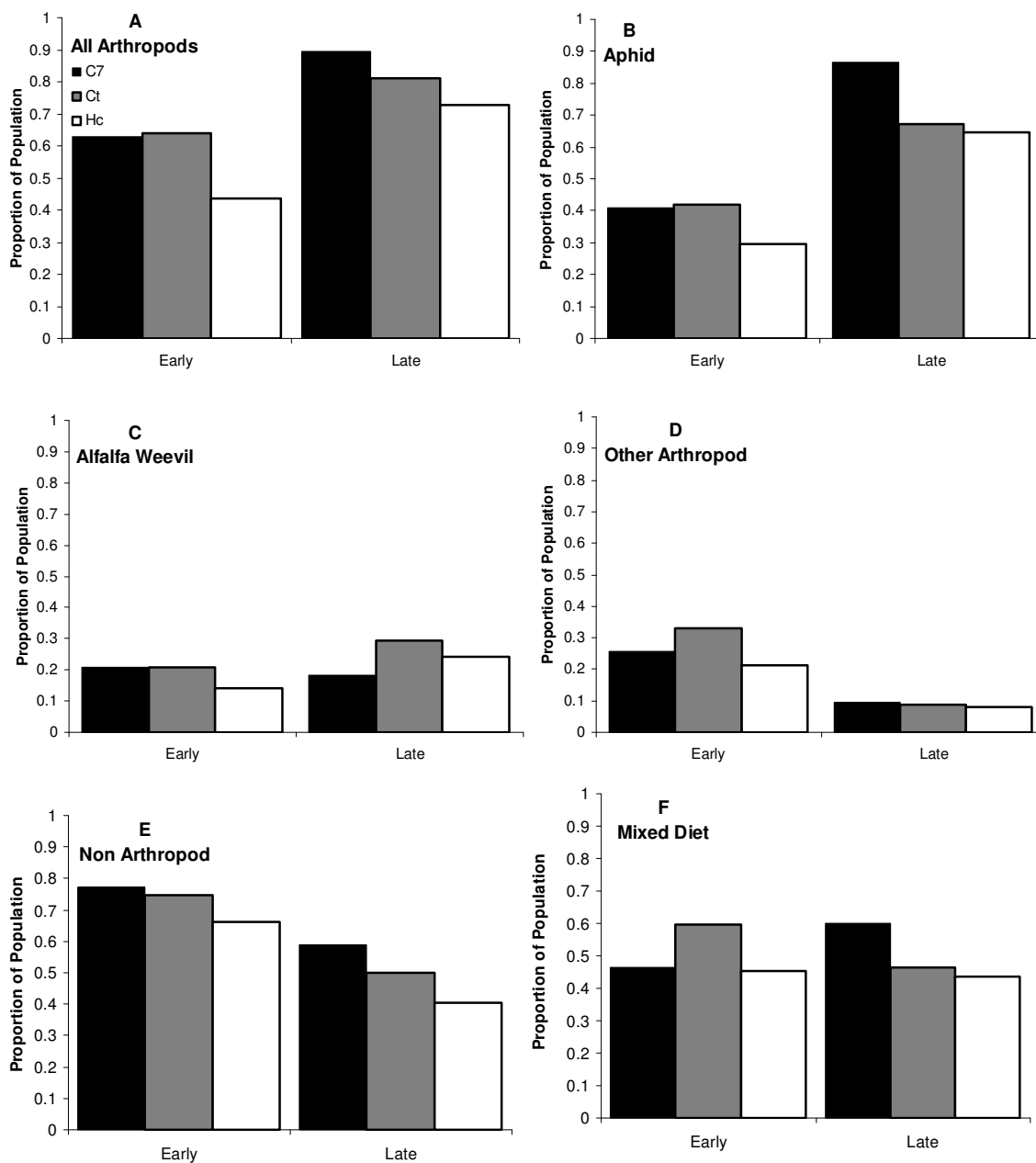


Fig. 3.4. Food use in spring of 2004 by female lady beetles of three species: *Coccinella septempunctata* (C7), *C. transversoguttata* (Ct), and *Hippodamia convergens* (Hc). Results are shown for (A) all arthropod prey combined, (B) aphid, (C) weevil, (D) other arthropod, (E) non-arthropod, and (F) mixed diet. The category “All arthropod prey combined” includes aphids, weevils, and all other unidentified arthropods. “Other arthropod prey” includes only unidentified arthropods, with aphids and weevils excluded. “Non-arthropod” includes all non-arthropod food such as pollen and fungi. “Mixed diet” is based on individuals with dissected frass pellets that contained more than one of these categories.

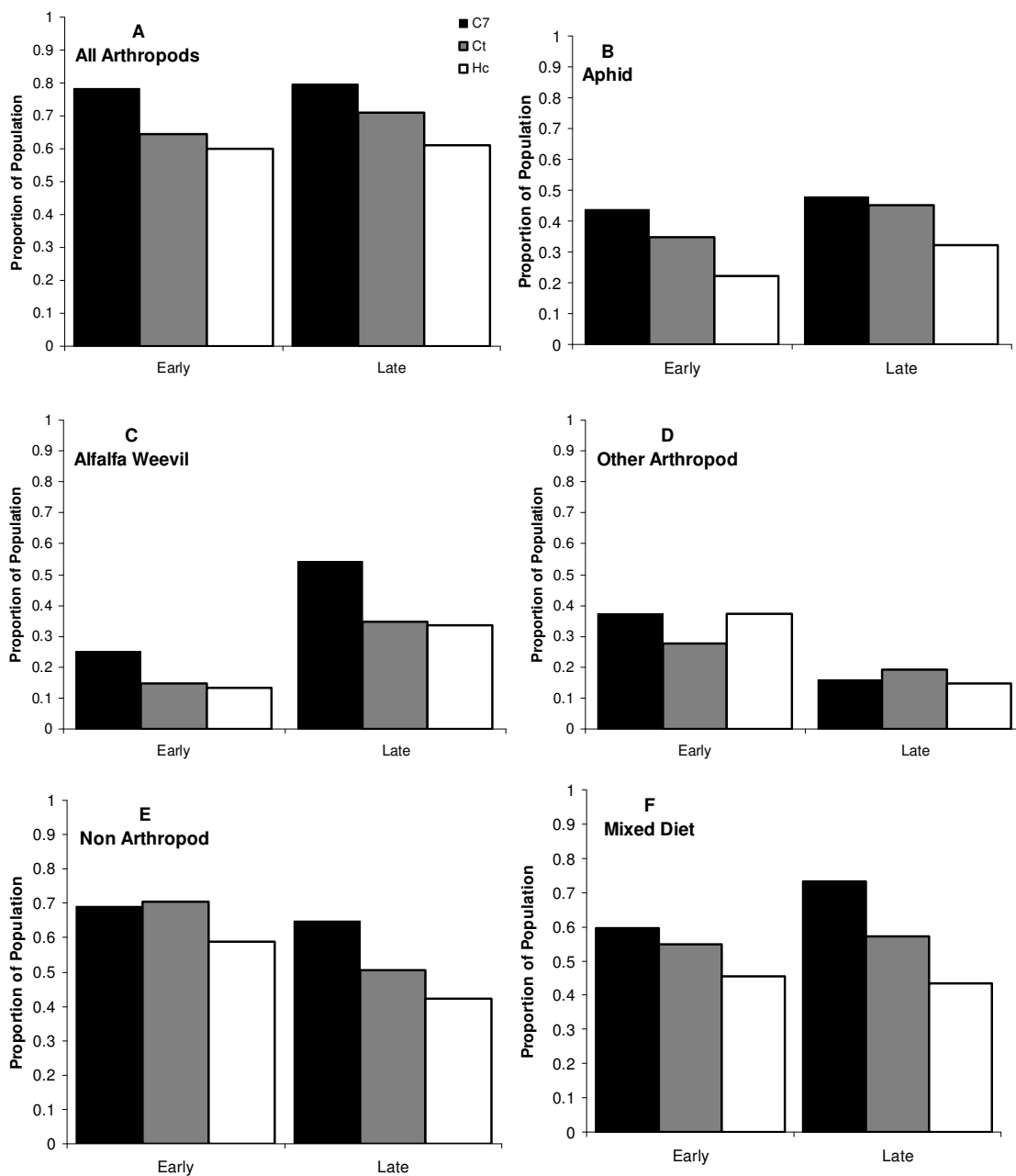


Fig. 3.5. Food use in spring of 2005 by female lady beetles of three species: *Coccinella septempunctata* (C7), *C. transversoguttata* (Ct), and *Hippodamia convergens* (Hc). Results are shown for (A) all arthropod prey combined, (B) aphid, (C) weevil, (D) other arthropod, (E) non-arthropod, and (F) mixed diet. The category “All arthropod prey combined” includes aphids, weevils, and all other unidentified arthropods. “Other arthropod prey” includes only unidentified arthropods, with aphids and weevils excluded. “Non-arthropod” includes all non-arthropod food such as pollen and fungi. “Mixed diet” is based on individuals with dissected frass pellets that contained more than one of these categories.

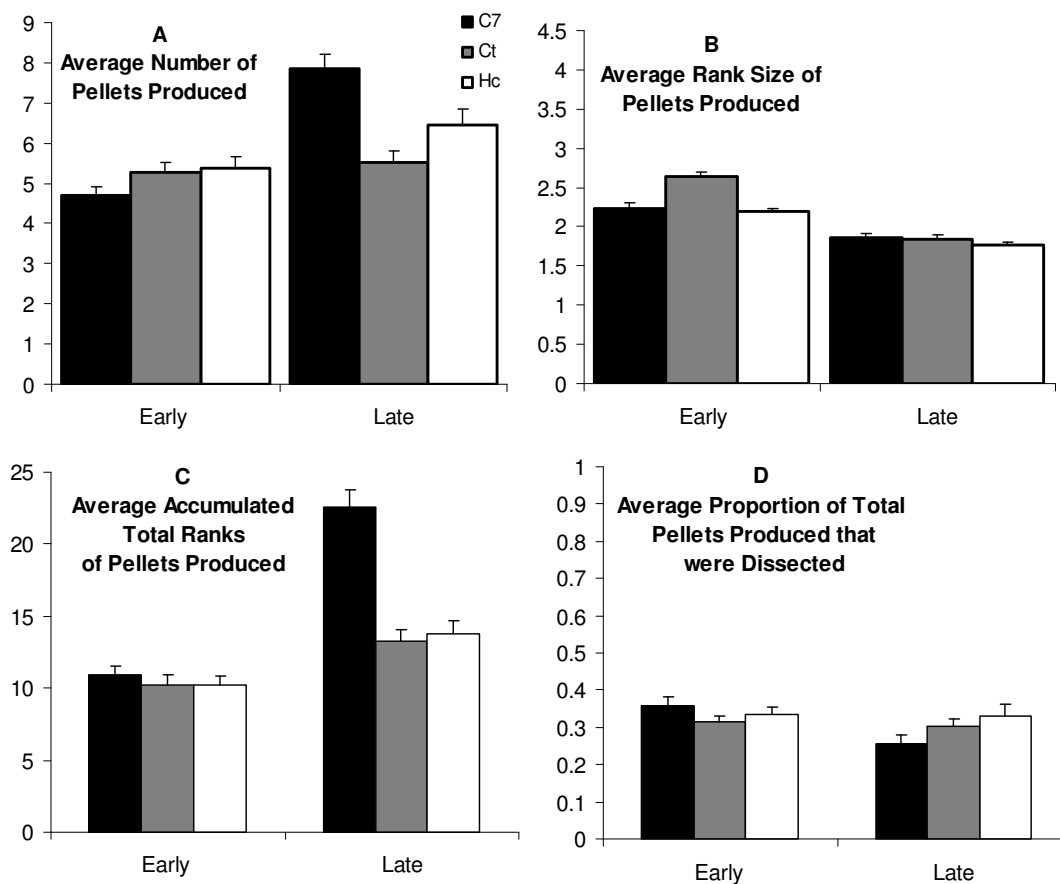


Fig. 3.6. Attributes of frass pellets produced by females of the three lady beetle species collected early and late in spring 2005 from alfalfa fields: (A) the average number of frass pellets produced by an individual; (B) the average size (estimated by rank) of frass pellets produced, including those pellets not dissected; (C) the average sum total of ranks of frass pellets produced; (D) the proportion of the total amount of frass produced that was dissected (this proportion was determined only for those individuals whose frass contained prey or food fragments, and one pellet was selected for dissection from each individual).

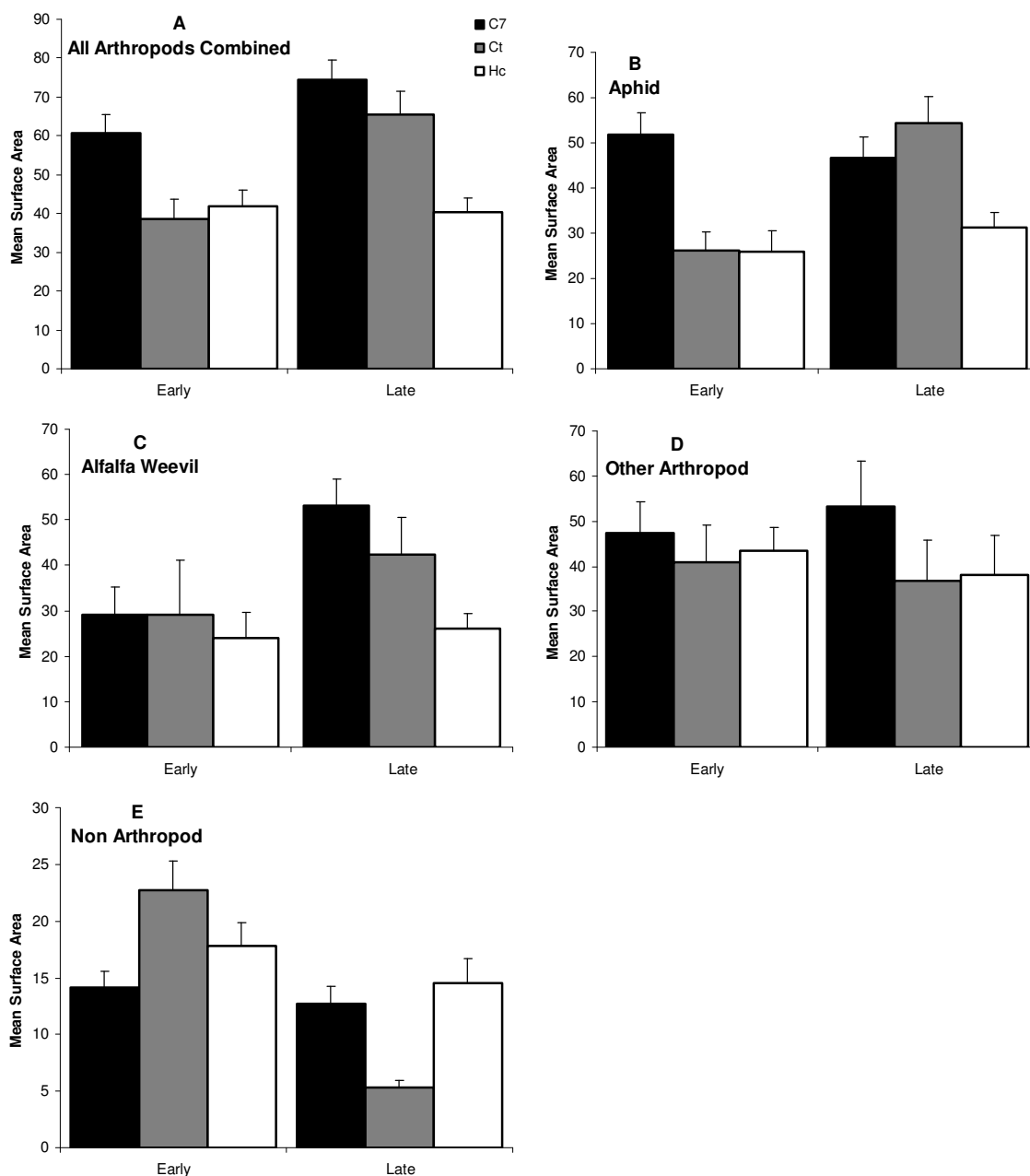


Fig. 3.7. Mean surface area (mm²) of prey in dissected frass pellets from females of *C. septempunctata* (C7), *C. transversoguttata* (Ct), and *H. convergens* (Hc) that were collected early or late spring in 2005 from alfalfa fields. Results are shown for (A) all arthropod prey combined, (B) aphids, (C) weevils, (D) other arthropods (i.e., not including aphids and weevils), and (E) non-arthropod food. For each category, data are taken only from those frass pellets containing that type of food.

CHAPTER 4

CONCLUSIONS

Aphidophagous lady beetles have consistently been shown to rely on multiple sources of food, displaying a diet that is often highly polyphagous in nature (Hodek 1973). Nonetheless, their preferred preys, aphids, are required by many species to facilitate growth and development of immature stages, as well as maturation of eggs and oviposition by adult females (Hodek 1996, Dixon 2000). Limited size of aphid populations in many habitats may necessitate the reliance of lady beetles on other sources of food in order to survive (Hodek 1973). The extent to which other sources of food are used can differ among lady beetle species and may offer another explanation for why certain species of lady beetles come to dominate over others in particular environments.

Alfalfa fields (*Medicago sativa* L.) in northern Utah provide habitat to a number of native North American lady beetle species, but over the past decade, an introduced species, *Coccinella septempunctata* L., has come to dominate (Evans 2004). Many native species still utilize this habitat, but in much lower numbers than in the past (Evans 2004). Alfalfa provides many foods for lady beetles as alternatives and supplements to aphids, including plant pollen, fungal spores, as well as other arthropod prey. During recent years in which there has been the rise in dominance by *C. septempunctata*, northern Utah alfalfa fields have been characterized by low aphid densities, likely requiring this complex of lady beetle species to utilize these other sources of food extensively.

Many methods can be used to determine the dietary breadth of polyphagous predators (reviewed by Sunderland 1987, Powell et al. 1996, Symondson 2002, Harwood and Obrycki 2005). This research sought to develop the seldom used technique of frass

analysis to compare the diets of *C. septempunctata* with those of native lady beetles in the alfalfa habitat. Specifically, *C. septempunctata* was compared with *C. transversoguttata richardsoni* Brown and *Hippodamia convergens* Guérin-Méneville, the next most abundant lady beetle species that occur in alfalfa fields of northern Utah. Frass analysis is well suited to use with lady beetles for a number of reasons. Lady beetles are biting/ chewing predators that ingest indigestible fragments of their food that will be voided in their frass. Additionally, the technique does not require the predator to be killed. Frass analysis has been used to differentiate among many types of prey in the past and so can provide information about the dietary breadth of these species (Putnam 1964). Also, as a relatively “low-tech” option for deciphering the dietary components of lady beetles, it does not require excessive time or monetary commitment to be successful.

Before the technique of frass analysis could be applied in the field to study natural diets of lady beetles, laboratory experiments were necessary to determine how long prey fragments would take to pass through the gut, and to ascertain whether key prey items could be distinguished from one another from such fragments in frass pellets. Multiple lady beetle species were tested with two common prey items from alfalfa, pea aphids and alfalfa weevil larvae, to determine how long to hold the lady beetles for frass production. Pea aphids (*Acyrtosiphon pisum* [Harris]) are a key prey resource for lady beetles in the alfalfa habitat. Additionally, lady beetles have been shown to readily utilize alfalfa weevil larvae (*Hypera postica* [Gyllenhall]) as prey (Evans and England 1996, Kalaskar and Evans 2001; see also references as cited in Evans 2004). Because alfalfa weevil larvae can reach high densities in the field, it was expected that they would make up a large part of the lady beetles’ diets.

Lady beetle adults were found, with only few exceptions, to stop producing frass pellets containing prey fragments within 48 hours after removal from prey when held at a constant temperature (20°C) in the lab. When fed aphids, almost all individuals produced frass containing fragments of their prey (80-97%), but after feeding on weevils, a much lower percentage of adults produced frass containing weevil fragments (29-71%). This was likely due to the lady beetles taking predominantly liquid meals from their weevil prey. This suggests that frass analysis for natural populations of lady beetles in alfalfa fields may result in an underestimate of weevil consumption. Nonetheless, the numbers of lady beetles found to consume aphids and weevils based on frass analysis can be used to obtain a minimum estimate of their use of these types of prey in alfalfa fields. Pea aphids, alfalfa weevil larvae, and *C. septempunctata* larvae were fed to *C. septempunctata* adults to determine if these types of prey produced distinct fragments in the frass pellets of adult lady beetles. Each type of prey produced distinctive fragments in the frass.

Frass analysis was then tested during a short field experiment. For this experiment, plots were set up in an alfalfa field and sprayed with sugar-water to enhance retention of lady beetles (Evans and England 1996), thereby increasing capture rates for frass analysis. Half of the plots were seeded with greenhouse-reared aphids to increase densities. Frass analysis was then used to determine the diets of *C. septempunctata* adults collected over four days from the plots. Higher percentages of individuals collected in the high aphid density plots were found to have consumed aphids than those from the low aphid density plots. Thus, frass analysis could distinguish local differences in prey availability; as aphid densities naturally change during the season, frass analysis might be

able to track this shift. High percentages of individuals were found to feed on alfalfa weevil larvae as well, whether in areas of high or low aphid density. Additionally, it was possible to distinguish other types of prey in the frass of the lady beetles, including Thysanoptera, Collembola, pollen, and fungi. Many lady beetles were found to rely on a mixed diet of different types of prey, rather than rely on a single type, even in the high aphid density plots.

After this initial characterization and development of frass analysis, the technique was used to determine the diets of three lady beetle species (*C. septempunctata*, *C. transversoguttata*, and *H. convergens*) in spring alfalfa fields, prior to the first cutting of hay in each of two years. The spring is typically characterized by low densities of pea aphids, an abundant source of secondary prey, the alfalfa weevil larvae, and by the presence of other arthropods, pollen, and fungal sources. The lady beetle species could then be compared for similarities in their use of different food types. Overall, high proportions of all three species were found to have fed on some type of food, either arthropod or non-arthropod, and most were found to have consumed multiple types of food. As the season progressed, the percentage utilizing aphids and weevils increased, which paralleled the increase in aphid and weevil densities. A corresponding decrease in the percentages utilizing other types of arthropods and pollen and fungi also occurred as the season progressed.

In general, higher percentages of *C. septempunctata* than the two native lady beetle species were found to feed on all types of prey, especially weevils and aphids. All three lady beetle species consumed similar types of arthropod prey, including Collembola and Thysanoptera (unidentifiable fragments also were similar in general between the

species). Cannibalism or intraguild predation of coccinellid larvae was rare, with an extremely low, but similar incidence of occurrence for all three species. Additionally, the types of pollen and fungi found were similar for the three species.

During the second year of sampling the spring crop of alfalfa, the amount of frass produced by individual lady beetles was ranked by size, and the total surface area of each type of prey found in the frass was measured with the aid of a microscope. In general, individuals of *C. septempunctata* consumed greater quantities of prey cuticle than did individuals of native species, and they also produced, on average, larger amounts of frass. The two native species tended to consume greater quantities of non-arthropod food, however. The trend towards greater consumption of arthropod prey, greater output of frass, and generally higher percentages of *C. septempunctata* adults consuming a wide variety of prey, may explain the rise of this introduced species to dominance in alfalfa habitats. While all three species (*C. septempunctata*, *C. transversoguttata*, and *H. convergens*) are similar in their choice of prey and food resources in the spring alfalfa habitat, *C. septempunctata* appears to be more successful in finding and consuming prey than its native counterparts. In the past, this species has been shown to tolerate and frequent habitats with low aphid densities (Triltsch and Freier 1998, Evans 2004). This tolerance of low aphid densities, and rise to dominance particularly in northern Utah alfalfa fields, may come in part because of *C. septempunctata's* greater ability to search for and find food, particularly aphids, in comparison to these other species.

Though not without its problems, frass analysis is well suited to documenting the dietary breadth of coccinellids as well as assessing the similarities and differences among species. It is possible to distinguish a wide variety of prey, but only if the lady beetle

consumes and voids fragments of that prey. Hence, certain types of prey such as arthropod eggs, will not be documented because of the lack of sclerotized parts for the lady beetle to consume (Triltsch 1999). Additionally, prey from which the lady beetle tends to take only liquid meals will either be underestimated or absent from a diet assessed in this manner. An additional problem with the technique is that it can be hard to identify all of the prey fragments present, as found by Putnam (1964) who reported that less than 10% of prey fragments could be assigned to arthropod order. Still, the technique can be useful as a comparison for particular kinds of prey, including such common agricultural pests as aphids, alfalfa weevil larvae, thrips, and mites (Putnam 1964). One asset of this method is that the lady beetles do not need to be killed for assays, and after frass is produced, they can be released back into the environment or used in further laboratory experiments. Because of the beneficial nature of lady beetles, the ability to return them to the environment where they can control agricultural pests is desirable. Additionally, frass analysis (with sufficient development) can provide quantitative comparisons between lady beetle species, as in this study where the surface area of prey fragments in frass preparations was assessed. Frass analysis also provides a low cost, and efficient way to assess the diets of lady beetles, and could provide direction as to which prey types to test when more expensive, and time-intensive methods are utilized. In summary, the results of this study illustrate that frass analysis can be a valuable, cost-effective approach to learn more about the diets of polyphagous insect predators under natural conditions.

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APPENDICES

Appendix A.
Photographs from Prey Indicator Experiment
and Field Experiment (Chapter 2)

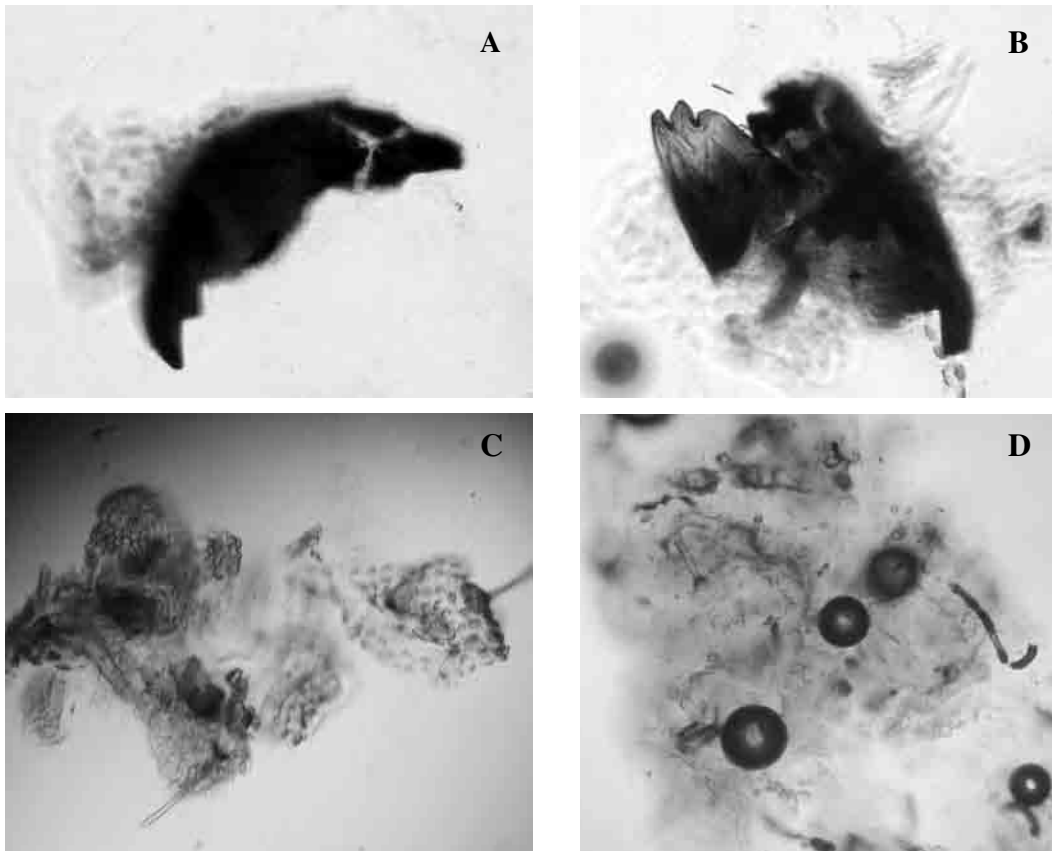


Fig. A.1. Photographs of alfalfa weevil larvae cuticle from frass dissected during prey indicator experiment, Chapter 2. Panels are as follows: (A) Head capsule, (B) Mandible with portion of head capsule and cuticle in background, (C) Cuticle with setae attached, (D) Cuticle with setae attached.

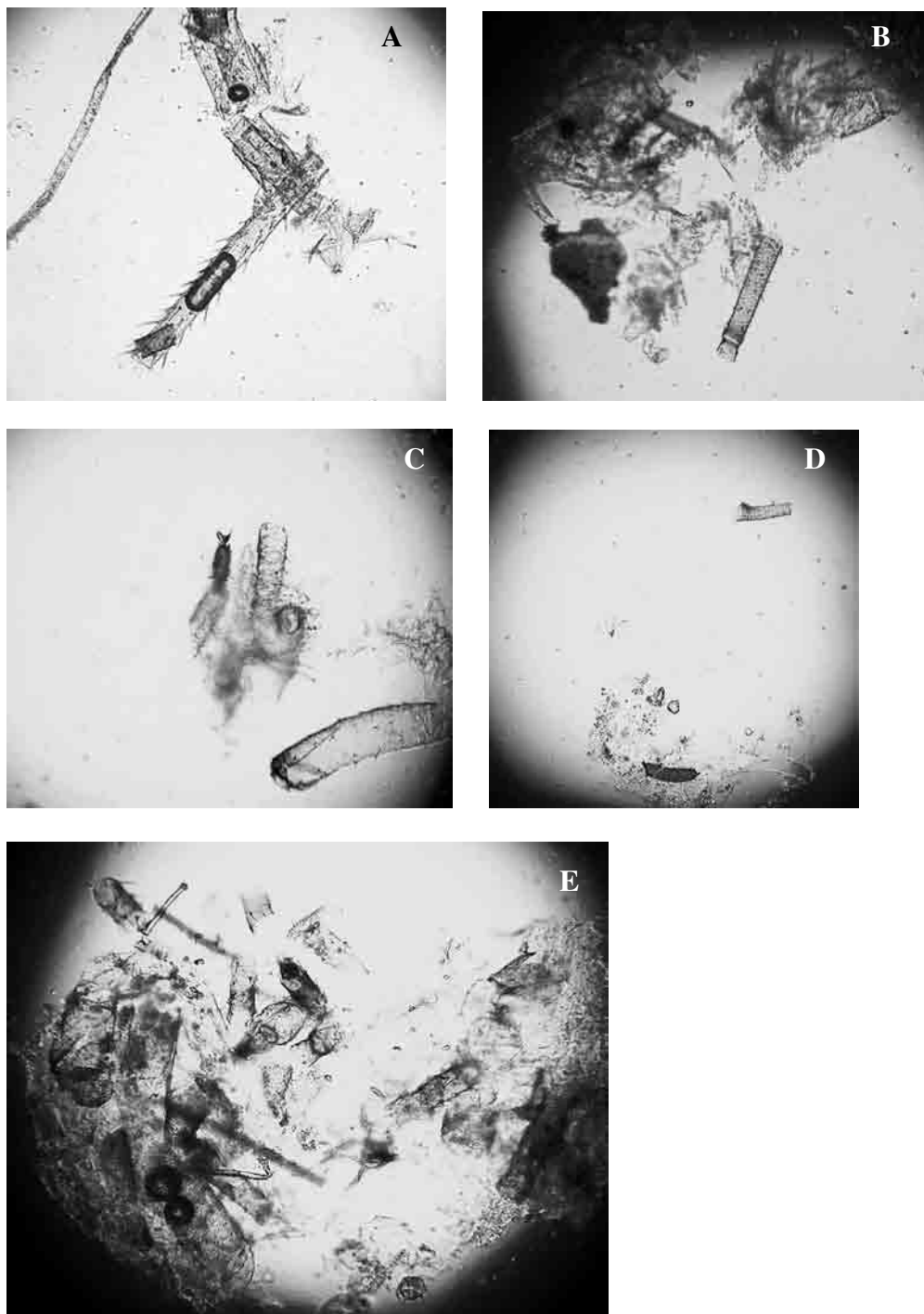


Fig. A.2. Photographs of aphid cuticle from frass dissected during prey indicator experiment, Chapter 2. Panels are as follows: (A) sections of legs, (B) sections of legs and cuticle from the aphid body, (C) sections of legs and final tarsal claw, (D) cornicle in upper right corner and final tarsal claw in lower middle, (E) assortment of cuticle from body parts including portion of mouth near center.

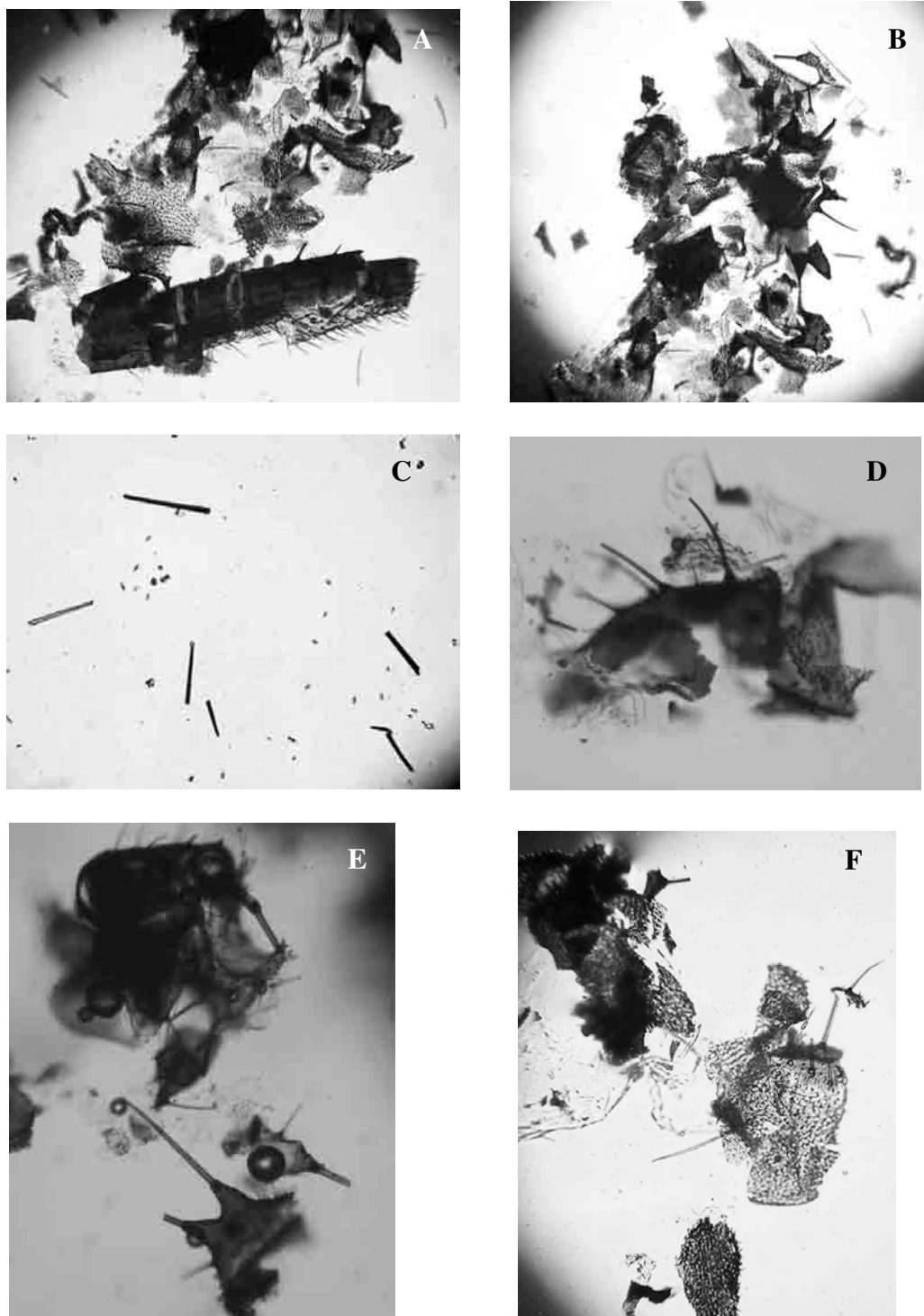


Fig. A.3. Photographs of conspecific larvae (*Coccinella septempunctata*) cuticle from frass dissected during prey indicator experiment, Chapter 2. Panels are as follows: (A) portion of leg and cuticle from body, (B) general cuticle from body, (C) setae detached from body, (D) spiny cuticle from dorsal surface of body, (E) spiny cuticle from dorsal surface of body, (F) general cuticle from body, some with setae attached.

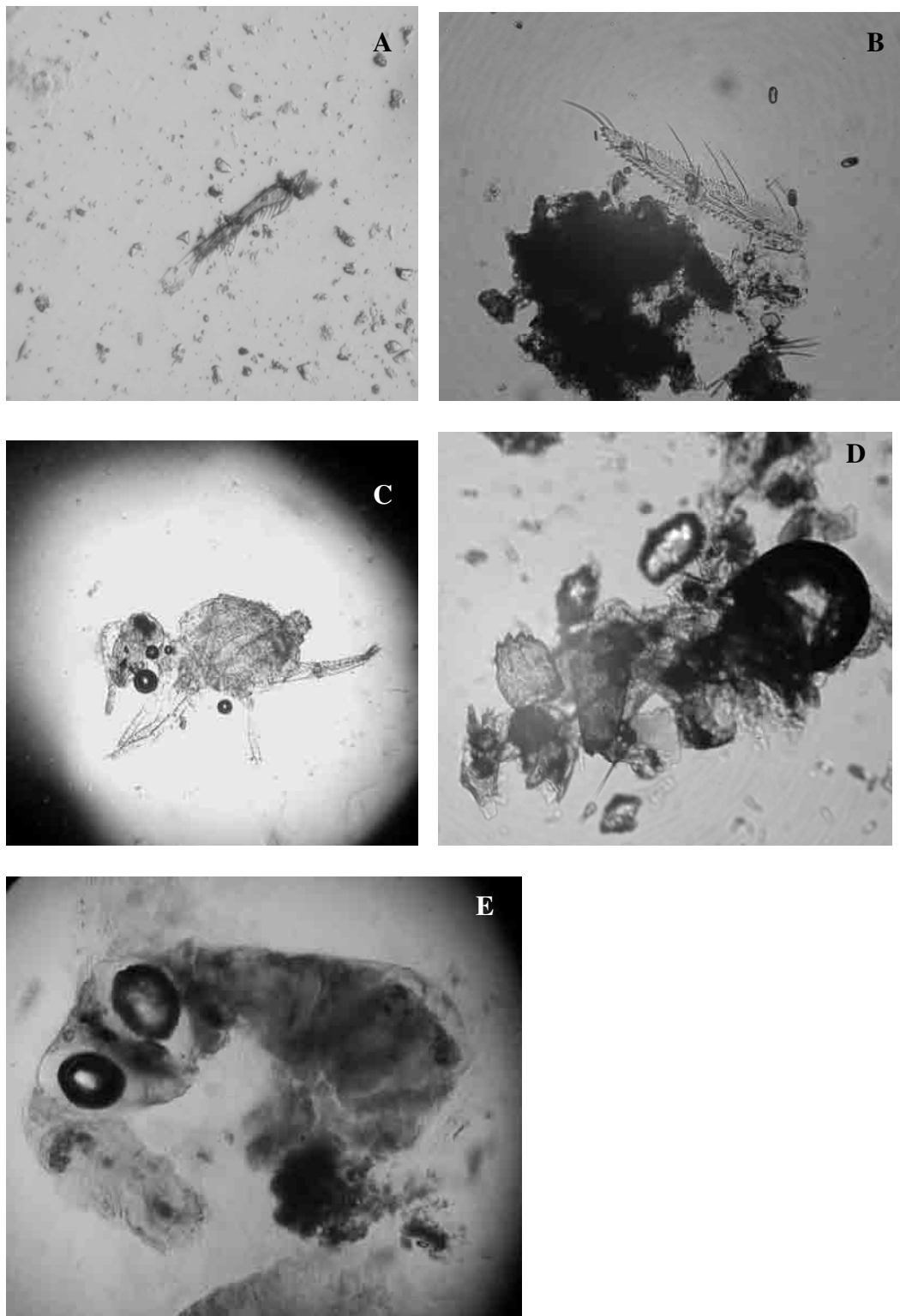


Fig. A. 4. Photographs of dissected frass pellets from field experiment, Chapter 2. Panels are as follows: (A) possible thrips wing, (B) possible thrips wing, (C) almost intact collembolan, (D) assorted cuticle and non-alfalfa weevil mandible, (E) appearance of almost empty frass pellet showing appearance of peritrophic membrane.

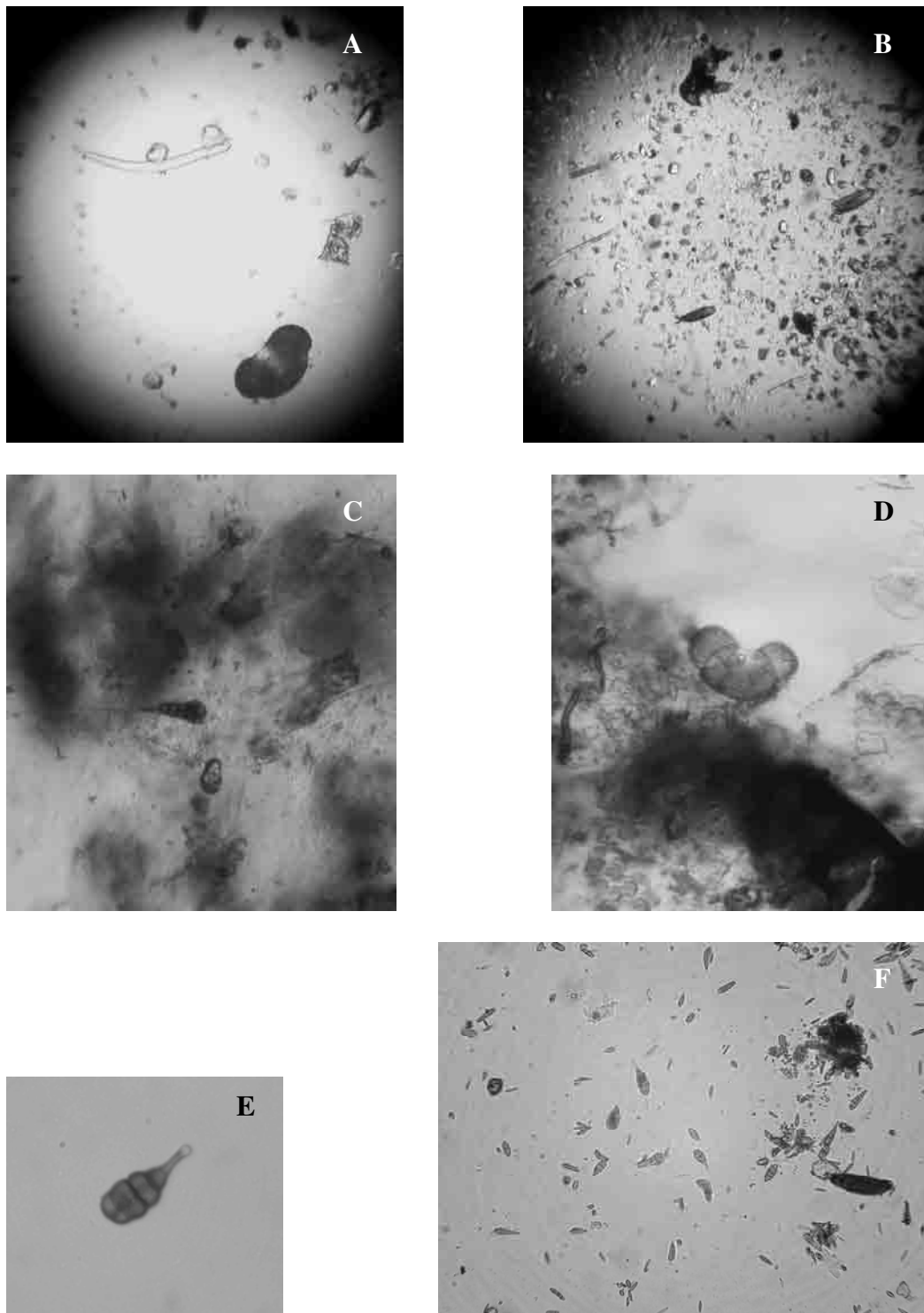


Fig. A.5. Photographs of dissected frass pellets from field experiment, Chapter 2, showing non-arthropod food types. Panels are as follows: (A) assortment of pollen, largest in field of view from genus *Pinus*, (B) an assortment of pollen types as well as amorphous particles and final tarsal claw of aphid, (C) fungi -- likely *Alternaria*, as well as alfalfa weevil cuticle, (D) pollen from genus *Pinus* as well as cuticle from alfalfa weevil, (E) fungi -- likely *Alternaria*, (F) an assortment of fungi.

Appendix B.
Complete Results of ANOVA performed, including sums of squares, and mean squares
(Chapter 2 and Chapter 3).

Table B.1. Complete results from repeated measures ANOVA on first gut-clearing experiment, Chapter 2. Type III sums of squares were used. Time indicates days one and two, at 24 and 48 hours after removal from pea aphid prey. Sex indicates female and male C7.

	df	Sums of Squares	Mean Square	F-value	P-value
Sex	1, 34	0.53	0.53	0.27	0.60
Time	1, 34	268.96	268.96	97.78	<0.0001
Time x Sex	1, 34	0.63	0.63	0.23	0.64

Table B.2. Complete results from repeated measures ANOVA on second gut-clearing experiment, Chapter 2. Time indicates days 1, 2, and 3 at 24, 48, and 72 hours after removal from prey. Lady beetle species included are as follows: C7, Ct, Hc, Hq, and Hax. Prey species included are pea aphids and alfalfa weevil larvae. Type III sums of squares were used, and Huynh-Feldt Epsilon corrections of p-values for within subject effects were utilized for sphericity of data.

	df	Sums of Squares	Mean Square	F-value	P-value	Huynh-Feldt Corrected P-value
Lady beetle species	4, 301	527.68	131.92	3.83	0.0047	N/A
Prey species	1, 301	673.89	673.89	19.56	<0.0001	N/A
Lady beetle species x prey species	4, 301	556.80	139.20	4.04	0.0033	N/A
Time	2, 602	23268.23	11634.11	279.01	<0.0001	<0.0001
Time x Lady beetle species	8,602	957.03	119.63	2.87	0.0039	0.013
Time x Prey species	2, 602	1034.64	517.32	12.41	<0.0001	0.0004
Time x Lady beetle species x Prey species	8, 602	1515.44	189.43	4.54	<0.0001	0.0004

Table B.3. Complete results from one-way ANOVA on prey density data from field census, Chapter 3. Time period indicates early and late spring periods.

Effect of Time Period	df	Sums of Squares	Mean Square	F-value	P-value
2004 Aphid Densities	1, 98	13.59	13.59	260.11	<0.0001
2005 Aphid Densities	1, 118	7.51	7.51	34.56	<0.0001
2004 Weevil Densities	1, 8	9.57	9.57	10.40	0.012
2005 Weevil Densities	1, 9	27.05	27.05	18.50	0.002

Table B.4. Complete results of two-way ANOVA (time [early vs. late 2005] x species [C7, Hc, and Ct]) for attributes of frass pellets produced by individual lady beetle females, collected from alfalfa fields during field census (Chapter 3). This includes the average number of frass pellets produced, average rank size of frass pellets produced, average sum total of ranks for size of frass pellets produced, and proportion of the total amount of frass that was dissected. The proportion of the total amount of frass dissected was only determined for those individuals whose frass pellet contained some type of prey or food. All effects are from Type III sums of squares. C7 indicates *Coccinella septempunctata*, Ct indicates *C. transversoguttata*, and Hc indicates *Hippodamia convergens*.

		df	Sums of Squares	Mean Square	F-value	P-value
Average number of pellets produced	Time x Species	2, 884	298.41	149.20	14.73	<0.0001
	Time	1, 884	386.77	386.77	38.18	<0.0001
	Species	2, 884	66.01	33.01		
Average rank size of pellets produced	Time x Species	2, 884	9.08	4.54	9.81	<0.0001
	Time	1, 884	21.21	21.21	45.86	<0.0001
	Species	2, 884	66.42	33.21		<0.0001
Average accumulated total ranks of pellets produced	Time x Species	2, 884	3471.90	1735.95	24.52	<0.0001
	Time	1, 884	5670.56	5670.56	80.09	<0.0001
	Species	2, 884	4553.43	2276.72	32.16	<0.0001
Proportion of total dissected	Time x Species	2, 664	0.37	0.18	2.94	0.54
	Time	1, 664	0.27	0.27	4.38	0.037
	Species	2, 664	0.094	0.047	0.75	0.47

Table B.5. Complete results of two-way ANOVA (time [early vs. late 2005] x species [C7, Hc, and Ct]) for mean surface area consumed of different types of prey (Chapter 3). Prey types are as follows: all arthropod prey combined, aphids, alfalfa weevil larvae, other arthropods (not including aphids or weevils), and non-arthropod food (includes pollen and fungi). C7 indicates *C. septempunctata*, Ct indicates *C. transversoguttata*, Hc indicates *H. convergens*. Data for each type of prey was only included if the individual had consumed that type of prey. All effects are from Type III sums of squares.

		df	Sums of Squares	Mean Square	F-value	P-value
All Arthropods	Time x Species	2, 805	112.09	56.05	5.21	0.0056
	Time	1, 805	223.35	223.35	20.77	<0.0001
	Species	2, 805	457.10	228.55	21.25	<0.0001
Aphids	Time x Species	2, 325	91.01	45.50	5.43	0.0048
	Time	1, 325	59.42	59.42	7.09	0.0081
	Species	2, 325	137.76	68.88	8.22	0.0003
Weevils	Time x Species	2, 256	2.50	1.25	0.93	0.40
	Time	1, 256	11.06	11.06	8.24	0.0044
	Species	2, 256	5.17	2.59	1.93	0.15
Other Arthropods	Time x Species	2, 220	11.78	5.59	0.50	0.61
	Time	1, 220	0.038	0.038	0	0.95
	Species	2, 220	29.44	14.72	1.25	0.29
Non-Arthropods	Time x Species	2, 516	28.29	14.14	11.20	<0.0001
	Time	1, 516	36.17	36.17	28.65	<0.0001
	Species	2, 516	3.75	1.87	1.48	0.23

