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Frass Analysis of Diets of Aphidophagous Lady Beetles (Coleoptera: Coccinellidae) in Utah Alfalfa Fields

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ABSTRACT Aphidophagous lady beetles enhance their foraging success in natural settings by consuming other types of food in addition to aphids. Frass analysis was used to examine natural diets of female lady beetles in fields of alfalfa (*Medicago sativa* L.) in northern Utah. The first (spring) alfalfa crop was censused in 2004 and 2005 to determine the diet of female adults of the introduced *Coccinella septempunctata* L., and two native species, *C. transversoguttata richardsoni* Brown, and *Hippodamia convergens* Guerin. The proportion of females of the three lady beetle species that fed on pea aphids [*Acyrthosiphon pisum* (Harris)] and alfalfa weevil larvae [*Hypera postica* (Gyllenhal), an abundant alternative prey] increased from early to late season during the first crop. A corresponding seasonal decrease occurred in the proportion of females consuming other types of arthropods (e.g., thrips and collembolans) and nonarthropod food (pollen and fungal spores). Overall, frass analysis indicated that the diets of *C. septempunctata* and the two native species in alfalfa were similar in their inclusion of a broad variety of foods. The study shows that frass analysis can provide a good overview of the diets of lady beetles in natural settings.

KEY WORDS *Coccinella*, gut analysis, *Hippodamia*, predation, pollen

Insect predators tend to be polyphagous in natural settings (Hagen et al. 1999). Although they may focus on particular prey, these predators seem to optimize their foraging by consuming many other types of prey as well. Consequently, it can often be difficult to determine the diets of insect predators in the field. Many techniques (reviewed by Sunderland 1987, Powell et al. 1996, Symondson 2002, Harwood and Obrycki 2005) have been used to address this challenge. 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, including enzyme-linked immunosorbent assays (ELISA) and polymerase chain reaction (PCR) to detect prey-specific biochemicals.

An additional technique is the use of fecal dissection or frass analysis (Conrad 1959, Putman 1964, Lawton 1970, Thompson 1978, Honěk 1986, Powell et al. 1996). This is similar to gut dissection, except that the analysis is on prey remains once they have been voided by the predator and thus the predator need not be killed. This method is only possible if the organism consumes prey containing indigestible fragments. In the past, this technique has been used with odonate nymphs (Lawton 1970, Thompson 1978) and coccinellids (Putman 1964). In the study presented here, frass analysis was used to determine the feeding habits of adult aphidophagous lady beetles within alfalfa fields (*Medicago sativa* L.) of northern Utah.

Aphidophagous lady beetles feed on many foods in addition to preferred aphids. In supporting larval growth and development and reproduction by adults, aphids serve as essential prey for these predators (Hodek 1962, 1996). Other foods are also important, however, because aphid populations are ephemeral (Agarwala et al. 1998) and likely also because mixed diets are optimal nutritionally (Soares et al. 2004, Mayntz et al. 2005). Alternative arthropod prey or plant foods (Hodek 1962, 1996) sustain aphidophagous lady beetles when aphids are often in short supply, such that these predators can reproduce rapidly when sufficient numbers of aphids are subsequently consumed (Hemptinne and Desprets 1986, Evans and Gunther 2005).

Species of aphidophagous lady beetles differ in their use of the many types of foods in different habitats. One frequent choice is pollen (e.g., from crop plants as well as weeds and native vegetation). 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, Lundgren et al. 2005). Other aphidophagous lady beetles regularly consume pollen as an alternative food (Putman 1964, Hemptinne and Desprets 1986, Triltsch 1999, Lundgren et al. 2004, Ricci et al. 2005, Lundgren

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Table 1. Numbers (*N*) of adult females of lady beetles collected from alfalfa fields and frass pellets dissected (one per female) for the early and late sampling periods in 2004 and 2005 and percentages of lady beetles for which dissected frass pellets contained food fragments

Sampling period	*C. septempunctata*		*C. transversoguttata*		*H. convergens*	
	N	Percent with food fragments in frass	*N*	Percent with food fragments in frass	*N*	Percent with food fragments in frass
2004 Early	183	89.3	67	82.1	71	81.7
2004 Late	187	94.7	58	87.9	62	77.4
2005 Early	156	82.7	115	76.5	167	71.3
2005 Late	153	81.7	135	73.3	161	64.6

2009). 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, Evans 2009). Additional foods consumed include sugars (e.g., nectar; Pemberton and Vandenberg 1993), and spores and conidiospores of common fungi (Putman 1957, 1964, Triltsch 1999, Ricci et al. 2005, Ricci and Ponti 2005).

Alfalfa fields are exploited by many aphidophagous lady beetle species throughout the world (Neuenschwander et al. 1975, Hodek and Honěk 1996, Elliott et al. 2002, Grez et al. 2008). Alfalfa fields offer these predators both aphids and many alternative foods, including other 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)]; the abundant larvae during the first crop of alfalfa are fed on by many lady beetle species (Kalaskar and Evans 2001, Evans 2004, and references within). Both weevil larvae and pea aphids [*Acyrthosiphon pisum* (Harris)] increase in number and availability to lady beetles during the growth of the first crop of alfalfa (Evans and England 1996, Evans 2004).

The aim of this study was to use frass analysis to assess the diets of common lady beetle species within alfalfa fields in northern Utah during the first (spring) crop when lady beetles are especially abundant. Within Utah, up to a dozen species of native lady beetles plus the introduced *Coccinella septempunctata* L. can be found in alfalfa fields. Contents of frass pellets produced by adult lady beetles were examined for presence of aphids and alternative prey (especially the highly abundant alfalfa weevil larvae). In particular, two central questions were addressed: (1) to what extent do these lady beetles feed on a broad variety of foods in alfalfa, in addition to their preferred aphid prey?, and (2) do the diets of these lady beetle species change over the season?

Materials and Methods

Field Sites. In both 2004 and 2005, lady beetle adults were sampled in two alfalfa fields in Cache County, UT, throughout the first (spring) crop. Fields were located at the Utah Agricultural Experiment Station Animal Science and Caine Dairy farms in 2004 and at the Wellsville North and Cache Junction farms in 2005.

Sampling Procedures. Censuses were conducted from 26 April to 31 May 2004 and from 13 May to 16 June 2005, beginning when alfalfa reached a height of 20 cm and ending at the first cutting. Given the proximity of the two fields each year, and the similarity between them in seasonal patterns of abundance of aphids, weevils, and lady beetles, frass pellet data from the two fields were combined for analysis in each year of the study. Data were divided each year between an early period and a late period (i.e., first and second halves of the census period) to examine possible changes with time in the lady beetles' use of foods.

To obtain sufficient numbers of individuals for analysis, areas within fields of alfalfa were sprayed lightly with sucrose solution, a known arrestant for lady beetles (Evans and Swallow 1993, Evans and Richards 1997). Such spraying raises concern that feeding behavior of lady beetles in treated areas might be altered. Previous laboratory studies (E.W.E., unpublished data) indicated that lady beetles consumed the same amount of one major prey in this study, alfalfa weevil larvae, regardless of whether prey individuals had or had not been sprayed with sucrose solution (the solution was applied in the laboratory to mimic coating of weevils with sugar as would occur in the field for exposed individuals on sprayed foliage). This field study was therefore undertaken on the working premise that spraying with sucrose solution could critically enhance sample size without substantial artifactual effect on determination of natural, field diet.

In 2004, an area (1.5 by 3 m) was sampled in each field at 4- to 10-d intervals (with the location of the area being moved between intervals). In 2005, three to five areas (1.5 by 3 m each) were sampled at these intervals (again with areas being relocated between intervals). Twenty-four hours before areas were first sampled, they were sprayed with 1.5 liters of a 15% sucrose water solution. From collections made over the next 1–5 d (depending on weather), adults of the following three species of lady beetles were obtained to quantify and compare food habits: *C. septempunctata, C. transversoguttata richardsoni* Brown, and *Hippodamia convergens* Guerin. Only females (the most voracious sex; Hemptinne et al. 1996) were examined for their food habits (see Table 1 for sample sizes).

Frass Pellet Analysis. In the laboratory, females were placed individually into small (5.5 cm diameter) petri dishes and held in an incubator at 20°C, 16 L: 8

D. A drop of 15% sucrose water solution was added to each dish to prevent starvation. Beetles were held for 48 h (previous laboratory studies of digestion rates indicated that almost all fragments were voided from the beetles' guts during this 48-h period; Davidson 2008). The largest pellet produced by each individual during this 48-h holding period was selected for dissection and analysis (sample sizes for numbers of pellets dissected are given in Table 1).

Pellets were placed individually in the well of a depression glass slide with a drop of 20% sodium hydroxide. When softened, the pellets were teased apart and inspected microscopically at 100× magnification. In both years, contents were scored as to whether fragments of the following foods were present: aphids, alfalfa weevil larvae, pollen and fungi (of various species combined), and arthropods other than aphids and weevils. The distinction between cuticular fragments from alfalfa weevils, pea aphids, and other kinds of arthropods was determined in preliminary studies in which lady beetles were fed particular prey (Davidson 2008). Pollen and fungi could be distinguished from arthropod prey based on their shape and illustrations from Moore et al. (1991), Triltsch (1999), Agrios (2005), and Ricci et al. (2005). Because so many types of pollen and fungal spores could be found in frass pellets, all were combined and scored as a single category (nonarthropod foods).

Identification of most arthropods other than aphids and weevil larvae (e.g., Hemipterans and various coleopteran and lepidopteran larvae) from fragments was problematic without further diagnostic laboratory preparation. These fragments were therefore combined into a single "other arthropod" category for quantitative analysis. This category also included cuticular fragments that could be identified as from lady beetle larvae, thrips, and Collembola (because such fragments were few in number, however, they were simply included in the category of other arthropods).

In 2005, amounts of fragments of each food type present were quantified on dissection of the largest pellet produced by each female. For each of the three species, this largest pellet represented ≈30% of the total estimated amount of frass produced by a female when held for 48 h after collection in both the early and late sampling periods. The total amount of all fragments combined for a given food type was estimated by using a microscope fitted with an eyepiece reticle. The reticle provided a gridded central field of view divided into 100 (1 mm^2) squares (21-mm-diameter Meiji Technology MA283/05; Meiji Techno America, Santa Clara, CA); this gridded area covered the entire well of the depression glass slide that held fragments of the dissected pellet. The mean amount of each food type was estimated for each lady beetle species by determining the mean number of 1-mm^2 squares containing fragments of that food type.

Statistical Analysis. All statistical analyses were performed using SAS version 9.1 (SAS Institute 2003). Presence versus absence data for different categories of prey in frass pellets from 2004 and 2005 were analyzed with χ^2 tests of independence. Amount of prey within frass from 2005 was analyzed using two-way analysis of variance (ANOVA; type III sums of squares), with species and time (early or late sampling period) as main effects. Each food category was analyzed individually by ANOVA for only those individuals that had consumed that category; the analyses were thus intended to address the issue of how much food was consumed, given that the predator fed on a particular category of food (χ^2 analyses in turn addressed the issue of the frequency with which predators fed on a particular food category). 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.

Results and Discussion

Frass analysis showed that females of *C. septempunctata*, *C. transversoguttata*, and *H. convergens* consume a wide variety of foods in first crop (spring) alfalfa, including pollen and fungal spores. Throughout the spring in 2004 and 2005, a large 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 (Fig. 1). In addition to cuticular fragments of aphids, cuticular fragments of alfalfa weevil larvae were found very commonly in the frass of females of all three species (Fig. 1). Prominent among other arthropods also consumed by the three lady beetle species were thrips, previously well documented as alternative food for adult lady beetles (Putman 1964, Triltsch 1997, 1999, Ricci and Ponti 2005). Individuals of all three predator species frequently consumed more than one category of food over a short period of time, as reflected in the varied contents of a single frass pellet. Approximately one half of the pellets dissected for each year contained more than one category of prey.

The three species of lady beetles were broadly similar in their patterns of food use, as reflected in the contents of their frass pellets (Fig. 1). For all three lady beetle species combined, frass analysis showed changes in diet as spring progressed. In both years, an increase from the early to the late period was detected in the proportion of frass pellets containing aphids (Fig. 1; 2004: $\chi^2 = 102.49$, df = 1, $P < 0.0001$; 2005: $\chi^2 = 6.56$, df = 1, $P = 0.01$). This pattern was statistically significant for all three species individually in 2004 (*C. septempunctata:* $\chi^2 = 83.70$, df = 1, $P < 0.0001$; *C. transversoguttata:* $\chi^2 = 8.10$, df = 1, $P = 0.004$; *H. convergens:* $\chi^2 = 16.27$, df = 1, $P < 0.0001$), and for *H. convergens* ($\chi^2 = 4.26$, df = 1, $P = 0.04$) but not *C. septempunctata* ($\chi^2 = 0.53$, df = 1, $P = 0.47$) or *C. transversoguttata* ($\chi^2 = 2.79$, df = 1, $P = 0.09$) in 2005.

Also in both years, a decrease from the early to the late period for all three species combined was detected in the proportion of frass pellets containing arthropods other than aphids and weevils (2004: $\chi^2 = 31.12$, df = 1, $P < 0.0001$; 2005: $\chi^2 = 38.77$, df = 1, $P < 0.0001$) and nonarthropod foods (2004: $\chi^2 = 29.25$, df = 1, $P < 0.0001$; 2005: $\chi^2 = 15.92$, df = 1, $P < 0.0001$).

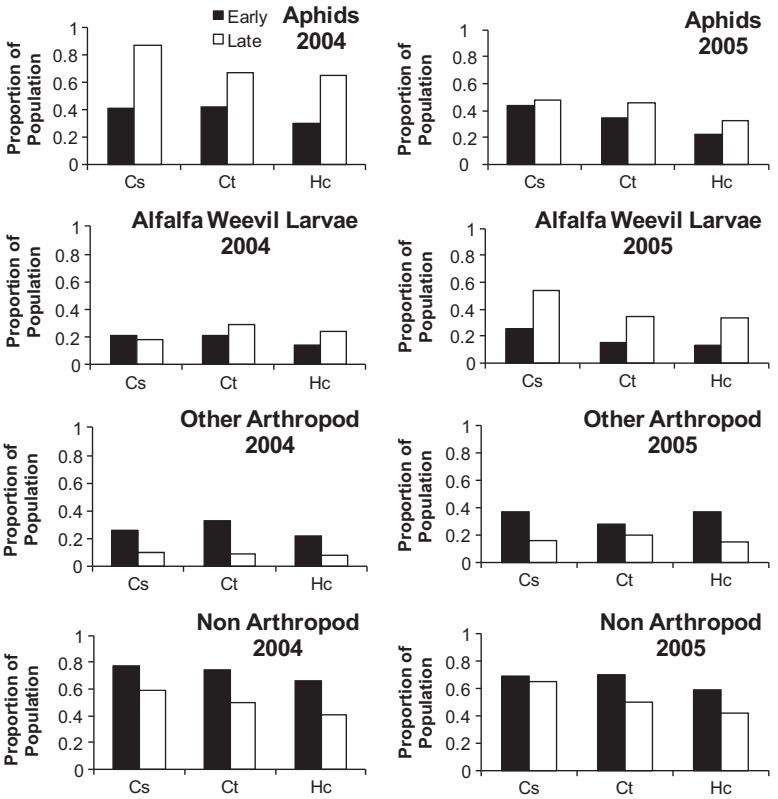

Fig. 1. Proportion of females of *C. septempunctata* (Cs), *C. transversoguttata* (Ct), and *H. convergens* (Hc) from alfalfa fields in early (dark bars) and late (white bars) sampling periods during the first (spring) crop in 2004 and in 2005, that produced frass with fragments of aphids, alfalfa weevil larvae, arthropods other than aphids and weevil larvae, and nonarthropod foods (fungi and pollen).

This pattern concerning arthropods other than aphids and weevils was statistically significant for all three species individually in 2004 (*C. septempunctata:* $\chi^2 = 16.47$, df = 1, $P < 0.0001$; *C. transversoguttata:* $\chi^2 = 10.76$, df = 1, $P = 0.001$; *H. convergens:* $\chi^2 = 4.42$, df = 1, $P = 0.036$), and for *C. septempunctata* ($\chi^2 = 18.30$, df = 1, $P < 0.0001$) and *H. convergens* ($\chi^2 = 20.92$, df = 1, $P < 0.0001$) but not *C. transversoguttata* ($\chi^2 = 2.56$, df = 1, $P = 0.11$) in 2005. The pattern concerning nonarthropod foods was also statistically significant for all three species individually in 2004 (*C. septempunctata:* $\chi^2 = 14.08$, df = 1, $P = 0.0002$; *C. transversoguttata:* $\chi^2 = 8.11$, df = 1, $P = 0.004$; *H. convergens:* $\chi^2 = 8.92$, df = 1, $P = 0.003$), and it was statistically significant for *C. transversoguttata* ($\chi^2 = 10.38$, df = 1, $P = 0.001$) and *H. convergens* ($\chi^2 = 8.87$, df = 1, $P = 0.003$) but not *C. septempunctata* ($\chi^2 = 0.72$, df = 1, $P = 0.40$) in 2005.

An increase was detected in 2005 ($\chi^2 = 57.20$, df = 1, $P < 0.0001$), but not in 2004 ($\chi^2 = 0.46$, df = 1, $P = 0.50$), for all three species combined from the early to the late period in the proportion of frass pellets containing alfalfa weevil larvae. The increase was statistically significant for each of the three species individually in 2005 (*C. septempunctata:* $\chi^2 = 27.66$, df = 1, $P < 0.0001$; *C. transversoguttata:* $\chi^2 = 13.08$, df = 1, $P = 0.0003$; *H. convergens:* $\chi^2 = 19.10$, df = 1, $P < 0.0001$) but not in 2004 (*C. septempunctata:* $\chi^2 = 0.39$, df = 1, $P = 0.53$; *C. transversoguttata:* $\chi^2 = 1.18$, df = 1, $P = 0.28$; *H. convergens:* $\chi^2 = 2.22$, df = 1, $P = 0.14$).

Seasonal changes in diets of lady beetles have been reported previously by others (Triltsch 1997, 1999; Ricci et al. 2005). The increase in the presence of aphid and weevil fragments in frass pellets observed as spring progressed likely reflects the greater availability of these prey species in late than in early spring (Evans and England 1996, Evans 2004). Even during the late sampling period in 2004 and 2005, a large proportion of alfalfa weevil larvae were early (first and second) instars (Davidson 2008); the abundance of small, readily handled weevil prey likely promoted weevil consumption by the lady beetles. The decrease in the presence of nonarthropod foods and fragments of arthropods other than weevils and aphids in frass pellets as spring progressed likely reflects reduced use of these foods with increased availability of the two primary prey species in first crop alfalfa, pea aphids, and alfalfa weevil larvae. 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, although aphids were consistently used throughout the year (Ricci et al. 2005).

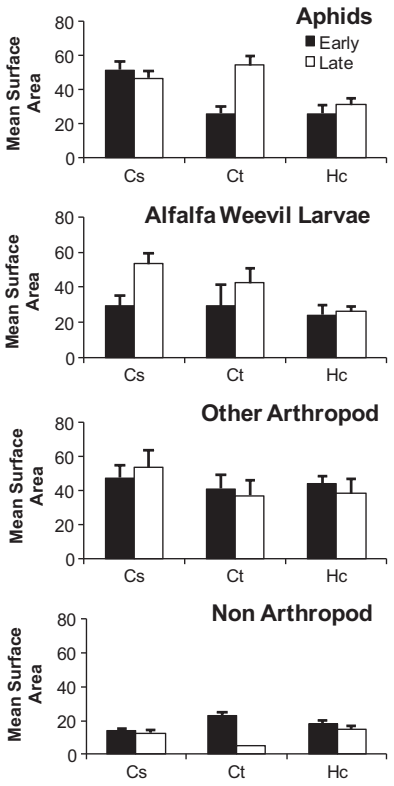

Fig. 2. Amount of food fragments in dissected frass pellets from lady beetle females, quantified as mean surface area (mm^2) occupied by fragments when viewed through the microscope (as explained in Methods and Materials). Means ± SE for females of *C. septempunctata* (Cs), *C. transversoguttata* (Ct), and *Hippodamia convergens* (Hc) that were collected early (dark bars) or late (white bars) in the first (spring) crop in 2005 from alfalfa fields, are shown for frass pellets containing aphids, alfalfa weevil larvae, arthropods other than aphids or weevil larvae, and nonarthropod food (fungi or pollen).

Pollen in lady beetle frass most likely came from flowering plants in the alfalfa fields or on field edges. These sources included *Capsella bursa-pastoris* L. Medic, *Cardaria draba* L. Desv., *Cirsium* spp., *Erodium cicutarium* L. L'Her. ex Ait., *Isatis tinctoria* L., *Taraxacum officinale* Weber, *Veronica biloba* L., and *Tragopogon dubius* Scop., as well as various grasses (Poaceae) and alfalfa. In addition, pollen grains from pine trees (as occurred near the fields) were found regularly in the frass of all lady beetle species. In addition to the well-known pollinivore *Coleomegilla maculata* (Lundgren et al. 2004, 2005), many lady beetle species consume pollen (Lundgren 2009). Many lady beetle species also regularly consume fungi throughout adulthood (Putman 1964, Triltsch 1999, Ricci et al. 2005, Ricci et al. 2005). Among fungi found in frass in this study, spores of *Alternaria* and *Puccinia* were most common.

Instances of cannibalism and intraguild predation were very rare, with frass from only a few individuals containing cuticular fragments that could have been from coccinellid larvae, and there was no tendency for this to occur more frequently in the frass of *C. septempunctata* versus native species. This concurs with Triltsch (1999), who concluded that cannibalism occurs predominantly between larval stages of lady beetles in alfalfa, with adults rarely consuming larval stages (note, however, that instances of egg predation would not be detected by frass analysis in this study; Triltsch 1997). Preliminary laboratory studies of frass from consumption of larval lady beetles indicated that such consumption by adults in the field, had it occurred, would have provided diagnostic cuticular fragments in frass analysis (Davidson 2008).

Frass pellets that contained prey fragments differed considerably among species and time period in 2005 in the quantities of different food categories that they contained (Fig. 2; Table 2). In many cases, frass pellets of a given species contained similar amounts of a given type of food in the early and late sampling periods. In only one case did the quantity clearly decline with season: frass pellets of *C. transversoguttata* contained much less nonarthropod food in the late versus the early period. Marked seasonal increases occurred in the quantities of aphids and weevils contained in frass pellets of *C. transversoguttata* females. A strong increase from the early to late period occurred also in the quantity of weevils contained in frass pellets of *C. septempunctata* females. No clear change from early to late period in the amount of any food type in frass pellets was observed for *H. convergens* females (Fig. 2).

An additional study is needed to determine how frass contents vary depending on differences in consumption and digestion among lady beetle species eating a variety of foods (similar caveats apply to the results of PCR and ELISA analyses; Symondson 2002, Harwood and Obrycki 2005, Sheppard and Harwood 2005). Initial laboratory studies showed, for example, that *C. septempunctata*, *C. transversoguttata*, and *H.*

Table 2. Results of two-way ANOVA (time [early versus. late 2005] × species [*C. septempunctata*, *C. transversoguttata*, and *H. convergens*]) for amount of food fragments in frass for those individuals that had consumed a given type of food

Category of food	Time			Species			Time × species		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
Aphids	7.09	1,325	0.0081	8.22	2,325	0.0003	5.43	2,325	0.0048
Weevils	8.24	1,256	0.0044	1.93	2,256	0.15	0.93	2,256	0.40
Other arthropods	0.01	1,220	0.95	1.25	2,220	0.29	0.5	2,220	0.61
Nonarthropods	28.65	1,516	<0.0001	1.48	2,516	<0.0001	11.2	2,516	<0.0001

All effects are from type III sums of squares.

convergens females consistently produced diagnostic fragments of aphids in their frass when they preyed on pea aphids but did so less consistently when they preyed on alfalfa weevil larvae (from which they sometimes took only a liquid meal; Davidson 2008). Thus, females of all three species very likely consumed alfalfa weevils even more frequently in the alfalfa fields studied here than is indicated by the observed high frequencies of frass pellets containing weevil fragments. This supports the inference drawn previously from field observations (Kalaskar and Evans 2001, Evans 2004, and references within) that alfalfa weevil larvae are important components of lady beetle diets in spring alfalfa.

The most general aim of this study was to determine how much three species of lady beetles use the many sources of food available to them in the alfalfa habitat. Frass analysis proved very useful, because 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 additional 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). When such prey are likely to constitute a large portion of the diet, or when specific species of prey are of special interest, molecular methods of diet determination (e.g., ELISA and PCR) may be more appropriate. However, if indigestible fragments are consumed by the predator, frass analysis can be performed quickly and with minimal cost, in contrast to these other methods, and can thereby yield a broad description of predator diet. Additionally, there is no need to sacrifice the insect. The results of this 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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References Cited

Agarwala, B. K., S. Bhattacharya, and P. Bardhanroy. 1998. Who eats whose eggs? Intra-versus inter-specific interactions in starving ladybird beetles predaceous on aphids. Ethol. Ecol. Evol. 10: 361–368.

Agrios, G. N. 2005. Plant pathology, 5th ed. Elsevier Academic, Burlington, MA.

Conrad, M. S. 1959. The spotted lady beetle, *Coleomegilla maculata* (DeGeer), as a predator of Eur. corn borer eggs. J. Econ. Entomol. 52: 843–847.

Davidson, L. N. 2008. Diets of ladybird beetles (Coleoptera: Coccinellidae) in Utah alfalfa fields. MS thesis, Utah State University, Logan, UT.

Elliott, N. C., R. W. Kieckhefer, G. J. Michels, and K. L. Giles. 2002. Predator abundance in alfalfa fields in relation to aphids, within-field vegetation, and landscape matrix. Environ. Entomol. 31: 253–260.

Evans, E. W. 2004. Habitat displacement of North American ladybirds by an introduced species. Ecology 85: 637–647.

Evans, E. W. 2009. Lady beetles as predators of insects other than Hemiptera. Biol. Control 51: 255–267.

Evans, E. W., and J. G. Swallow. 1993. Numerical responses of natural enemies to artificial honeydew in Utah alfalfa. Environ. Entomol. 22: 1392–1401.

Evans, E. W., and S. England. 1996. Indirect interactions in biological control of insects: pests and natural enemies in alfalfa. Ecol. Applic. 6: 920–930.

Evans, E. W., and D. R. Richards. 1997. Managing the dispersal of ladybird beetles (Coleoptera: Coccinellidae): use of artificial honeydew to manipulate spatial distributions. Entomophaga 42: 93–102.

Evans, E. W., and D. I. Gunther. 2005. The link between food and reproduction in aphidophagous predators: a case study with *Harmonia axyridis* (Coleoptera: Coccinellidae). Eur. J. Entomol. 102: 423–430.

Grez, A. A., T. Zaviezo, S. Diaz, B. Camousseight, and G. Cortes. 2008. Effects of habitat loss and fragmentation on the abundance and species richness of aphidophagous beetles and aphids in experimental alfalfa landscapes. Eur. J. Entomol. 105: 411–420.

Hagen, K. S., N. J. Mills, G. Gordh, and J. A. McMurtry. 1999. Terrestrial arthropod predators of insect and mite pests, pp. 383–503. *In* T. S. Bellows and T. W. Fisher (eds.), Handbook of biological control. Principles and applications of biological control. Academic, San Diego, CA.

Harwood, J. D., and J. J. Obrycki. 2005. Quantifying aphid predation rates of generalist predators in the field. Eur. J. Entomol. 102: 335–350.

Hemptinne, J. L., and A. Desprets. 1986. Pollen as a spring food for *Adalia bipunctata*, pp. 29–35. *In* I. Hodek (ed.), Ecology of Aphidophaga. Academia Press, Prague, Czech Republic.

Hemptinne, J. L., A.F.G. Dixon, and G. Lognay. 1996. Searching behaviour and mate recognition by males of the two-spot ladybird beetle, *Adalia bipunctata.* Ecol. Entomol. 21: 165–170.

Hodek, I. 1962. Essential and alternative foods in insects. Proc. 11th Int. Congr. Entomol. 2: 698–699.

Hodek, I. 1996. Food relationships, pp. 143–238. *In* I. Hodek and A. Honěk (eds.), Ecology of Coccinellidae. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Hodek, I., and A. Honěk. 1996. Ecology of Coccinellidae. Kluwer Academic, Dordrecht, The Netherlands.

Honěk, A. 1986. Production of faeces in natural populations of aphidophagous coccinellids (Col.) and estimation of predation rates. J. Appl. Entomol. 102: 467–476.

Kalaskar, A., and E. W. Evans. 2001. Larval responses of aphidophagous lady beetles (Coleoptera: Coccinellidae) to weevil larvae versus aphids as prey. Ann. Entomol. Soc. Am. 94: 76–81.

Lawton, J. H. 1970. Feeding and food energy assimilation in larvae of the damselfly *Pyrrhosoma nymphula* (Sulz.) (Odonata: Zygoptera). J. Anim. Ecol. 39: 669–689.

Lundgren, J. G. 2009. Relationships of natural enemies and non-prey foods. Springer, Dordrecht, The Netherlands.

Lundgren, J. G., A. A. Razzak, and R. N. Wiedenmann. 2004. Population responses and food consumption by predators *Coleomegilla maculata* and *Harmonia axyridis* (Coleoptera: Coccinellidae) during antithesis in an Illinois cornfield. Environ. Entomol. 33: 958–963.

Lundgren, J. G., A. Huber, and R. N. Weidenmann. 2005. Quantification of consumption of corn pollen by the predator *Coleomegilla maculata* (Coleoptera: Coccinellidae) during anthesis in an Illinois cornfield. Agric. For. Entomol. 7: 1–8.

Mayntz, D., D. Raubenheimer, M. Salomon, S. Toft, and S. J. Simpson. 2005. Nutrient specific foraging in invertebrate predators. Science 307: 111–113.

Moore, P. D., J. A. Webb, and M. E. Collinson. 1991. Pollen analysis. Blackwell Scientific Publications, Oxford, United Kingdom.

Neuenschwander, P., K. S. Hagen, and R. F. Smith. 1975. Predation on aphids in California's alfalfa fields. Hilgardia 43: 53–78.

Pemberton, R. W., and N. J. Vandenberg. 1993. Extrafloral nectar feeding by ladybird beetles (Coleoptera: Coccinellidae). Proc. Entomol. Soc. Wash. 95: 139–151.

Powell, W., M. P. Walton, and M. A. Jervis. 1996. Populations and communities, pp. 223–292. *In* M. Jervis and N. Kidd (eds.), Insect natural enemies: practical approaches to their study and evaluation. Chapman & Hall, London, United Kingdom.

Putman, W. L. 1957. Laboratory studies on the food of some Coccinellids (Coleoptera) found in Ontario peach orchards. Can. Entomol. 89: 572–579.

Putman, W. L. 1964. Occurrence and food of some Coccinellids (Coleoptera) in Ontario peach orchards. Can. Entomol. 96: 1149–1155.

Ricci, C., and L. Ponti. 2005. Seasonal food of *Ceratomegilla notata* (Coleoptera: Coccinellidae) in mountain environments of Northern Italian Alps. Eur. J. Entomol. 102: 527–530.

Ricci, C., L. Ponti, and A. Pires. 2005. Migratory flight and pre-diapause feeding of *Coccinella septempunctata* (Coleoptera) adults in agricultural and mountain ecosystems of Central Italy. Eur. J. Entomol. 102: 531–538.

SAS Institute. 2003. PROC user's manual, version 9.1. SAS Institute, Cary, NC.

Sheppard, S. K., and J. D. Harwood. 2005. Advances in molecular ecology: tracking trophic links through predator-prey food webs. Funct. Ecol. 19: 751–762.

Soares, A. O., D. Coderre, and H. Schanderl. 2004. Dietary self-selection behaviour by the adult of the aphidophagous ladybeetle *Harmonia axyridis* (Coleoptera: Coccinellidae). J. Anim. Ecol. 73: 478–486.

Sunderland, K. D. 1987. A review of methods of quantifying invertebrate predation occurring in the field. Acta Phytophath. Entom. Hung. 22: 13–34.

Symondson, W.O.C. 2002. Molecular identification of prey in predator diets. Mol. Ecol. 11: 627–641.

Thompson, D. J. 1978. Prey size selection by larvae of the damselfly, *Ischnura elegans* (Odonata). J. Anim. Ecol. 47: 769–785.

Triltsch, H. 1997. Gut contents in field sampled adults of *Coccinella septempunctata* (Col: Coccinellidae). Entomophaga 42: 125–131.

Triltsch, H. 1999. Food remains in the guts of *Coccinella septempunctata* (Coleoptera: Coccinellidae) adults and larvae. Eur. J. Entomol. 96: 355–64.

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