BioControl **47:** 1–21, 2002.

Host plants affect predator fitness via the nutritional value of herbivore prey: Investigation of a plant-aphid-ladybeetle system

K.L. GILES[1*], R.D. MADDEN[1], R. STOCKLAND[1], M.E. PAYTON[2] and J.W. DILLWITH[1]
Departments of [1] Entomology and Plant Pathology, and of [2] Statistics, Oklahoma State University, Stillwater, Oklahoma, USA;
[*] author for correspondence; e-mail: kgiles@okstate.edu

Received 28 June 2000; accepted in revised form 24 April 2001

Abstract. The interactions among host plants (*Medicago sativa* L., cv. 'OKO8' and *Vicia faba* L., cv. 'Windsor'), aphid prey (*Acyrthosiphon pisum* Harris, Homoptera: Aphididae), and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) preimaginal biology were evaluated. Interactions were measured over a range of limiting daily prey levels (1.2 mg–16.4 mg) from each host plant colony. Compared with *A. pisum* reared on *V. faba*, *A. pisum* reared on *M. sativa* stored significantly more fatty acids which resulted in a 1.17-fold increase in available calories for developing *C. septempunctata*. The increased survival, decreased developmental times, and larger size of *C. septempunctata* supplied with *A. pisum* reared on *M. sativa* clearly demonstrate host plant effects at the third trophic level. At low very limiting daily prey levels, *A. pisum* reared on *M. sativa* were more suitable prey for *C. septempunctata* survival, development, and adult size than *A. pisum* reared on *V. faba*. *Coccinella septempunctata* survival ratios (larval), developmental times, and adult size converged (were not statistically different) between host plants at higher daily *A. pisum* levels. These convergence's support the hypothesis that there were quantitative differences in the nutritional value of aphids, as influenced by differences in fatty acids and subsequent nutritional levels (calories), between aphids reared on separate plant hosts. The observed tritrophic interactions appear to be modulated by the biochemical response of *A. pisum* to host plants.

Key words: fatty acids, insect predator, tritrophic interactions, *Acyrthosiphon pisum*, *Coccinella septempunctata*, *Medicago sativa*, *Vicia faba*

Introduction

The effects of plants on entomophagous insects have been well documented in tritrophic interaction studies (Starks et al., 1972; Rice and Wilde, 1989; Kareiva and Sahakian, 1990; van Emden and Wratten, 1990; Campbell et al., 1992; Souissi and Le Ru, 1997; Obrycki and Kring, 1998; Bottrell et al., 1998; Giles et al., 2000). Altered prey population levels, as well as altered

access to prey or changes in prey acceptability or suitability can dramatically influence natural enemies. The biochemical contents of plants may result in toxic or nutritionally poor prey and may increase mortality, slow developmental rates, decrease growth rates, and reduce fecundity of natural enemies. Plants have been shown to alter third trophic level dynamics in many ecological systems, however, the mechanisms of many tritrophic interactions are unknown (Kareiva and Sahakian, 1990; Power, 1992; Hodek, 1993; Hodek and Honěk, 1996; Bottrell et al., 1998; Giles et al., 2000).

The storage of nutritional contents (primarily fatty acids) in aphids is influenced by plant species or cultivar (Dillwith et al., 1993). Therefore, when evaluating the suitability of aphid prey, monitoring changes in their nutritional value among host plants is an essential component of tritrophic interaction studies (Giles et al., 2000). Pea aphids, *Acyrthosiphon pisum* Harris (Homoptera: Aphididae), store up to six times more myristic acid when feeding on *Medicago sativa* L. (alfalfa) versus *Vicia faba* L. (faba bean) (Bergman et al., 1990; Neese, 1995; Giles et al., 2000). This increased fatty acid storage increases the total quantitative nutritional value (calories) of *A. pisum* on *M. sativa*, and may influence survival, development, and reproduction of Coccinellidae predators. For example, Bashir (1973) observed that when myristic acid levels in artificial diets were increased, *Olla abdominalis* Say (Coleoptera: Coccinellidae) developed faster, adults were larger, and females were more fecund.

The suitability of *A. pisum* for the survival and development of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) has been well demonstrated (Obrycki and Orr, 1990; Phoofolo and Obrycki, 1995; Hodek and Honěk, 1996; Obrycki et al., 1998). Little information exists, however, relating host plant effects on *A. pisum* to the biology of *C. septempunctata*. The objective of this study was to investigate interactions between host plants (*M. sativa* or *V. faba*) and *A. pisum* fatty acid storage, and the subsequent effects on *C. septempunctata* survival, sex ratio, development, and size (elliptical body area). In particular, we attempted to examine the role of *A. pisum* nutritional content (calories) as affected by fatty acid storage between host plants. We hypothesized that the elevated caloric content (as affected by increased levels of fatty acids) of aphids from the *M. sativa* colony would result in higher survivorship for *C. septempunctata*, faster development, and larger adults compared with *C. septempunctata* supplied with aphids from the *V. faba* colony. We addressed this hypothesis by examining whether differences in survival, development, and size of *C. septempunctata* supplied with *A. pisum* from the two host plants were the result of quantitative differences in the nutritional value of aphids. Quantitative differences in the nutritional value of *A. pisum* between host plants would represent a similar composition of

nutrients, but differing levels of nutritional energy sources such as myristic acids. Alternatively, qualitative differences in the nutritional value of *A. pisum* between host plants would be the result of the absence of essential nutrients, or the presence of a toxin in the aphid derived from the host plant. We addressed this question by supplying *C. septempunctata* larvae a range of limiting (sub-optimal) daily prey levels (mg of *A. pisum* per day) from colonies reared on *M. sativa* or *V. faba*. If survival ratios, development times, and adult size for *C. septempunctata* supplied with *A. pisum* reared on *M. sativa* versus *V. faba* were different at low very limiting daily prey levels but similar at higher less limiting daily prey levels, the nutritional value of *A. pisum* between host plants would likely be quantitative. Additionally, we further confirmed the effects of altered myristic acid levels by inducing increased levels within *A. pisum* reared on *V. faba* (by decreasing colony temperature) and evaluating the subsequent effects on *C. septempunctata* survival, development, and size.

Materials and methods

Aphid colonies

An *A. pisum* colony was maintained on *V. faba* cultivar 'Windsor' at 22 °C and a photoperiod of 16:8 (L:D) h. Aphids from this original colony served as the infestation source for a colony maintained on *M. sativa* cultivar 'OKO8' at 22 °C and a photoperiod of 16:8 (L:D) h. Using aphids from the original colony, a third colony was established on *V. faba* at 10 °C and a photoperiod of 16:8 (L:D) h. All colonies were maintained in separate environmental chambers.

Three separate analyses were conducted to evaluate the nutritional value of aphids among colonies. During the study, aphid fatty acid profiles were quantified for each 22 °C colony (n = 11–12 samples of 10 aphids each) and the 10 °C colony (n = 4 samples of 10 aphids) using methods described by Bergman et al. (1991). Also, an isoperibol calorimeter (Model 1261, Parr Instruments Co., Moline, IL) was used to quantify caloric content of aphids from all three colonies (n = three 5 g samples from each colony). Additionally, proximate analysis (A.O.A.C., 1990) was used to quantify percentage protein for aphids from each colony (three pooled 2–5 g samples from each colony).

C. septempunctata *feeding studies*

All feeding studies were conducted in a table-top environmental chamber maintained at 24 °C and a photoperiod of 16:8 (L:D) h. Adult *C. septempunctata* were collected from North-central Oklahoma wheat fields and

separated into mating pairs. Thirteen pairs were maintained in half-pint cardboard containers covered with fine mesh. Pairs were provided daily with an unlimited supply of *A. pisum* reared on *V. faba*, moist cotton, and a supplementary wheat-honey-yeast mixture. Egg masses from each mating pair were collected daily, and separated into 10 ml glass vials stopped with cotton.

Effects of A. pisum *maintained on* M. sativa *or* V. faba *at 22 °C*

A factorial study was conducted to evaluate the effects of increasing daily levels of *A. pisum* reared on *M. sativa* or *V. faba* on the preimaginal biology of *C. septempunctata*. Upon eclosion, first instar *C. septempunctata* were individually isolated into 10 ml cotton stopped glass vials and assigned to one of 10 daily aphid treatments. Daily prey levels from the 22 °C *M. sativa* colony were (mean ± SE) 1.2 ± 0.03 , 2.2 ± 0.06, 4.3 ± 0.12, 8.2 ± 0.18, or 16.4 ± 0.28 mg (live fresh weight) of late instar apterous *A. pisum*. Daily prey levels from the 22 °C *V. faba* colony were (mean ± SE) 1.2 ± 0.06 , 2.2 ± 0.05, 4.3 ± 0.09, 8.2 ± 0.06, or 16.4 ± 0.24 mg (live fresh weight) of late instar apterous *A. pisum*. Aphids were weighed using a digital microbalance. From 57–60 individuals were assigned to each treatment; treatments were replicated across parental lines (mating pairs).

In order to isolate the effects of differences in prey nutritional value between host plant colonies, an upper limit of 16.4 mg of *A. pisum* was chosen because it represented an adequate but suboptimal diet for *C. septempunctata*. This suboptimal diet would allow high survival and quick developmental rates but eliminate the confounding effects of satiation (Obrycki et al., 1998; Giles et al., 2000).

Newly eclosed larvae were supplied with their assigned daily *A. pisum* levels by placing aphids in close proximity to individual *C. septempunctata* in an effort to eliminate the effects of searching. Consumption of aphids was not directly monitored during our study, however, no *A. pisum* were present 24 h after supplying aphids to late instar *C. septempunctata* for all treatments. All *C. septempunctata* larvae were systematically checked daily between 1400 and 1700 h for molting, death, pupation, and adult emergence. Emerging adults were supplied with water for 1 d before measuring body area. We used the procedure of Obrycki et al. (1998) to estimate adult size [elliptical body area; $mm^2 = (\Pi \times 1/2$ (body length) $\times$ 1/2 (body width)].

Effects of A. pisum *maintained on* V. faba *at 10 °C*

A separate study was conducted to evaluate the effects of *A. pisum* maintained at 10 °C on *V. faba* on the preimaginal biology of *C. septempunctata*. We hypothesized that the elevated caloric content (as affected by increased levels of fatty acids) of aphids from the 10 °C colony will result in higher survivorship for *C. septempunctata*, faster development, and larger adults

compared with *C. septempunctata* supplied with aphids from the 22 °C *V. faba* colony. Upon eclosion, first instar *C. septempunctata* were individually isolated into 10 ml cotton stopped glass vials and assigned to one of two limiting daily *A. pisum* treatments. The daily prey level (live fresh weight) from the 10 °C *V. faba* colony was exactly 4.0 mg of late instar apterous *A. pisum*. For comparison, the daily prey level from the 22 °C *V. faba* colony was also exactly 4.0 mg of late instar apterous *A. pisum*. From 19–22 individuals were assigned to each treatment; treatments were replicated across 5 Coccinellidae parental lines. Experimental procedures were identical to those previously described. To ensure comparable values in adult elliptical body area measurements to those observed for previous treatments, we measured a representative female ratio (0.167; 1 male: 5 females) from each 4.0 mg treatment similar to ratios observed for the 4.3 mg treatments from *M. sativa* and *V. faba* at 22 °C (0.158 to 0.174).

Statistical analysis

All statistical analyses were performed using SAS version 6.12 (SAS Institute, 1996). The significance level chosen for all statistical analyses was $p = 0.05$. Myristic acid and total fatty acid contents were compared among aphids from each colony by analysis of variance (PROC MIXED). The Mixed Procedure is appropriate for experiments with unequal replications and unequal variances among treatments. Caloric content from bomb calorimetry measures was compared among aphids from each colony by analysis of variance (PROC ANOVA).

Effects of A. pisum *maintained on* M. sativa *or* V. faba *at 22 °C*

Ratios of larval survival, pupal survival, larval + pupal survival, and females were compared among and between treatments using chi-square analyses (PROC FREQ). Larval, pupal, and preimaginal developmental times (days), and adult elliptical body area (mm^2) of *C. septempunctata* were analyzed by analysis of variance (PROC MIXED) with host plant and daily prey level (mg per day) as fixed factors. Preliminary analysis revealed no significant effects of parental line or sex on developmental times or adult body area, therefore data were pooled for analysis. Treatment means (LSMEANS) were compared by the least significance difference test (STDERR PDIFF).

For each host plant, linear relationships between larval and preimaginal developmental times, and adult body area and mg of aphids per day were analyzed by regression analysis (PROC GLM). Additionally, to investigate the effects of observed differences in caloric content between aphid colonies as affected by differences in fatty acid levels, two separate regression analyses independent of host plant were conducted. First, linear relationships between

larval, preimaginal developmental times, and adult body area and mg of aphids per day were analyzed by regression analysis (PROC GLM). Secondly, for comparison, after converting the independent variable to calories for aphids from each colony, linear relationships between larval, preimaginal developmental times, and adult body area and calories per day were analyzed by regression analysis (PROC GLM).

Effects of A. pisum *maintained on* V. faba *at 10 °C*

Ratios of larval survival, pupal survival, and larval + pupal survival were compared between treatments using Chi-square analyses (PROC FREQ). Larval, pupal, preimaginal developmental times (days), and adult body area (mm^2) of *C. septempunctata* were compared between treatments using *t*-tests. Survivorship, development, and adult size data from the 4.0 mg treatments (aphids from 10 °C and 22 °C *V. faba* colonies) were plotted for visual comparison, but not included in regression analyses.

Voucher specimens

Voucher specimens (*C. septempunctata* adults) are deposited in the Department of Entomology and Plant Pathology Museum at Oklahoma State University, Stillwater.

Results

Fatty acid and caloric content of aphid colonies

Significant differences were observed for myristic acid (F = 38.3, df = 2, 24, $p < 0.001$) and total fatty acid content (F = 53.2, df = 2, 24, $p < 0.001$) among aphid colonies (Table 1). For aphids reared on *M. sativa* at 22 °C, average (± SE) myristic acid content was 11.5 ± 1.9 μg per mg aphid fresh weight, whereas average total fatty acid content was 16.1 ± 2.2 μg per mg aphid fresh weight. The average (± SE) myristic acid and total fatty acid content for aphids reared on *V. faba* at 22 °C were 1.8 ± 0.3 and 6.4 ± 0.4 μg per mg aphid fresh weight, respectively. For aphids reared on *V. faba* at 10 °C, average (± SE) myristic acid content was 25.5 ± 1.9 μg per mg aphid fresh weight, whereas average total fatty acid content was 39.7 ± 2.6 μg per mg aphid fresh weight. Additionally, aphid caloric content, as measured by bomb calorimetry, varied significantly (F = 259.0, df = 2, 6, $p < 0.001$) among colonies (Table 1). For aphids reared on *M. sativa* at 22 °C, *V. faba* at 22 °C, and *V. faba* at 10 °C, the average (± SE) calories per mg of fresh aphid weight were 1.195 ± 0.009, 1.021 ± 0.029, and 1.340 ± 0.028, respectively. Among

Table 1. Daily aphid diets, estimated lipid content, and calories for each treatment

Host plant	Temp. °C[a]	mg per day	Myristic acid μg[b]	Fatty acid μg[b]	Calories[c]
A. pisum					
Experimental diet treatments					
M. sativa	22	1.2 ± 0.03	13.8	19.3	1.434
V. faba	22	1.2 ± 0.06	2.1	7.7	1.225
M. sativa	22	2.2 ± 0.06	25.3	35.3	2.629
V. faba	22	2.2 ± 0.05	3.9	14.1	2.245
M. sativa	22	4.3 ± 0.12	49.4	69.0	5.138
V. faba	22	4.3 ± 0.09	7.6	27.6	4.388
M. sativa	22	8.2 ± 0.18	94.1	131.6	9.799
V. faba	22	8.2 ± 0.06	14.6	52.5	8.369
M. sativa	22	16.4 ± 0.28	188.3	263.3	19.597
V. faba	22	16.4 ± 0.24	29.1	105.1	16.737
Additional treatments					
V. faba	10	4.0	102.0	158.8	5.362
V. faba	22	4.0	7.1	25.6	4.082

[a]Temperature of aphid colonies.
[b]Myristic acid and total fatty acid calculated using average mg of aphids per day and results of lipid analysis (μg per mg aphid).
[c]Calculated from results of bomb calorimetry.

aphid colonies, proximate analysis of pooled samples revealed that *A. pisum* protein levels were nearly equivalent; aphids reared on *M. sativa* at 22 °C contained 10.9 percent protein, whereas aphids reared on *V. faba* contained 10.6 and 11.3 percent protein for 22 °C and 10 °C colonies, respectively.

Survival and female ratios

Effects of A. pisum *maintained on M. sativa or V. faba at 22 °C*

When comparing *C. septempunctata* survival on aphids from the 22 °C colonies (*M. sativa* versus *V. faba*), chi-square analyses indicated significant differences among all treatments in larval survival ratios, pupal survival ratios, and larval + pupal survival ratios (Table 2). Larval and larval + pupal survival ratios quickly increased before reaching a plateau between the 8.2 and 16.4 mg daily prey levels from each host plant (Figures 1A and 1C). At all daily prey levels, higher survivorship was observed for *C. septempunctata* supplied with *A. pisum* reared on *M. sativa* versus *V. faba* (Table 1; Figures 1A

Table 2. Ratio of *C. septempunctata* surviving the larval, pupal, and total preimaginal stages, and ratio of females at 24 °C resulting from increasing daily levels of *A. pisum* reared on either *M. sativa* or *V. faba* at 22 °C

Variable	Daily prey level of *A. pisum* (mg / day) from each host plant										Ratio comparisons		
	1.2		2.2		4.3		8.2		16.4		χ^2	df	p
	M.s.[a]	*V.f.*[b]	*M.s.*	*V.f.*	*M.s.*	*V.f.*	*M.s.*	*V.f.*	*M.s.*	*V.f.*			
Larval	0.017	0	0.333	0.050[c]	0.817	0.617	0.897	0.864	0.930	0.867	272.2	9	0.001
Pupal	0	—	0.600	0.333	0.837	0.676	1.000	0.882	1.000	0.846	93.9	8	0.001
Larval + Pupal	0	0	0.200	0.017	0.683	0.417	0.897	0.763	0.930	0.733	263.9	9	0.001
Female	—	—	0.167	0	0.158	0.174	0.540	0.325	0.780	0.432	78.4	7	0.001
N[d]	60	60	60	60	60	60	58	59	57	60	—	—	—

[a]Pea aphids reared on *M. sativa*.
[b]Pea aphids reared on *V. faba*.
[c]Paired underlined values represent significant differences ($p < 0.05$) for 2×2 χ^2 tests between host plants at each mg level.
[d]Total number of *C. septempunctata* larvae per treatment at beginning of experiment.

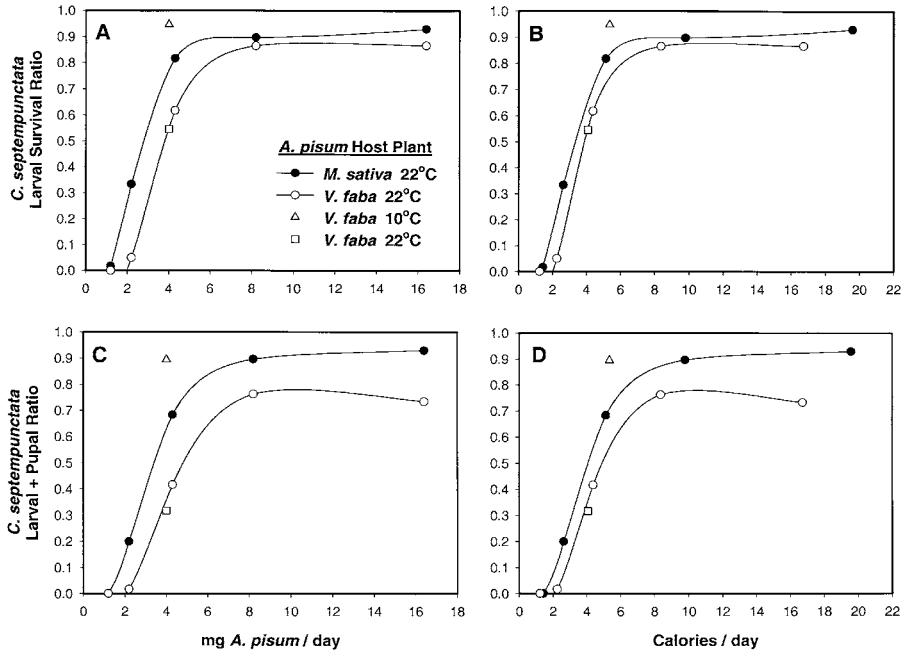

Figure 1. Survival ratios for *C. septempunctata* at 24 °C supplied with increasing daily levels of *A. pisum* reared on *M. sativa* or *V. faba* at 22 °C, or on *V. faba* at 10 °C. The relationships between (A) larval survival and mg of *A. pisum* per day, (B) larval survival and calories per day, (C) larval + pupal survival and mg of *A. pisum* per day, and (D) larval + pupal survival and calories per day are shown. Survival ratios for the additional *C. septempunctata* supplied with 4.0 mg per day of *A. pisum* from the 22 °C or 10 °C *V. faba* colonies are plotted in graphs A–D.

and 1C). Comparisons of larval survival ratios between host plants at similar daily prey levels indicated significant differences ($\chi^2 > 5.9$; df = 1, $p < 0.015$) at the 2.2 and 4.3 mg levels (Table 2). For pupal survival ratios, significant differences were observed between host plants at the 8.2 and 16.4 mg daily prey levels ($\chi^2 > 6.4$; df = 1, $p < 0.011$; Table 2). Significant differences for larval + pupal survival between host plants were observed at the 2.2, 4.3, and 16.4 mg levels ($\chi^2 > 7.9$; df = 1, $p < 0.005$), but not at the 8.2 mg level ($\chi^2 = 3.7$; df = 1, $p = 0.055$; Table 2).

Chi-square analysis indicated significant differences among all treatments in the ratio of females (Table 2). Increased ratios of females were observed for larvae supplied with aphids from the *M. sativa* colony at higher prey densities. Comparisons of female ratios between host plants at similar daily prey levels indicated significant differences ($\chi^2 > 4.1$; df = 1, $p < 0.041$) at the 8.2 and 16.4 mg levels (Table 2).

Table 3. ANOVA results (Mixed Procedure, SAS) for *C. septempunctata* developmental time (days) and adult size (eliptical body area) reared at 24 °C on increasing daily levels of *A. pisum* (prey level) from two host plant colonies maintained at 22 °C

Response variable	Test of fixed effects			
	Source of variation	df	F	*p*
DEVELOPMENTAL TIME				
Larval	Host plant	1, 309	48.55	<0.001
	Prey level	4, 309	260.50	<0.001
	Host plant × Prey level	3, 309	6.78	<0.001
Pupal	Host plant	1, 265	0.07	0.795
	Prey level	3, 265	1.11	0.345
	Host plant × Prey level	3, 265	3.21	0.024
Total preimaginal	Host plant	1, 265	34.26	<0.001
	Prey level	3, 265	342.48	<0.001
	Host plant × Prey level	3, 265	12.33	<0.001
ADULT BODY AREA (mm^2)[a]				
Size	Host plant	1, 268	19.72	<0.001
	Prey level	3, 268	181.95	<0.001
	Host plant × Prey level	2, 268	0.67	0.515

Host plants were *M. sativa* and *V. faba*. Daily prey levels from *M. sativa* and *V. faba* were (means) 1.2 , 2.2, 4.3, 8.2, or 16.4 mg per day of *A. pisum*.
[a]Calculated using equation for an ellipse [Π × 1/2 (body length) × 1/2 (body width)]. Additional replications represent individuals developing to the adult stage but with missing data on developmental.

Effects of A. pisum *maintained on* V. faba *at 10 °C*

Chi-square analysis indicated significant differences between treatments (4.0 mg *A. pisum* per day from *V. faba* colonies at 10 °C versus 22 °C) in larval survival, pupal survival, and larval + pupal survival ($\chi^2 > 5.8$; df = 1, $p < 0.015$; Figure 1). For *C. septempunctata* supplied with 4.0 mg of aphids per day from the 10 °C *V. faba* colony, 8 of 17 (0.471) were females, whereas 2 of 7 (0.286) developed into females on a diet of 4.0 mg of aphids per day from the 22 °C *V. faba* colony. Chi-square analysis did not indicate significant differences between these female ratios ($\chi^2 = 0.7$; df = 1, $p = 0.404$).

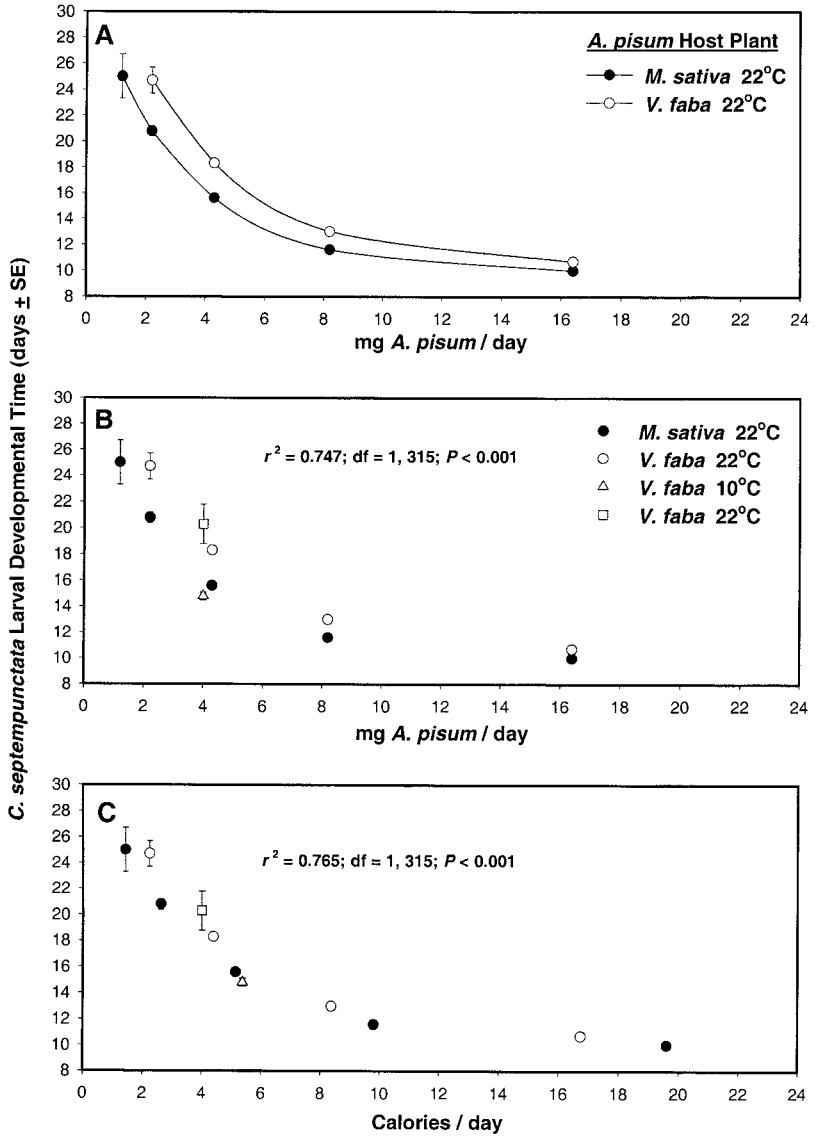

Figure 2. Larval developmental times (days, means ± SE) for *C. septempunctata* at 24 °C supplied with increasing daily levels of *A. pisum* reared on *M. sativa* or *V. faba* at 22 °C, or on *V. faba* at 10 °C. Shown are (A) the relationships between larval development time and mg of *A. pisum* per day from each 22 °C host plant colony, (B) regression data points (means ± SE) and statistics for the relationship between larval development time and mg of *A. pisum* per day, and (C) regression data points (means ± SE) and statistics for the relationship between larval development time and calories per day. Means ± SE development times for the additional *C. septempunctata* supplied with 4.0 mg per day of *A. pisum* from the 22 °C or 10 °C *V. faba* colonies are plotted in graphs B and C, but were not included in regression analysis.

Development

Effects of A. pisum *maintained on* M. sativa *or* V. faba *at 22 °C*

For *C. septempunctata* supplied with aphids from the two 22 °C colonies, larval and preimaginal developmental times were different among daily prey levels and between host plants, and significant interactions between host plants and daily prey levels were detected (Table 3; Figures 2A and 3A). The developmental times of *C. septempunctata* decreased quadratically (non-linearly as a decelerating curve) as daily aphid levels from *M. sativa* (larval: $r^2 = 0.795$; df = 1, 172; $p < 0.001$; preimaginal: $r^2 = 0.800$; df = 1, 155; $p < 0.001$) and *V. faba* (larval: $r^2 = 0.789$; df = 1, 140; $p < 0.001$; preimaginal: $r^2 = 0.824$; df = 1, 112; $p < 0.001$) increased (Figures 2A and 3A).

At all daily prey levels, larval developmental times were faster for *C. septempunctata* supplied with aphids from the *M. sativa* colony ($p < 0.042$; Figure 2), however, at the 16.4 mg daily prey level, preimaginal developmental times were similar between host plants ($p = 0.479$; Figure 3). The quadratic relationships between developmental times (independent of host plant) and mg of aphids per day from the 22 °C colonies were highly significant (Figures 2B and 3B). After converting the independent variable to calories from each 22 °C colony, the quadratic relationships for developmental times (independent of host plant) were slightly more precise and highly significant (Figures 2C and 3C).

For *C. septempunctata* supplied with aphids from the two 22 °C colonies, pupal developmental times which ranged from 5.1 to 6.0 days were not different among daily prey levels or between host plants, but an interaction between host plant and daily prey level was detected (Table 3). At the 16.4 daily prey level, pupal developmental times were slightly lower ($p = 0.017$) for *C. septempunctata* supplied with aphids from *V. faba* (5.1 ± 0.1 days) versus *M. sativa* (5.5 ± 0.1 days).

Effects of A. pisum *maintained on* V. faba *at 10 °C*

Larval and preimaginal developmental times (days ± SE) for *C. septempunctata* supplied with 4.0 mg of aphids from the 10 °C *V. faba* colony were shorter than those supplied with aphids from the 22 °C *V. faba* colony (*t*-test, $p < 0.004$; Figures 2B and 3B). Average pupal developmental times ranged from 5.0 to 6.0 days but were not significantly different between treatments (*t*-test, $p < 0.380$).

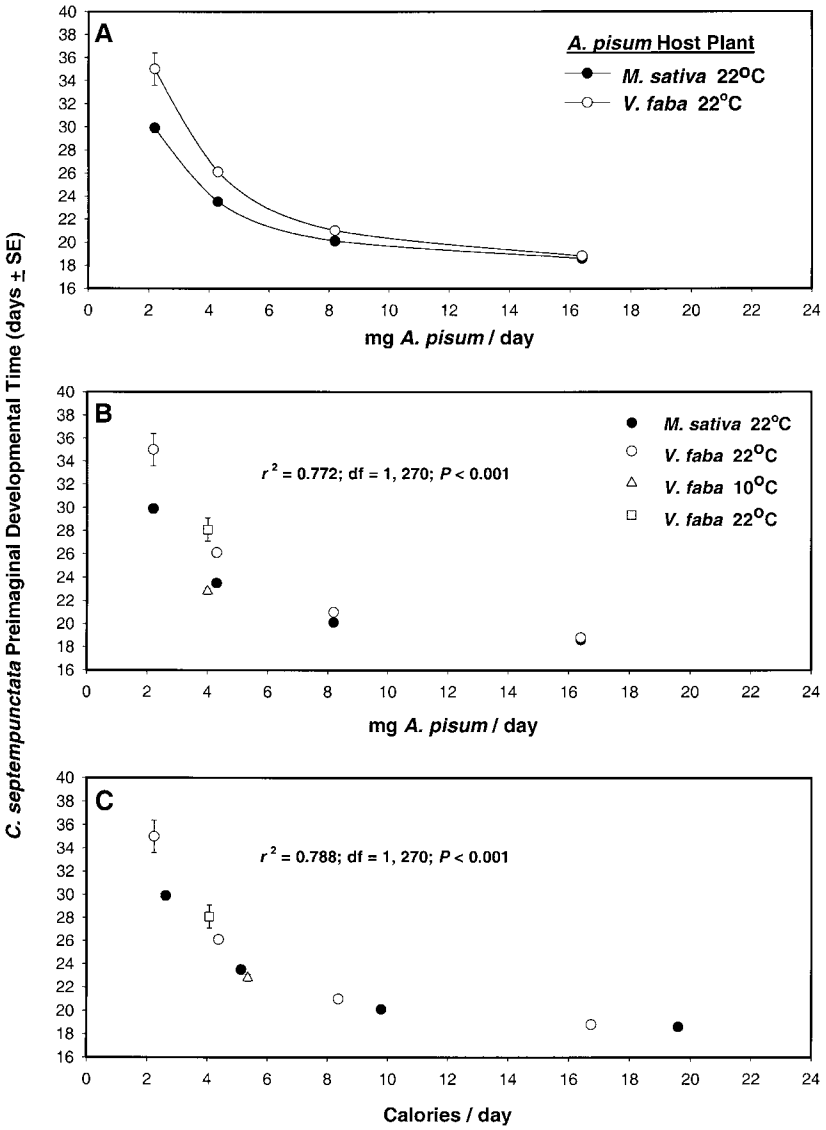

Figure 3. Preimaginal developmental times (days, means ± SE) for *C. septempunctata* at 24 °C supplied with increasing daily levels of *A. pisum* reared on *M. sativa* or *V. faba* at 22 °C, or on *V. faba* at 10 °C. Shown are (A) the relationships between preimaginal development time and mg of *A. pisum* per day from each 22 °C host plant colony, (B) regression data points (means ± SE) and statistics for the relationship between preimaginal development time and mg of *A. pisum* per day, and (C) regression data points (means ± SE) and statistics for the relationship between preimaginal development time and calories per day. Means ± SE preimaginal development times for the additional *C. septempunctata* supplied with 4.0 mg per day of *A. pisum* from the 22 °C or 10 °C *V. faba* colonies are plotted in graphs B and C, but were not included in regression analysis.

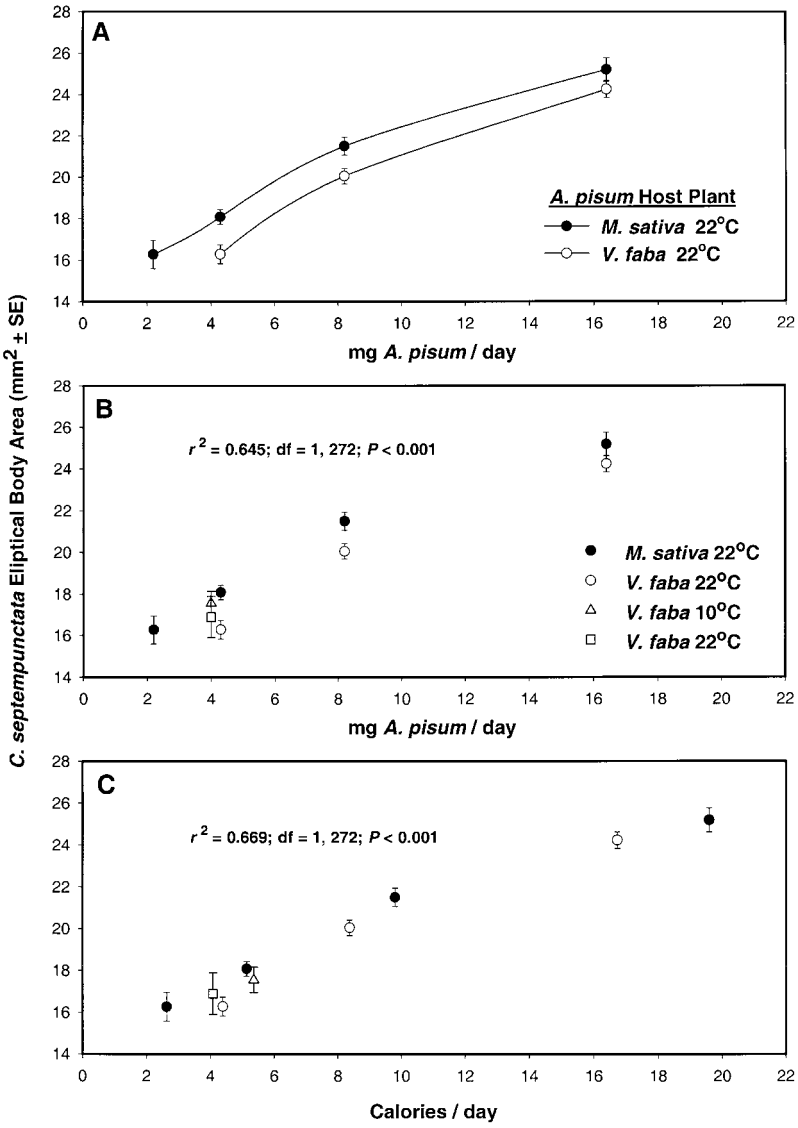

Figure 4. Elliptical body area (means ± SE) for *C. septempunctata* at 24 °C supplied with increasing daily levels of *A. pisum* reared on *M. sativa* or *V. faba* at 22 °C, or on *V. faba* at 10 °C. Body area was calculated using equation for an ellipse [Π × 1/2 (body length) × 1/2 (body width)]. Shown are (A) the relationships between body area and mg of *A. pisum* per day from each 22 °C host plant colony, (B) regression data points (means ± SE) and statistics for the relationship between body area and mg of *A. pisum* per day, and (C) regression data points (means ± SE) and statistics for the relationship between body area and calories per day. Means ± SE body areas for the additional *C. septempunctata* supplied with 4.0 mg per day of *A. pisum* from the 22 °C or 10 °C *V. faba* colonies are plotted in graphs B and C, but were not included in regression analysis.

Adult elliptical body area

Effects of A. pisum *maintained on* M. sativa *or* V. faba *at 22 °C*
For *C. septempunctata* supplied with aphids from the two 22 °C colonies, adult elliptical body area (mm^2) differed among daily prey levels and between host plants, but a significant interaction between host plant and daily prey level was not detected (Table 3; Figure 4A). The body area of *C. septempunctata* increased quadratically (non-linearly as a negatively accelerating curve) as daily aphid levels from *M. sativa* ($r^2 = 0.699$; df = 1, 151; $p < 0.001$) and *V. faba* ($r^2 = 0.640$; df = 1, 118; $p = 0.007$) increased (Figure 4A). At the 4.3 mg and 8.2 mg daily prey levels, body area was larger for *C. septempunctata* supplied with aphids from the *M. sativa* colony ($p < 0.007$; Figure 4), however, at the 16.4 mg daily prey level, body area was similar between host plants ($p = 0.070$; Figure 4).

The quadratic relationship between adult elliptical body area (independent of host plant) and mg of aphids per day from the 22 °C colonies was highly significant (Figure 4B). After converting the independent variable to calories from each 22 °C colony, the quadratic relationship for adult body area (independent of host plant) was slightly more precise and highly significant (Figure 4C).

Effects of A. pisum *maintained on* V. faba *at 10 °C*
The average adult elliptical body area ($mm^2 \pm$ SE) for *C. septempunctata* (1:5 male:female ratios) supplied with 4.0 mg of aphids from the 10 °C *V. faba* colony was similar to the average body area of beetles supplied with aphids from the 22 °C *V. faba* colony (*t*-test, $p < 0.586$; Figure 4B).

Discussion

Effects of A. pisum *maintained on* M. sativa *or* V. faba *at 22 °C:*
Interactions between daily prey level and host plant

Acyrthosiphon pisum reared on *V. faba* cultivar 'Windsor' is considered an excellent food item for *C. septempunctata*, allowing complete development and reproduction (Obrycki and Orr, 1990; Phoofolo and Obrycki, 1995; Hodek and Honěk, 1996; Obrycki et al., 1998). As previously documented, *C. septempunctata* exhibits reduced survivorship and delayed development when supplied with sub-optimal levels of *A. pisum* reared on *V. faba* (Obrycki et al., 1998). However, no studies have investigated survivorship and development of *C. septempunctata* supplied with sub-optimal levels of *A. pisum* from separate host plants.

In our study, survivorship on *A. pisum* from the *M. sativa* colony was consistently higher than *C. septempunctata* supplied with aphids from *V. faba*. Similar to results reported by Obrycki et al. (1998), larval, pupal, and larval + pupal survivorship increased as daily prey level increased, and this increase was most notable between the 2.2 mg and 4.3 mg daily prey levels from both host plants (Table 2; Figure 1).

We replicated daily aphid treatments across parental lines and therefore would expect similar sex ratios among treatments. However, female ratios declined as daily prey levels decreased indicating differences in survival between sexes (Table 2). As daily prey levels from each host plant increased, the effects of differences in survival (primarily pupal) between sexes are more clearly demonstrated (i.e., larger number of individuals for χ^2 analysis). For *C. septempunctata* supplied daily with 16.4 mg of *A. pisum* reared on *M. sativa* and *V. faba*, female ratios were 0.780 and 0.432, respectively. These differences could be due to the nutritional differences between aphid colonies, and the subsequent effect on female survival at the pupal stage.

The decreased developmental times of *C. septempunctata* supplied with low daily levels of *A. pisum* reared on *M. sativa* clearly demonstrate host plant effects at the third trophic level. For *C. septempunctata* supplied with *A. pisum* from either *M. sativa* or *V. faba*, the minimum times required for preimaginal development were statistically similar between host plants (16.4 mg/day). But, at the 2.2 mg daily prey level, preimaginal development times were 5 days different between host plants (Figure 3). The non-linear relationships (decelerating curves) between daily prey level and developmental times are similar to the inverse relationship for developmental rates (negatively accelerating curves) reported for other insect predators (Baumgaertner et al., 1981; Mills, 1981; Jervis and Kidd, 1996; Giles et al., 2000).

Adult *C. septempunctata* elliptical body area increased as daily prey level increased from both host plants, however, adults were generally larger when supplied with *A. pisum* from *M. sativa* (Figre 4). These differences in size could have important effects on populations size; Sundby (1968) documented that smaller *C. septempunctata* females are less fecund. For *C. septempunctata* supplied with *A. pisum* from either *M. sativa* or *V. faba*, the largest elliptical body areas were statistically similar between host plants (16.4 mg/day). But, at the 4.3 mg daily prey level, elliptical body areas were statistically different between host plants (Figure 4). Previous studies have documented a linear relationship between prey consumption and growth (dry weight gain) for Coccinellidae and other insect predators (Mills, 1981; Jervis and Kidd, 1996). In our study, adult elliptical body area increased non-linearly (quadratically) (Figure 4).

The differences in *A. pisum* nutritional value between the 22 °C colonies (*M. sativa* or *V. faba*) would be quantitative if nutritional components were similar in composition, but with differing levels between prey. Qualitative differences would result from the absence of essential nutritional components, or the presence of toxins in the prey derived from host plants (van Emden and Wratten, 1990; Price, 1997; Giles et al., 2000). If survival ratios, developmental times, and adult size of *C. septempunctata* were different between host plants at very limiting daily prey levels but statistically similar at higher less limiting daily prey levels, and a significant interaction between daily prey levels and host plants was detected, differences in the nutritional value of *A. pisum* are likely quantitative. Potentially, these quantitative differences would be correlated with differences in the observed fatty acid and caloric content of *A. pisum* between colonies. Qualitative differences would be evident if differences in survival, development, and adult size occurred at both very limiting and higher less limiting daily prey levels (Giles et al., 2000).

The observed differences in larval survivorship of *C. septempunctata* supplied with *A. pisum* reared on *M. sativa* versus *V. faba*, suggest quantitative differences in the nutritional value of aphids between host plants. The convergence of larval survival ratios between host plants at higher daily prey levels supports this conclusion (Table 2; Figure 1A). However, after incorporating pupal survivorship, the ratios of *C. septempunctata* surviving at the 16.4 mg daily prey level were different between host plants (Figure 1C). These differences between host plants appear to be influenced by differences in survival between sexes. Potentially, the limiting daily prey level range used in this study is not appropriate for quantifying *C. septempunctata* pupal survival between sexes. Alternatively, differences in larval + pupal survival between host plants may reflect unmeasured qualitative differences in the nutritional value of aphids from each colony. Further studies at higher daily prey levels would resolve this question.

Larval and preimaginal developmental times, and adult elliptical body area of *C. septempunctata* converged between host plants as prey level increased, further suggesting quantitative differences in the nutritional value of aphids between host plants (Figures 2, 3 and 4). These responses have been observed for other Coccinellidae supplied with the same daily *A. pisum* treatments used in this study, but not for the lacewing *Chrysoperla rufilabris* Burmeister (K. L. Giles, unpublished data; Giles et al., 2000).

Effects of altered A. pisum *caloric content*

We hypothesized that the observed differences in *C. septempunctata* survival, development, and adult size would be correlated with differences in the

observed myristic acid and caloric content of *A. pisum* between the 22 °C colonies. After converting the independent variable from mg to calories for aphids from each colony, *C. septempunctata* larval survival is nearly identical (Figures 1A and 1B), but larval + pupal survivorship is different between colonies at both low and high levels (Figures 1C and 1D). As previously mentioned, we may not have used the prey range necessary to fully address pupal survival.

Larval and larval + pupal survival ratios from the additional replications of 4.0 mg per day of *A. pisum* from the 22 °C *V. faba* colony fit as predicted before and after converting for caloric content (Figure 1). The high caloric content of aphids from the 10 °C *V. faba* colony, resulted in very high larval and larval + pupal survivorship for the 4.0 mg treatment (Figure 1). Unknown additional nutritional benefits (in addition to increased myristic acid) or simply a lack of replications may have caused this extremely high survival ratio for such a low daily prey level.

After converting the independent variable from mg to calories, r^2 values increased slightly for larval and preimaginal development, and adult elliptical body area (Figures 2, 3 and 4). Larval and preimaginal developmental times from the additional replications of 4.0 mg per day of *A. pisum* from the 22 °C *V. faba* colony fit as predicted before and after converting for caloric content (Figures 2 and 3). The high caloric content of aphids from the 10 °C *V. faba* colony, resulted in very short development times for the 4.0 mg treatment. After converting the independent variable from mg to calories, larval and preimaginal development times for the 4.0 mg treatment from the 10 °C *V. faba* colony closely matched the set of data points representing prey from the 22 °C colonies (Figures 2 and 3). Possibly due to the high levels of variability associated with only 6 individuals, adult body area measurements were not distinguishable between the 4.0 mg treatments (10 °C versus 22 °C *V. faba* colonies; Figures 4B and 4C).

Converting the independent variable to calories from mg improved the predictability of larval survival, larval and preimaginal development times, and adult elliptical body area (Figures 1–4). The slightly more precise relationships associated with calories provide further evidence (along with convergence of larval survival, development, and adult elliptical body area at high prey levels) that the observed differences in *C. septempunctata* survival, development, and adult size are correlated with differences in the observed myristic acid and caloric content of *A. pisum* between the 22 °C (*M. sativa* versus *V. faba*) colonies. This evidence also suggests that the observed tritrophic interactions are modulated by the biochemical response of *A. pisum* to host plants.

Evaluating tritrophic interactions

Though little studied, successful development of predacious insects is thought to be dependent primarily on quantitative proportions of nutrients (Thompson, 1999; Thompson and Hagen, 1999). The observed nutritional differences for *A. pisum* between host plants (*M. sativa* versus *V. faba*) are the result of altered biochemical processes, primarily the storage of myristic acid (Bergman et al., 1990; Dillwith et al., 1993; Neese, 1995). The mechanisms responsible for these altered biochemical processes have not been identified, however, high levels of myristic acid storage are consistent for *A. pisum* reared on alfalfa in laboratory colonies and those collected from alfalfa fields (J. W. Dillwith, unpublished data).

Our study supports the hypothesis that quantitative differences in the nutritional value of *A. pisum*, as influenced by altered myristic acid storage and subsequent differences in caloric content between aphids reared on separate plant hosts, were primarily responsible for differences in *C. septempunctata* larval survival, larval and preimaginal development, and adult elliptical body area. Our conclusion is supported by the convergence of larval survival ratios, development times, and body areas at higher prey levels, and the slightly more precise relationships associated with converting the independent variable to calories. The fact that we were able to increase survivorship, and decrease development times by increasing *A. pisum* myristic acid levels and calorie content within host plant (10 °C *V. faba* colony) further supports the idea that observed tritrophic interactions are modulated by the biochemical response of *A. pisum*. Increased myristic acid in aphid prey likely benefit developing ladybeetles because of higher levels of a common energy source for insects. However, future studies should investigate what if any significance myristic acid has on predators, beyond its caloric contribution.

Quantifying ecological relationships among host plants, aphid biochemical responses, and Coccinellidae biology is essential for understanding the mechanisms of tritrophic interactions. For tritrophic interaction studies, we recommend the inclusion of sub-optimal prey levels as variables (Weiser and Stamp, 1998; Giles et al., 2000). Indeed, several studies have demonstrated that naturally occurring Coccinellidae populations develop on sub-optimal diets (Obrycki et al., 1997; 1998). Additionally, to avoid incorrect claims of antibiosis during tritrophic interaction studies, nutritional analysis of prey should be standard procedure. Because host plants have been shown to influence storage of nutritionally important contents in aphids, nutritional contents of prey need to be quantified in order to fully address the mechanisms of tritrophic interactions (Dillwith et al., 1993; Giles et al., 2000). Quantification of fatty acid and amino acid levels, and concentrations of essential minerals and toxins along with accompanying experimental studies utilizing artificial

diets will aid in identifying the role of nutritionally important molecules for insect predators.

Acknowledgments

We thank J. Frazier, J. Bryant, A. Mackay, and R. Fuentes for technical help. We also thank T. Royer and N. Elliott for their critical review of this manuscript. This work was approved for publication by the Director, Oklahoma Agricultural Experiment Station, Stillwater.

References

A.O.A.C., 1990. *Official Methods of Analysis*, 15th ed. Association of Official Analytical Chemists. Arlington, VA.

Bashir, M.O., 1973. *Effect of Nutrition on Development and Reproduction of Aphidophagous Coccinellids with Special Reference to Olla Abdominalis (Say).* PhD Dissertation. University of California, Berkeley. 171 pp.

Baumgaertner, J.U., A.P. Gutierrez and C.G. Summers, 1981. The influence of aphid prey consumption on searching behavior, weight increase, developmental time, and mortality of *Chrysopa carnea* (Neuroptera: Chrysopidae) and *Hippodamia convergens* (Coleoptera: Coccinellidae) larvae. *Can. Entomol.* 113: 1007–1014.

Bergman, D.K., R.M. Edwards, R.C. Berberet and J.W. Dillwith, 1990. Fatty acid composition of pea aphids on two hosts. In: *Proc. Aphid-Plant Interactions: Populations to Molecules*. Stillwater, OK. MP-132. p. 295.

Bergman, D.K., J.W. Dillwith and R.C. Berberet, 1991. Spotted alfalfa aphid, *Therioaphis maculata*, fatty acids relative to the condition and susceptibility of its host. *Arch. Insect Biochem. Physiol.* 18: 1–12.

Bottrell, D.G., P. Barbosa and F. Gould, 1998. Manipulating natural enemies by plant variety selection and modification: a realistic strategy. *Annu. Rev. Entomol.* 43: 347–367.

Campbell, R.K., D.K. Reed, J.D. Burd and R.D. Eikenbary, 1992. Russian wheat aphid and drought stresses in wheat: tritrophic interactions with *Diaeretiella rapae* and plant resistance. In: S.B.J. Menken, J.H. Visser and P. Harrewijn (eds.), *Proc. 8th Int. Symp. Insect-Plant Relationships*. Kluwer Academic Publishers, Dordrecht pp. 297–298.

Dillwith, J.W., P.A. Neese and D.L. Brigham, 1993. Lipid biochemistry in aphids. In: D.W. Stanley-Samuelson and D.R. Nelson (eds.), *Insect Lipids: Chemistry, Biochemistry, and Biology.* University of Nebraska Press, Lincoln and London. pp. 389–434.

Giles, K.L., R.D. Madden, M.E. Payton and J.W. Dillwith, 2000. Survival and development of *Chrysoperla rufilabris* (Neuroptera: Chrysopidae) supplied with pea aphids (Homoptera: Aphididae) reared on alfalfa and faba bean. *Environ. Entomol.* 29: 304–311.

Hodek, I., 1993. Habitat and food specificity in aphidophagous predators. *Biocontrol Science and Tech.* 3: 91–100.

Hodek, I. and A. Honěk, 1996. *Ecology of Coccinellidae.* Kluwer Academic Publishers, Dordrecht.

Jervis, M. and N. Kidd, 1996. *Insect Natural Enemies: Practical Approaches to Their Study and Evaluation.* Chapman and Hall, London.

Kareiva, P. and R. Sahakian, 1990. Tritrophic effects of a simple architectural mutation in pea plants. *Nature.* 345: 433–434.

Mills, N.J., 1981. Some aspects of the rate of increase of a coccinellid. *Ecol. Entomol.* 6: 293–299.

Neese, P.A., 1995. *Fatty acid Metabolism in the Pea Aphid, Acyrthosiphon Pisum (Harris): Regulation in Relation to Plant Host.* PhD Dissertation. Oklahoma State University, Stillwater, OK.

Obrycki, J.J. and C.A. Orr, 1990. Suitability of three prey species for nearctic populations of *Coccinella septempunctata*, *Hippodamia variegata*, and *Propylea quatuordecimpunctata* (Coleoptera: Coccinellidae). *J. Econ. Entomol.* 83: 1292–1297.

Obrycki, J.J., A.M. Ormord and K.L. Giles, 1997. Partial life table analysis for larval *Coleomegilla maculata* (Degeer) and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). *J. Kansas. Entomol. Soc.* 70: 339–346.

Obrycki, J.J., K.L. Giles and A.M. Ormord, 1998. Interactions between an introduced and indigenous coccinellid species at different prey densities. *Oecologia* 117: 279–285.

Obrycki, J.J. and T.J. Kring, 1998. Predaceous coccinellidae in biological control. *Annu. Rev. Entomol.* 43: 295–321.

Phoofolo, M.W. and J.J. Obrycki, 1995. Comparative life-history studies of nearctic and palearctic populations of *Coccinella septempunctata* (Coleoptera: Coccinellidae). *Environ. Entomol.* 24: 581–587.

Power, M., 1992. Top-down and bottom-up forces in food webs: do plants have primacy? *Ecology* 73: 733–746.

Price, P.W., 1997. *Insect Ecology.* John Wiley & Sons, New York.

Rice, M.E. and G.E. Wilde, 1989. Antibiosis effect of sorghum on the convergent lady beetle (Coleoptera: Coccinellidae), a third-trophic level predator of the greenbug. *J. Econ. Entomol.* 82: 570–573.

SAS Institute, 1996. Version 6.12. SAS Institute, Cary, NC.

Souissi, R. and B. Le Ru, 1997. Effect of host plants on fecundity and development of *Apoanagyrus lopezi*, and endoparasitoid of the cassava mealybug, *Phenacoccus maihoti*. *Entomol. Exp. Appl.* 82: 235–238.

Starks, K.J., R. Muniappan and R.D. Eikenbary, 1972. Interaction between plant resistance and parasitism against the greenbug on barley and sorghum. *Ann. Entomol. Soc. Am.* 65: 650–655.

Sundby, R.A., 1968. Some factors influencing the reproduction and longevity of *Coccinella septempunctata* Linnaeus [(Coleoptera: Coccinellidae)]. *Entomophaga* 13: 197–202.

Thompson, S.N., 1999. Nutrition and culture of entomophagous insects. *Annu. Rev. Entomol.* 44: 561–592.

Thompson, S.N. and K.S. Hagen, 1999. Nutrition of entomophagous insects and other arthropods. In: T.S. Bellows and T.W. Fisher (eds.), *Handbook of Biological Control: Principles and Applications of Biological Control.* Academic Press, San Diego.

van Emden and S.D. Wratten, 1990. Tritrophic interactions involving plants in the biological control of aphids. In: *Proc. Aphid-plant Interactions: Populations to Molecules.* Stillwater, OK. MP-132. pp. 29–44.

Weiser, L.A. and N.E. Stamp, 1998. Combined effects of allelochemicals, prey availability, and supplemental plant material on growth of a generalist insect predator. *Entomol. Exper. Appl.* 87: 181–189.