



Mutualistic and antagonistic trophic interactions in canola: The role of aphids in shaping pest and predator populations



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HIGHLIGHTS

- Complex food webs can influence when and where pests become problematic in cropland.
- Aphids attract mutualistic ants and a suite of predators; these interactions influence the entire arthropod community.
- Aphids, ants, and predator populations were positively correlated with one another.
- The presence of aphids and its associated predatory arthropod community were negatively associated with imported cabbageworms.
- Small aphid populations in cropland may be important for maintaining reduced levels of key crop pests.

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ABSTRACT

Aphids have important effects on the abundance and occurrence of tending ants, predators, and pests in agronomic systems, and DNA-based gut content analysis can aid in establishing predator–prey interactions. The purpose of this study was to determine how the presence of aphids, ants, and pest individuals interact within canola (syn. oilseed rape) fields. Using seasonal data from canola fields, the relationships among ants, aphids and pest individuals were determined, along with the use of PCR techniques in order to amplify aphid DNA and confirm food web links on predators who consume aphids. We determined that aphid presence positively influences the number of ants and predators in a community, and diminishing aphid populations over the growing season were associated with declines in both ants and predators. These reduced populations of predators and aphids may have provided the opportunity for a key pest, *Pieris rapae* to build populations as the season ensued. This research suggests that complex interactions among herbivores and shared predators contribute to pest outbreaks.

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1. Introduction

The green peach aphid (*Myzus persicae* [Sulzer]; Hemiptera: Aphididae) is a worldwide pest that occurs on a wide variety of crops (Desneux and Ramirez-Romero, 2009), including canola (syn. oilseed rape; *Brassica napus* L. Brassicales: Brassicaceae) (Farooq and Tasawar, 2008). Canola plants are most susceptible to damage by aphids during bud formation and through the late flowering stage (Berlandier, 2014). Large aphid infestations in early spring can cause wilting, abortion of flowers, and reduced pod setting in canola, which can result in yield losses (Berlandier, 2014). The increase in canola production in recent years in the

Midwestern states of North America has accompanied an increase of green peach aphid populations (Weiss et al., 2013). Like other aphids, green peach aphids feed on the phloem of their host plants (Mitter, 1958; Lundgren, 2009; Lach et al., 2010; Yao, 2014) that is rich in carbohydrates and poor in other nutrients and amino acids, resulting in the production of sugary honeydew (van Emden et al., 1969; Lach et al., 2010). This honeydew often acts as a food source for many ant species, resulting in the formation of mutualistic interactions.

In return for honeydew, tending ants often move aphids to new plant tissues (Hölldobler and Wilson, 1990; Gonzalez Hernandez et al., 1999; Finlayson et al., 2009), reduce debris buildup causing fewer disease outbreaks (Bach, 1991), and protect the aphids from potential predators (Way, 1963; Stadler and Dixon, 2008). Those aphid colonies that are tended by ants often see an increase in population numbers (Way, 1963; Cushman and Addicott, 1989; Del-Claro and Oliveira, 1993; Fischer and Shingleton, 2001;

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Dibble, 2009) due in part to reduced predation and enhanced feeding (Bristow, 1984; Del-Claro and Oliveira, 2000; Flatt and Weisser, 2000). These mutualistic interactions often have important effects on other species that are present within the shared system and food web.

The imported cabbageworm (*Pieris rapae* L.; Lepidoptera: Pieridae) oviposits and feeds only on those plant species that produce glucosinolates (Hopkins and van Loon, 2001), one of which is canola. Complex interactions of competing herbivores and predators present on potential host plants can alter when and where a female *P. rapae* lays her eggs. Aphid presence on potential host plants does not deter oviposition by these butterflies (Layman and Lundgren, in press), creating scenarios where the two herbivores share individual host plants. Since these two insects often occur on different parts of the plant (Harcourt, 1963) they have minimal contact with one another. Ants and other predators, however can affect both where eggs are laid, and overall survival of offspring. Some butterflies use visual cues to determine if predacious ants are present on potential host plants, and alter their oviposition preference accordingly (Sendoya et al., 2002). The presence of other predacious insects also alter egg laying behavior by changing chemical cues on plants and forcing females to search for enemy free space to ensure survival of their offspring (Gilbert, 1979; Holloway and Herbert, 1979; Price et al., 1980). Many ants are considered generalist predators (Hölldobler and Wilson, 1990) and often consume eggs and larvae of many insect species including those of *P. rapae* (Jones, 1987). Insect eggs are often considered as concentrated forms of nutrients due to their low water content (McNeil, 1971; Lundgren, 2009) making them high quality prey items for ants needed to maintain their colonies (Beattie, 1985). Predators can affect the maternal preference in combination with competitive herbivores by deterring females from laying eggs on those plants through the use of behavioral and pheromone interactions (Atsatt, 1981; Layman and Lundgren, in press).

A large, diverse variety of generalist predators inhabit *Brassica* crops, including canola, and contribute to reducing pests such as aphids and *P. rapae* larva numbers below economically damaging population levels (Gavolski et al., 2011). The main predators include various lady beetle species (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae), and several families of spiders (Araneae). However, additional research on the bioinventory of insects in canola is warranted (Gavolski et al., 2011).

Previous laboratory research has shown that aphids in the presence of a predator can affect pest numbers (M.L.L. unpublished data). In order to determine if these interactions still occur in a field setting, we assessed the insect community of canola along with key predators that aid in reducing aphid population numbers. We also assessed the effects that ants, aphids, and *P. rapae* have on one another with collected population data, along with aphid predation that was occurring in the field. We believe that that aphid populations support ant and predator populations, and in turn these populations aid in suppressing *P. rapae* populations.

2. Materials and methods

2.1. Plants

Prior to the beginning of the experiment canola was planted in 10 blocks (24 × 6 m each) between 3.7 and 18 m apart. Canola seed (an open pollinated line without insecticide, Monsanto Company, St. Louis, MO) was planted at a density of 7.3 kg/ha in these no-till blocks within spring wheat residue with a fertilizer of 70-30-30-20 NPKS (Nitrogen-Phosphorous, Potassium, Sulfur), row spacing of 19 cm and a depth of 2 cm. Blocks were sprayed twice with the herbicide Clethodim (Select Max[®], Valent, Walnut

Creek, CA) at 840 g/ha, and blended with alkyl aryl polyoxylkane ether (Induce[®], Helena Chemical Company, Collierville, TN) at 350 g/ha, and once with the herbicide Clopyralid (Stinger[®], Dow AgroSciences, Indianapolis, IN) at 280 g/ha in order to remove grasses. Each block was then divided into 4 plots (3 × 6 m each) with a 3.6 m canola buffer in between each of the plots. Each plot was then randomly assigned a treatment, in which 6 plants were introduced into each of the 40 plots resulting in 240 plants overall. Experimental canola plants were also sprouted in peat pellets (Jiffy Products, Shippagan NB, Canada), and then transferred to soil mix (4:2:1 parts vermiculite: peat moss: field soil) at the first leaf stage. Half of the plants were grown in an aphid-free greenhouse, while the other half were grown in a greenhouse infested with *M. persicae*. Plants were watered daily, the temperature was 27 °C, and a photoperiod of 16:8 h light: dark (L: D) until the end of the second growth stage (≈12.7 cm tall).

2.2. Insects

M. persicae were reared from an established colony on canola plants within one of the greenhouses. Field ants were surveyed to determine nest density surrounding the experimental blocks by counting ant nests in the four neighboring field margins of alfalfa around the blocks, and also in each of the 10 canola blocks.

2.3. Experimental design

Three treatments were used to determine the role of ants and aphids on within-plant insect community dynamics on canola plants: ants and aphids, ants and no aphids, and no ants and no aphids. We initially planned to use a 2 × 2 factorial design that also included no ants and aphids, but we were unsuccessful in excluding ants from the plants when aphids were present, and so the treatments with and without ants with aphids were combined (following statistical analyses that showed no difference between these two treatments). Aphid-infested plants produced in the greenhouse ($n = 120$) were examined, and the number of aphids per plant was culled to 50 using a vacuum. Half of the un-infested plants ($n = 60$ each treatment) were subjected to the same ant exclusion procedure. Potted plants representing each of the three treatments were then placed in the canola field, plants in this field were 55 d old at the time of insertion of the potted plants on July 8th until August 4th. Each plot was randomly assigned a treatment, and six plants of the same treatment were placed randomly within each plot. A bamboo stake was inserted next to each pot and used to support the developing potted plant. Any plant material touching the pot, plant, or bamboo stake was removed, and the bottom 7.5 cm of the bamboo stake coated with tanglefoot in the ant-excluded treatment.

Starting at 24 h, insect communities on each of the focal canola plants were tabulated. These community assessments were conducted 1, 3, 8, 13, 17, 22, and 27 d after placement; until the surrounding field plants developed pods. During each observation, plants were non-destructively examined and all insects on the plant were collected and stored at −20 °C in 70% ethanol. Aphids in the aphid-infested treatment were allowed to remain on the plants. Following these whole plant counts, each plot was swept 25 times each with a 40.6 cm diameter insect net, and samples were stored at −20 °C in 70% ethanol. Arthropods collected in the whole plant and sweep samples were identified to species level to determine overall diversity within canola. Putative predatory species were advanced for gut content analysis of aphid predation. After the surrounding plants began setting pods, insect sampling ceased and all potted plants were removed from the field, and clipped at the soil level.

2.4. PCR-based gut content analysis of aphid predation

DNA was extracted from whole predators using DNEasy® extraction kits for blood and tissue (product #69506, QIAGEN, Valencia, CA). Samples were macerated in ATL buffer using sterilized plastic pestles, then incubated for 3 h in proteinase K. All procedures followed kit instructions, and the resulting DNA extractions were stored at -20°C .

Forward (5'-TTGATACAAGATTGGGTCACCT-3') and reverse (5'-CCAAATCCTTTATTTCCATG-3') primers that amplify a *M. persicae*-specific DNA sequence were optimized to intensify a 212 base pair region of the COI gene of *M. persicae* based on sequences published by Harper et al. (2005). *Myzus persicae* DNA within the stomachs of predators was amplified using qPCR reactions containing 12.5 μL $2 \times$ Quantitect SYBR Green PCR Master Mix (product #214143, QIAGEN), 1 μL forward primer (225 nm), 1 μL reverse primer (225 nm), 1 μL template, and 9.5 μL sterilized water. Reaction conditions were run on a Stratagene MX3000P thermocycler (Stratagene, La Jolla, CA, USA) with an initial step of 95°C for 15 min, followed by 15 s at 94°C , a second step of 30 s at the annealing temperature of 55°C , 30 s at 72°C , and the third segment of 1 min at 95°C , 30 s at 55°C , and 30 s at 95°C for 40 cycles. Primer specificity was ensured by screening the primers against bleach surface-sterilized legs or wings from 48 non-target specimens (Table S1) collected from the canola fields; no non-target amplification was detected.

Fluorescence was measured at 492 nm at the annealing step of each PCR cycle. The fluorescence was adjusted to bring the base-line correction to normalized levels above background fluorescence for each plate. In each reaction a melt curve was created, and the melting temperature for each positive reaction was measured. The *M. persicae* amplicon melted at approximately 75.5°C , and this diagnostic peak was verified in each positive sample. Negative and positive control series were run with each plate in order to insure accurate results. Each plate contained five wells that were devoted to a positive control series (where 1 μL of *M. persicae* DNA was used as the template) and three wells devoted to a negative or no-template control (where 1 μL of sterilized water was used as template). The eight most abundant predator species collected in the plots were selected (>12 individuals) for DNA extraction and PCRs.

2.5. Data analysis

Statistical analyses and significance tests ($\alpha = 0.05$) were performed using Systat 13 (Systat Software Inc., San Jose, CA), and SigmaPlot 12 (Systat Software Inc., San Jose, CA). The summed number of aphids, ants, and *P. rapae* individuals found collected on the six focal plants were counted for each plot and log transformed. Repeated-measures ANOVA was used to determine if treatment and time affected the abundance patterns of these species; significantly different means were separated with Fisher's LSD test. The effects of time on the density of *P. rapae* and aphids in each plot (pooled across treatments) were described using linear regressions. To describe the seasonal trends in abundances between these two species, the resulting slopes of the lines generated in each plot were compared between these two species using ANOVA. The numbers of aphids, ants, and *P. rapae* individuals per plant were log-transformed, and contrasts in the abundances of each pair of species per plant per plot were generated using linear regression analyses. Species richness (number of species), evenness (J), and diversity (Shannon H) were calculated for the seasonal community in each plot and were compared among treatments using ANOVA.

Predator communities were segregated from the main community database. Sample C_t (concentration thresholds; the

concentration of the target DNA in the sample being tested) values per plot were compared among treatments with ANOVA. The mean proportion of specimens that were positive for aphid DNA per plot were compared among treatments and among predator species using two independent Kruskal Wallis non-parametric ANOVAs, and significantly different means were separated using Conover–Inman test. The frequency of predation and the quantity of aphid DNA (C_t) in predators were compared to the numbers of aphids, ants, and *P. rapae* in each plot using six independent linear regression analyses.

3. Results

3.1. Treatment effects on aphids, ants, and butterfly immatures

Aphid populations per plant were significantly different among treatments ($F_{2,37} = 48.18$, $P < 0.001$), and within treatments over time ($F_{5,10} = 19.84$, $P < 0.001$) (treatment \times time: $F_{5,10} = 15.49$, $P < 0.001$) (Fig. 1). As expected, aphids were significantly more abundant on the treatment where aphid populations were not removed weekly with seasonal sum means (SEM) values of 122.13 ± 0.27 , 0.13 ± 0.05 , and 0.29 ± 0.11 aphids per plant for the treatments ants and aphids, ants and no aphids, and no ants and no aphids respectively (Fig. 1). The number of ants per plant was significantly different among treatments ($F_{2,37} = 10.44$, $P < 0.001$), and within treatments over time ($F_{5,10} = 2.49$, $P = 0.03$) (treatment \times time: $F_{5,10} = 3.39$, $P < 0.001$). Specifically, the treatment that had aphids on the plants had a higher number of ants over the season compared to the aphid-free treatments (Fig. 1). Seasonal sum means were calculated as 0.14 ± 0.01 , 0.07 ± 0.02 , and 0.03 ± 0.01 ants per plant for the treatments with ants and aphids, ants and no aphids, and no ants and no aphids respectively. Finally, treatments ($F_{2,37} = 24.82$, $P < 0.001$) and time ($F_{5,10} = 6.59$, $P < 0.001$) (treatment \times time: $F_{5,10} = 8.15$, $P < 0.001$) affected the number of *P. rapae* individuals found per plant (Fig. 2). The *P. rapae* populations were composed of 96% eggs and 4% larvae. The seasonal trends in the number of aphids and *P. rapae* per plant were found to have significantly different slopes (pooled across treatments) ($F_{1,78} = 27.06$, $P < 0.001$); population trends over time were opposite one another for these two taxa.

Regression analysis found that the number of ants per plant was significantly predicted the number of aphids per plant (pooled across treatments) ($r^2 = 0.42$), and that the number of aphids per plant significantly predicted the number of *P. rapae* individuals per plant ($r^2 = 0.18$), and the number of ants and number of *P. rapae* per plant ($r^2 = 0.24$) (Fig. 2). Regression coefficients were positive for aphids and ants, and negative for the other two interactions.

3.2. Effects of insect community structure and predators on aphid populations

A total of 22,960 invertebrates representing 120 taxa were collected over the 2 month sampling period. Of these specimens, 5785 were collected in sweep samples, and our community descriptions were based upon this community. Twenty-one taxa (18% of taxa) represented 90% of the total specimens collected in sweeps. Of these, 10 species were in the Order Diptera. The mean diversity (Shannon H), evenness (J), and species richness per plot were 2.76 ± 0.03 , 0.82 ± 0.01 , and 29.25 ± 0.77 respectively. There were no significant differences among treatments in diversity ($F_{2,37} = 0.19$, $P = 0.83$), evenness ($F_{2,37} = 0.89$, $P = 0.42$), or richness ($F_{2,37} = 1.65$, $P = 0.21$).

The mean predator abundances varied across the growing season (Fig. 3). To best apply limited resources, we restricted gut analyses to the most abundant predator taxa collected in sweep

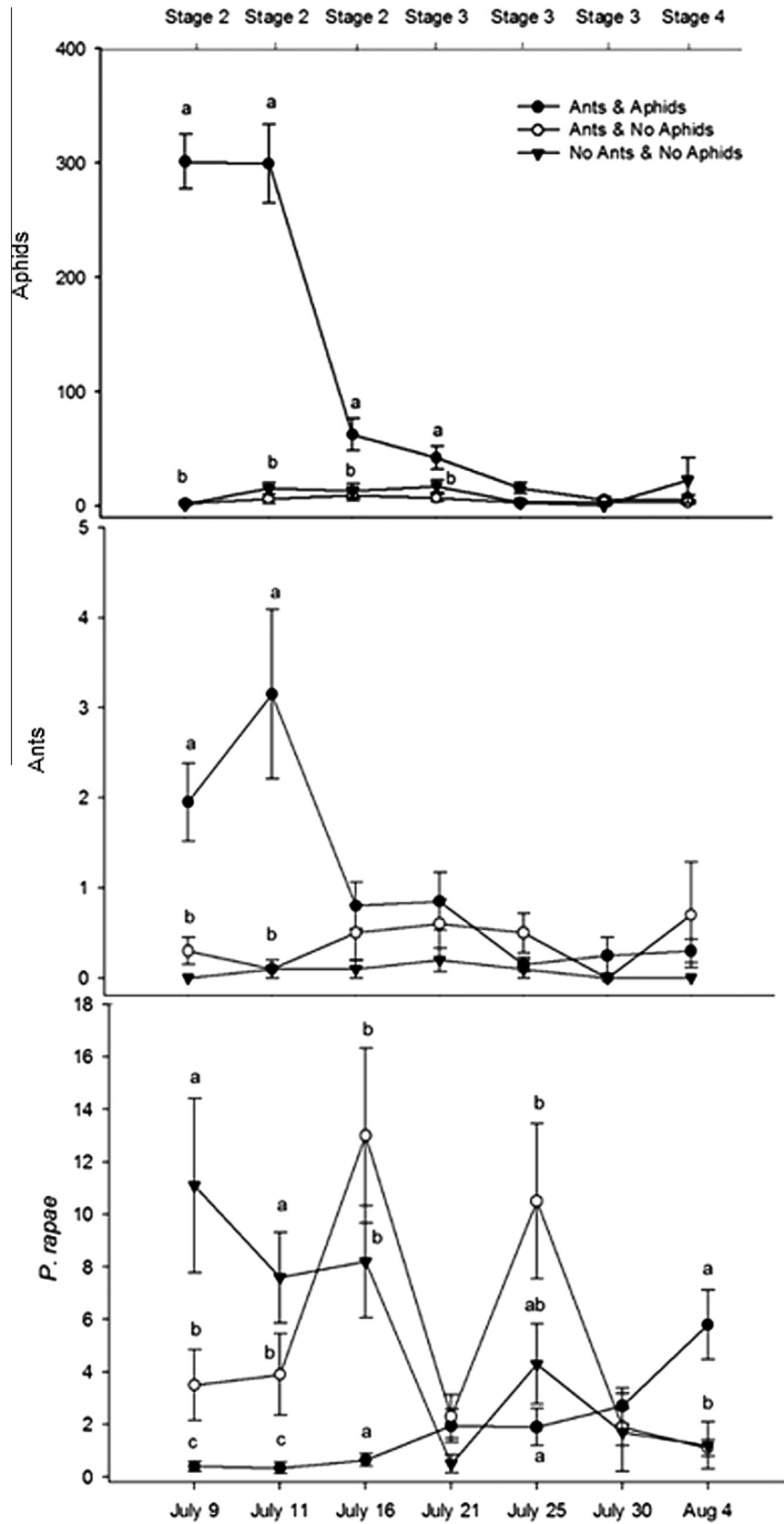


Fig. 1. The mean number of aphids, ants, and *P. rapae* individuals by date and treatment. Prior to the introduction into the field, canola host plants were inoculated with one of three treatments, ants and aphids (in which aphids were infested onto each of six canola plants [$n = 50$] and ants were allowed onto the plants), ants and no aphids, and no ants and no aphids in which no aphids were infested onto the plant and Tanglefoot® was applied to restrict ant movement onto the plant in the field. Each line represents a different elongation treatment with means \pm SEM at each date across the season. Stages listed across the top of the graph correlate with canola growth stages. Growth stage 2 is that of stem elongation, stage 3 is the development of the flower bud, and stage 4 is flowering. Points on the line topped with different letters are significantly different from one another for that date ($\alpha = 0.05$).

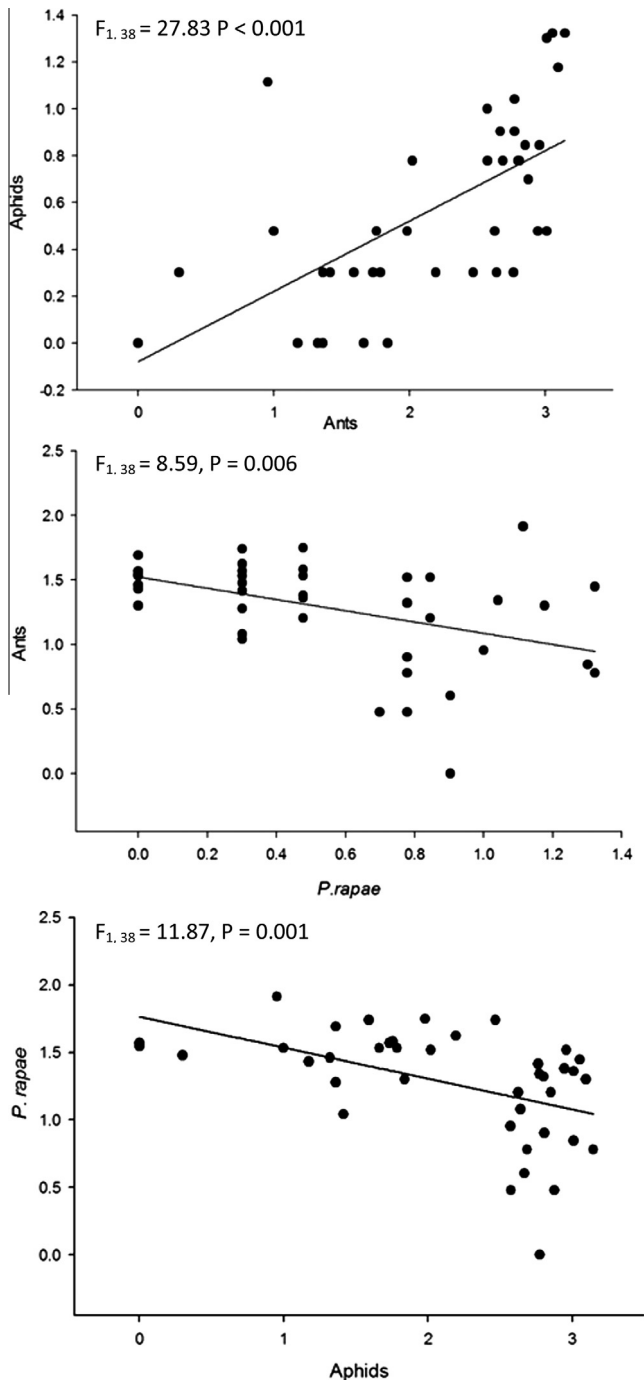


Fig. 2. Comparison of ant, aphid, and *P. rapae* individual presence on one another. A linear regression was completed to determine the strength of the relationship between the numbers of aphids found in the field in comparison to the number of ants and *P. rapae* found. Along with comparisons of the number of ants found and the number of *P. rapae* found.

samples and whole plant counts. Of these 826 predator specimens, more than 80% of specimens were represented by the predatory genera *Lasius* (25%), *Formica* (22%), *Tetragnatha* (16.8%), *Chrysoperla* larvae (9.4%), *Orius* (2.8%), *Mecaphesia* (2.1%), *Misumenops* (2.3%), and *Chrysoperla* adults (1.8%) (number of specimens tested are listed in Table 1). Predators ($24.64 \pm 2.18\%$ per plot) contained green peach aphid DNA in their stomachs, and there was no effect of treatment on the proportion of predators per plot with aphid remains in their stomachs (Kruskal–Wallis test

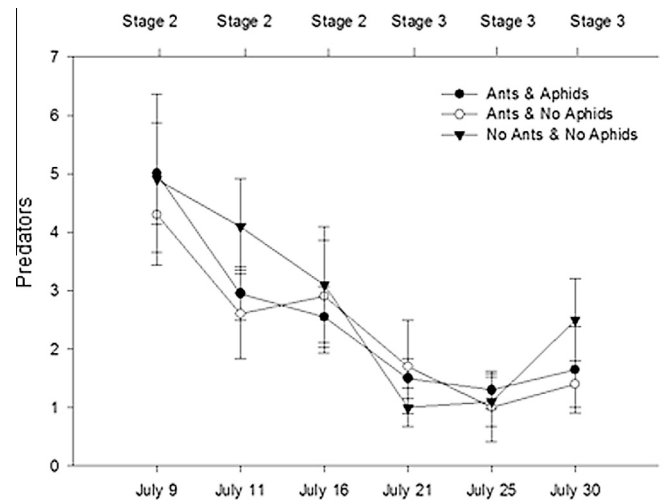


Fig. 3. Mean predator number across the growing season by treatment. Field sweeps were conducted in sample plots throughout the growing season and the number of predators found were counted for each treatment. Stages listed across the top of the graph correlate with canola growth stages. Growth stage 2 is that of stem elongation, and stage 3 is the development of the flower bud. Each dot on the graphed line represents the mean number of predators \pm SEM at each date across the season.

statistic = 0.75, $df = 2$, $P = 0.69$). Similarly, the amount of aphid DNA in the predator stomachs (C_t value) per plot was similar in all three treatments ($F_{2,37} = 4.72$, $P = 0.63$). The five predator species tested had similar quantities of aphid DNA in their guts (C_t values) (Table 1), and all but one species were unaffected by treatment. *Chrysoperla* sp. adults had significantly more aphid DNA in their stomachs when they were collected from the treatment with ants and no aphids; when collected from the treatment with ants and aphids there was an intermediate C_t value (Table 1). Predator species consumed aphids at significantly different frequencies (Kruskal–Wallis test statistic = 20.32, $df = 7$, $P = 0.005$) (Table 1). *Chrysoperla* sp. adults (Table 2) had a significantly higher frequency of predation than all other predators except *Formica*; *Chrysoperla* larvae and *Mecaphesia* had significantly lower frequencies of predation than *Chrysoperla* adults and the ant species (Table 2). Regression analysis found that the mean amount of aphid DNA in the predator stomachs (C_t) per plot was predictive of the number of ants ($r^2 = 0.35$; $F_{1,38} = 5.29$, $P = 0.03$) and aphids ($r^2 = 0.34$; $F_{1,38} = 4.94$, $P = 0.03$) per plant per plot, but was not predictive of *P. rapae* individuals ($r^2 = 0.06$; $F_{1,38} = 0.13$, $P = 0.73$). More specifically, increasing levels of aphid DNA in the predator guts were positively associated with increasing numbers of aphids (slope = 1.26) and ants (slope = 0.02) in a plot. Regression analysis revealed that the proportion of predators positive was not related to the number of ants ($r^2 = 0.17$; $F_{1,38} = 1.17$, $P = 0.29$), aphids ($r^2 = 0.02$; $F_{1,38} = 0.01$, $P = 0.92$), or *P. rapae* individuals ($r^2 = 0.18$; $F_{1,38} = 1.21$, $P = 0.28$) per plot.

4. Discussion

Aphids, predators, and ants were positively related with each other, and were negatively related with populations of *P. rapae*. These trends could have been driven by the interactions among species which drove the numerical patterns that we observed, or by climatic shifts throughout the growing season. Specifically, our data is in line with the hypothesis that aphids may have supported ants and predator populations, and these in turn suppress *P. rapae* populations.

Table 1

The role of aphid and ant infestations on predation frequencies and quantity of aphid DNA found in the guts of abundant predators. The mean and SEM C_t values, and proportion of positives for aphid DNA for each insect that tested positive by number of samples and by treatment, along with the proportion of insects for each genus that were found to be positive. Different letters within a column indicate significant difference from one another ($\alpha = 0.05$).

Treatment	<i>Orius insidiosus</i>	<i>Chrysoperla</i> adult	<i>Chrysoperla</i> larva	<i>Formica</i>	<i>Lasius</i>	<i>Tetragnatha</i>	<i>Misumenops</i>	<i>Mechapesia</i>	
<i>Mean C_t</i>									
Ants and aphids	37.32 ± 1.76	29.99 ± 2.9 b	37.35 ± 0.49	36.35 ± 0.45	38.75 ± 1.56	35.94 ± 0.55	37.68 ± 0	37.77 ± 0	
Ants and no aphids	36.72 ± 0.3	41.0 ± 5.88 a	36.02 ± 0.53	40.76 ± 1.35	36.09 ± 0.47	36.66 ± 0.35	0	38.89 ± 0	
No ants and no aphids	37.77 ± 0	23.45 ± 1.5 c	32.81 ± 1.72	35.71 ± 0.38	34.58 ± 0.4	37.11 ± 0.42	37.35 ± 1.52	0	
Statistics	$F_{(2,3)} = 0.03$, $P = 0.97$	$F_{(2,7)} = 1.41$ $P = 0.03$	$F_{(2,7)} = 2.07$, $P = 0.19$	$F_{(2,37)} = 1.54$, $P = 0.23$	$F_{(2,48)} = 1.09$, $P = 1.09$	$F_{(2,33)} = 0.64$, $P = 0.54$	$F_{(1,2)} = 0.01$, $P = 0.93$		
<i>Proportion positive</i>									
Ants and aphids	0.25 ± 0.13	0.5 ± 0.29 c	0.11 ± 0.25	0.25 ± 0.07	0.29 ± 0.07	0.2 ± 0.07	0.01 ± 0.01	0.2 ± 0.02	
Ants and no aphids	0.3 ± 0.2	0.63 ± 0.23 b	0.15 ± 0.08	0.15 ± 0.07	0.25 ± 0.11	0.22 ± 0.08	0	0.01 ± 0.01	
No ants and no aphids	0.1 ± 0.08	0.89 ± 0.11 a	0.19 ± 0.12	0.29 ± 0.11	0.14 ± 0.07	0.27 ± 0.07	0.3 ± 0.02	0	
Statistics	$F_{(2,14)} = 0.42$, $P = 0.66$	$F_{(2,8)} = 1.6$, $P = 0.05$	$F_{(2,29)} = 0.24$, $P = 0.79$	$F_{(2,32)} = 0.5$, $P = 0.61$	$F_{(2,34)} = 0.74$, $P = 0.49$	$F_{(2,32)} = 0.26$, $P = 0.78$	$F_{(2,13)} = 0.89$, $P = 0.43$	$F_{(2,7)} = 0.32$, $P = 0.74$	
C_t	37.19 ± 1.13	30.03 ± 4.01	35.91 ± 0.9	37.09 ± 0.8	37.27 ± 1.24	36.54 ± 0.45	38.33 ± 0.56	37.44 ± 1.25	Statistics $F_{7, 152} = 1.52$, $P = 0.16$
Proportion positive n (total N)	0.23 ± 0.09 a 0.09 ± 0.02 (24)	0.65 ± 0.14 b 0.06 ± 0.02 (17)	0.14 ± 0.04 a 0.29 ± 0.04 (72)	0.23 ± 0.05 a 0.73 ± 0.09 (183)	0.24 ± 0.05 a 0.45 ± 0.07 (212)	0.23 ± 0.04 0.58 ± 0.06 (145)	0.10 ± 0.07 a 0.08 ± 0.02 (18)	0.16 ± 0.09 a 0.07 ± 0.02 (22)	Statistics $F_{4, 122} = 4.12$, $P < 0.001$

Table 2

Frequencies of aphid predation by dominant predators in canola fields. Frequency of predation per canola plot was measured using gut content analysis. Means followed by different letters are significantly different from one another (Conover–Inman test; $\alpha = 0.05$).

Predator species	Frequency of predation; mean \pm SEM per plot (n)
<i>Chrysoperla</i> adult	0.65 \pm 0.14 (11) A
<i>Formica</i>	0.26 \pm 0.06 (26) AB
<i>Lasius</i>	0.21 \pm 0.04 (28) B
<i>Misomenops</i>	0.19 \pm 0.09 (15) BC
<i>Tetragnatha</i>	0.18 \pm 0.05 (28) BC
<i>Orius</i>	0.17 \pm 0.08 (14) BC
<i>Chrysoperla</i> larvae	0.15 \pm 0.03 (27) C
<i>Mecaphesia</i>	0.09 \pm 0.06 (11) C

Aphids and their honeydew are often food sources for many natural enemies and predators (van Emden et al., 1969). Ants in this study had the highest numbers on those treatments that had aphids associated with them (Fig. 1), and the number of ants was strongly associated with the number of aphids (Fig. 2). Ants often optimize aphid populations as both a source of sugar through the excreted honeydew (Mitter, 1958; Carroll and Janzen, 1973; Tschinkel, 2006; Lundgren, 2009; Lach et al., 2010; Yao, 2014), and food when prey becomes scarce (Way, 1954; Pontin, 1958; Cushman, 1991; Carabali-Banguero et al., 2013). Ant and predator densities were highest early on in the season when more food available in the form of aphids and the excreted honeydew. However, when the aphid population crashed, both the ant and predator densities also declined. Note that aphid and ant populations were only monitored on focal plants, and predators were collected throughout the plots. Our general perception is that ambient aphid populations throughout the plots likely followed the same demographic patterns as those on the aphid-infested plants; this would explain why predator numbers in all plots declined over the season at similar rates in the treatments where aphids were included and eliminated on the focal plants.

Assuming that aphids are a main food resource for both ants and predators, they could have over-used the aphid population causing this decline. Ants, aphid, and predator populations were never eliminated from the system; instead, low levels of activity were found throughout the remainder of the growing season (Fig. 1). As seen in the laboratory, ants will maintain a minimal population of aphids to accommodate the sucrose needs of the colony (M.L. unpublished data) after brood production. In contrast, if the ants' need for sugar resources or their foraging behavior were lessened, they may have reduced their attendance on the aphid colony, thereby exposing the aphids to predators.

These demographic trends could also have been caused by seasonal climate shifts, morphological or physiological changes within the host plant that had common effects on the insect community (and opposite effects on *P. rapae*). Climate is a major factor in insect activity, and many insects synchronize their life cycles in response to climatic cues during their life cycle (Parry, 1992). For many species, an increase in temperature results in increased metabolic rates (van Baaren et al., 2010). Increasing temperatures often allow for higher growth rates and reproduction in herbivorous insects (Bale et al., 2002); aphid populations, however, do not perform as well in high temperatures, instead have been seen to have quicker growth rates at temperatures below 90° (Tillman, 2007). This same trend is seen in predators, namely ants (Hölldobler and Wilson, 1990; Ruano et al., 2000). Using temperature data gathered from the weather station at the North Central Agricultural Research Center where the research took place, the average temperature for the seven sampling dates was calculated to be 26.43 °C, with an average relative humidity of 95%. The combination of the high temperature and high relative humidity could reduce the aphid population growth and ant foraging rates and

coupled with predation could be responsible for the population decline seen.

Seasonal progressions also change plant physiology and morphology, which could in turn affect the resident insect community associated with a plant (Kennedy, 1958; van Emden et al., 1969). Climate affects a plant's physiology (Prentice et al., 1992), as the season progresses environmental cues induce hormones that affect plant growth and development and tissue differentiation (Davies, 1995). Physiological changes can also occur with changes in seasonal availability of water and nutrient levels, creating surplus or stress issues that results in the altering of plant morphology (e.g., leaf thickness), along with changes in key nutrient levels required for growth (Peñuelas et al., 1994). Chlorophyll levels within leaves can be in these times of nutritional stress and can affect photosynthetic rates which results in slower growth and late blooming flowers (Peñuelas et al., 1994). These physiological changes can affect both abundance of insects, and their interactions with one another. Declines in aphids, ants, and predators could have been in response to the host changes as the season progressed (Figs. 1 and 3), however this was not tested.

Aphids were readily consumed by a variety of predators in canola, but this predation rate was unaffected by diminishing aphid densities as the season went on. The number of predators found per plot across the growing season continually decreased throughout the season (Fig. 3). It is possible that as the number of aphid prey decreased, predators dispersed to neighboring habitats with additional food resources. Regardless, higher numbers of aphids (and their associated ants) within a plot were associated with more aphid DNA found within the predator guts from that plot. Although all predators tested positive for aphid DNA, the frequency of predation was found to be significantly higher for one predator, adult *Chrysoperla*, versus nearly all of the other species tested (Table 2). *Chrysoperla* adults are regarded as primarily glucophagous (McEwen et al., 2001), feeding on pollen, nectar, and aphid honeydew (Tauber and Tauber, 1983). Even if these predators weren't consuming aphids directly, animal fecal material often contains DNA of its food (Klaassen et al., 2003; Marrero et al., 2009; Vincent et al., 2014), and honeydew consumption may explain the high detection rate in *Chrysoperla* gut samples. Regardless of their specific biologies, we found that all predator species tested had consumed *M. persicae* within canola fields, suggesting the important role of aphids in attracting predators into this system.

As the other insects in the system (predators, ants, and aphids) diminished, the number of *P. rapae* individuals found increased (Figs. 1 and 2). This pattern could have been driven by the phenology of the pest or by the removal of competitive barriers later in the season. *P. rapae* populations often increase over the season, peaking in mid- to late-August (Lundgren et al., 2002; Stefanescu, 1997, 2000). But the increase in *P. rapae* populations as the season progressed was only clearly evident in the treatment with aphids (and ants); in the treatments without aphids, the population levels of *P. rapae* remained constant (Fig. 2). This suggests that biological interactions on these plants were partially responsible for *P. rapae* population increases after aphids diminished. Aphids could affect *P. rapae* populations either through direct competition or through attracting predators of *P. rapae* eggs. Female *P. rapae* do not avoid aphids when making oviposition decisions (Layman and Lundgren, in press), which reduces the likelihood that aphids are influencing *P. rapae* through competition. But predators certainly influence the populations of *P. rapae* (Price et al., 1980; Bernays and Graham, 1988; Wiklund and Friberg, 2008; Layman and Lundgren, in press), under controlled conditions both ants and coccinellids consume large numbers of *P. rapae* eggs, and this predation is enhanced when aphids are present under laboratory conditions (M.L. unpublished data). The mutualistic interaction between the ants and aphids, or the increased foraging by other

predators induced by plant volatiles early in the season may have affected the oviposition rate or egg survival of the *P. rapae*. In this way, sustaining lower levels of aphids within cropland may be important in suppressing key pests like *P. rapae* through apparent competition with shared predators, and through their mutualistic interactions with ants.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biocontrol.2015.07.008>.

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