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**Increasing Trophic Complexity Influences Aphid Attendance by
Ants (Hymenoptera: Formicidae) and Predation¹**

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Increasing Trophic Complexity Influences Aphid Attendance by Ants (Hymenoptera: Formicidae) and Predation¹

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Abstract Species that are involved in multitrophic interactions are affected by the trophic levels that are above and below them in both indirect and direct ways. In this experiment, interactions among ants (*Formica montana* Wheeler; Hymenoptera: Formicidae), aphids (*Myzus persicae* [Sulzer]; Hemiptera: Aphididae), lepidopteran eggs (*Pieris rapae* [L.]; Lepidoptera: Pieridae), and lady beetles (*Coleomegilla maculata* DeGeer; Coleoptera: Coccinellidae) were examined on canola (*Brassica napus* L.; Brassicales: Brassicaceae) to determine whether aphids mediate interactions between ants and eggs and if aphid-tending ants will protect these species from predation by the lady beetles. The first trial examined how ant attendance affected aphid and egg survival over 72 h when these herbivores were present on their own or in combination. The second trial examined these same interactions when a lady beetle was included in the system. Ants consumed significantly more aphids when this prey was offered alone than when eggs were offered alongside. When lady beetles were present, ants did not consume aphids. Ants and lady beetles consumed nearly all of the eggs regardless of whether aphids were present or not. When a lady beetle was absent, ant attendance increased significantly, but only when eggs and aphids were present in the system together. Ants visited the aphids less frequently when a lady beetle was present in the system, possibly due to increased competition. This trend was consistent over time in all treatments. All of this suggests that ants can be an important source of pest management of other herbivores (and even of the aphids that they are attending) but that these interactions are mediated by whether an additional predator is present within the system.

Key words Coccinellidae, *Coleomegilla maculata*, food web, *Formica montana*, honeydew, *Myzus persicae*, *Pieris rapae*

Species are intertwined within complex interaction networks, reacting to and with a variety of plants, other insects, and the environment, making individual species responses nearly impossible to predict (Ives and Cardinale 2004). Organisms interact in at least three essential ways: competition, predation, and mutualism (Boucher et al. 1982). These interactions are important within natural communities and aid in regulating the abundance of insect populations (Bronstein 1998). The ability to predict when and where a particular insect will become abundant is often necessary, particularly in the framework of pest management.

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The aphid–ant mutualisms are a well-described relationship that has important ripple effects on other species within a food web such as increased competition for both food and habitat resources (Bronstein 1998). Aphids consume phloem contents from the host plant and excrete a sugar solution known as honeydew that is eaten by ants (Lundgren 2009; Mitter 1958; Yao 2014). Mutualisms between ants and aphids (and other homopterans) can be inconsistent (Bristow 1991) and, in part, are influenced by the relative resources present in the habitat (Carabali-Banguero et al. 2013), the species involved (Stadler and Dixon 2008), and the physiological status of the ant colony (Stadler and Dixon 2008). In return for sugar meals, tending ants often move aphids to optimal feeding positions on the plant or in response to changing environmental conditions, and even will clean aphid colonies of debris to reduce fungal growth (Finlayson et al. 2009; Gonzalez Hernandez et al. 1999; Hölldobler and Wilson 1990). The ants also protect the aphids from predators, and this protection has important implications for other species in the food web (Stadler and Dixon 2008). Specifically, ants influence herbivory rates on a plant by causing the aphid population to increase and through aggressive behavior toward other herbivores in the system (Cushman and Addicott 1989; Del-Claro and Oliveira 1993; Dibble 2009; Fischer and Shingleton 2001; Way 1963). By removing predators from species networks containing aphids, ants may indirectly affect aphid and other herbivore populations.

Lady beetles and aphid-tending ants often encounter one another as they search for a shared food source (Majerus et al. 2007). These interactions can be competitive or noncompetitive, depending on whether lady beetles attack the aphid population that is being tended by ants (Majerus et al. 2007). Typically, lady beetles prefer aphid populations that are not tended by ants in order to avoid competition (Majerus et al. 2007). If lady beetles choose to attack, they are usually chased away by ants while larvae are typically pushed off the plant or killed (Majerus et al. 2007). Lady beetles often assist in limiting the population growth of aphids, and not only cause increased mortality rates of aphids but also can induce avoidance behaviors which result in decreased feeding and reproduction (Nelson et al. 2004; Ness et al. 2009; Obrycki et al. 2009). Aphid-tending ants exhibit “ownership behavior” and become more aggressive toward lady beetles near where the tended aphids are located (Majerus et al. 2007; Way 1963).

The green peach aphid (*Myzus persicae* [Sulzer]; Hemiptera: Aphididae) is often tended by ants for the honeydew that they produce, including the ants of the *Formica montana* Wheeler (Hymenoptera: Formicidae) species (Henderson and Jeanne 1991). The ants occur on a variety of crops including the oilseed crop canola (*Brassica napus* L.) (Desneux and Romero 2009). Canola contains the chemical compound glucosinolate, which is a secondary metabolite and acts as a stimulant for both oviposition and feeding in more than 25 insect species (Hopkins et al. 2009). One of these is a primary pest of *Brassica* crops, the imported cabbageworm (*Pieris rapae* [L.]; Lepidoptera: Pieridae). Eggs laid by the adult female butterfly are frequently eaten by natural enemies such as lady beetles (Evans 2009) and other predators including a variety of ant species (Ashby 1974, Pfannestiel and Yeorgan 2002). Together these insects (aphids, ants, lady beetles, and lepidopteran larvae) interact with one another, along with the plant, to form complex, multitrophic interactions. To help understand these interactions, we addressed the general hypotheses that aphids mediate interactions between

imported cabbage worm eggs and ants, and that ants tending aphids protect the aphids and cabbageworm eggs from predation by lady beetles.

Materials and Methods

Plants. Experimental canola plants were sprouted in peat pellets (Jiffy Products, Shippagen, NB, Canada) and then transferred to a soil mix (4:2:1 parts vermiculite:peat moss:field soil) at the first leaf stage. Plants were watered daily, the temperature was 27°C, and the photoperiod was 16L:8D h. Plants were grown in a greenhouse until the end of the second leaf stage (\approx 12.7 cm), when they were transferred to the experiment.

Insects. A population of imported cabbageworm was produced from eggs that were obtained from Carolina Biological (Burlington, NC, USA) and reared from egg to adulthood on cabbage (var. 'Copenhagen Market Early,' *Brassica oleracea* L., Brassicales: Brassicaceae), canola (*B. napus*), and black rapeseed (*Brassica carinata*, A. Braun, Brassicales: Brassicaceae). Eggs laid on canola plants were separated from the oviposition plant using a damp paint brush at the time of the experimental trials. *Myzus persicae* were reared continuously on russet potato plants (\approx 38–50 cm in height) in the laboratory for 24–68 d prior to the treatment. Care was taken to ensure that similar-sized reproducing aphid adults were used when infesting treatments.

Nine *F. montana* ant colonies were collected from field margins at the USDA-ARS Research Farm in Brookings, SD (N 44.3064, W 96.7881) (voucher specimens left at the South Dakota State University Insect Museum). Each colony was housed in a separate 64 × 43 × 38-cm tub with a coating of Insect-a-slip-Fluon (2871C BioQuip®, Rancho Dominguez, CA, USA) along the top quarter of each tub to prevent ant escape. Each colony differed in the amount of soil in their respective tubs due to the size of the ant colony collected. Each colony contained 2 cotton water wicks in glass vials, tuna, and honey, which were changed every 48 h but then removed 24 h prior to the beginning of granting ants access onto the experimental plants. *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) were reared continuously for 6–12 mo on honey, water, and Lundgren's Super C Mac diet (Lundgren et al. 2011). Adult females (no males were used) used in the experiments were no older than 4 mo and were separated and starved 24 h prior to introduction on the experimental plants.

Experimental design. The two experimental trials were conducted in a climate-controlled room with a 16L:8D-h photoperiod at 27°C and approximately 30% relative humidity. Each of the two experiments compared four treatments arranged in an incomplete factorial design. Over the duration of the experiments, each ant colony was exposed in a random order to each treatment for a single observation ($n = 9$ observations per treatment). Care was taken with prey placement in order to distribute the prey evenly across all plant structures and avoid grouping of any one prey type. Each experiment lasted 3 d and began (depending on treatment) 24 h after either aphid infestation, egg infestation, or ant access. Each treatment was timed accordingly so they would begin and end concurrently. In the first experiment we examined the interactions between ants, aphids, and butterfly eggs. In the second experiment we examined whether lady beetles alter these interactions.

Experiment 1. We tested the hypothesis that ants affect two different herbivores (butterfly eggs or aphids) by monitoring the behavior of ants and the survival of aphids and butterfly eggs. The first treatment examined interactions between aphids and *P. rapae* eggs. Aphids and *P. rapae* eggs were placed onto a caged, potted canola plant. Each plant was enclosed in a 13.3 (diameter) × 34.3-cm (tall) plastic tube. The top of each tube was covered with mesh to prevent aphid escape, and the 2.5-cm hole drilled in the side of each cage was closed with a rubber stopper. The enclosed canola was infested with 50 aphids (previously weighed) for 24 h. After the aphids had established for 14 h, 40 *P. rapae* eggs were attached to the canola leaves with water and a fine paint brush, taking care that no eggs fell from the experimental plants during infestation. The eggs and aphids remained on the plant for 24 h. At the end of each trial, aphids were counted and weighed and eggs were counted.

The second treatment examined ant–aphid interactions. Aphids ($n = 50$) were placed on each potted canola plant for 24 h as described above. The entire caged plant was placed inside a plastic cage with a 2.5-cm hole drilled into the side, which was covered during aphid establishment. After the 24 h period, a 0.75-m bamboo stick (0.64-cm diameter) was placed between an ant colony and the caged aphids in such a way that the bridge only touched the plant and the soil in the ant colony. Ants were not allowed to visit the experimental plants prior to aphid infestation because aphids induce plant volatiles when they feed, which ants use to locate aphids. Counts of ants on the bridge and on the experimental plant were regularly recorded for 10 h on the first 3 d of the assay (specifically, we recorded ant attendance at 10:00 a.m. and at 12:00, 2:00, 4:00, and 8:00 p.m. each day. The number and weight of aphids were recorded at the end of the experiment.

The third treatment examined interactions between ants and *P. rapae* eggs. Canola plants were placed into the experimental cages described in the ant + aphid treatment for 24 h (except no aphids were added); at this time, the area between the ant colonies and the experimental cages were bridged with a bamboo stick for 24 h to allow ants to begin foraging on the experimental plants. Because *P. rapae* eggs are not known to have any volatile chemicals that induce foraging, ants were allowed to visit the experimental plants prior to egg introduction in order to deposit their chemical communication trails to stimulate foraging after eggs were added. After the ant-acclimation period, 40 *P. rapae* eggs (24–48 h old) were placed onto each canola plant with water and a fine paint brush. After infestation with *P. rapae* eggs, counts of active ants began at the aforementioned regular intervals for 3 d on the experimental plants and bridge. The ants that had colonized the arena by the final morning were counted. The number of eggs remaining on the plants in each cage at the end of the experiment was then recorded.

The final treatment examined the interaction between all three taxa (ants, aphids, and *P. rapae* eggs). Aphids ($n = 50$ per plant) were weighed and allowed to acclimate to a canola plant for 24 h. Next, the ant colony and a caged canola plant were bridged, and ants were allowed to forage on canola for 24 h. Following the ant acclimation period, 40 *P. rapae* eggs were placed onto each plant with water and a fine paint brush. After infestation of *P. rapae* eggs, counts of active ants began at regular intervals for 3 d. At the end of the trial, aphids were counted and weighed and eggs were counted.

Experiment 2. We tested two hypotheses. First, we hypothesized that adding an additional herbivore (butterfly eggs) to an aphid–ant mutualism would have no effect on the behavior of ants and the survival of aphids and butterfly eggs in the presence of an additional predator (lady beetle). Next, we hypothesized that adding a predator (lady beetle) would not alter the patterns in ant behavior and survival of aphids and butterfly eggs observed in the absence of the predator. The same four treatments as described above in Experiment 1 were repeated in Experiment 2, the difference being that a single female lady beetle was added to each cage directly before the counts occurred. Each trial followed the same format and lasted 3 d with observations at the same intervals as described above, 10 d after the completion of Experiment 1. At the end of each trial, aphids were counted and weighed and eggs were counted.

Data analysis. Statistical analyses and significance tests ($\alpha = 0.05$; we considered $\alpha = 0.07$ to be marginally significant) were performed with Systat 13 (Systat Software Inc., San Jose, CA). We compared the mean aphid weights, numbers of aphids remaining, and numbers of eggs remaining in each treatment using independent, nonparametric Kruskal-Wallis analysis of variance (ANOVA). Summed ant attendances per day were compared among the treatments using a Kruskal-Wallis nonparametric ANOVA. Significant differences among treatment means were separated using the Dwass-Steel-Christchlow-Fligner test, which is a multiple comparison based on pairwise rankings. Treatments with and without lady beetles were analyzed separately. The rate of ant attendance over time was examined independently for each treatment by examining the slope of the line fitted to the data. The number of ants in the arena per time period (15 periods from 5 times/d for 3 d) was used to calculate independent regression lines for each colony. The resulting averages of the nine slopes per treatment (one for each colony) were evaluated to determine whether the slopes were significantly different from zero using a one sample *t* test.

Results

Herbivore performance without lady beetles. Aphid weight and survival were significantly affected by the treatments without lady beetles. There were significant differences among the treatments in the number of aphids remaining at the end of the experiment (Kruskal-Wallis $\chi^2_2 = 8.17$, $P = 0.02$). Adding ants to the system reduced the number of aphids significantly as compared to the treatment without ants (Dwass-Steel-Christchlow-Fligner test statistic = 2.48, $P = 0.008$) (Fig. 1A). Adding the *P. rapae* eggs as a second herbivore into the system significantly reduced the predation of aphids that occurred by the ants versus when the aphids were the sole herbivore in the system (Dwass-Steel-Christchlow-Fligner test statistic = 5.41, $P < 0.001$). There were significant differences among the treatments in the aphid weights at the end of the experiment (Kruskal-Wallis $\chi^2_2 = 8.17$, $P = 0.02$). The mean (\pm SEM) aphid weight was 0.01 ± 0.01 , -0.005 ± 0.002 , and -0.003 ± 0.001 g for the treatments aphids and eggs; ants and aphids; and ants, aphids, and eggs, respectively. Pairwise comparisons between treatments revealed that aphid weight change was significantly different from the ant-free treatment versus from the

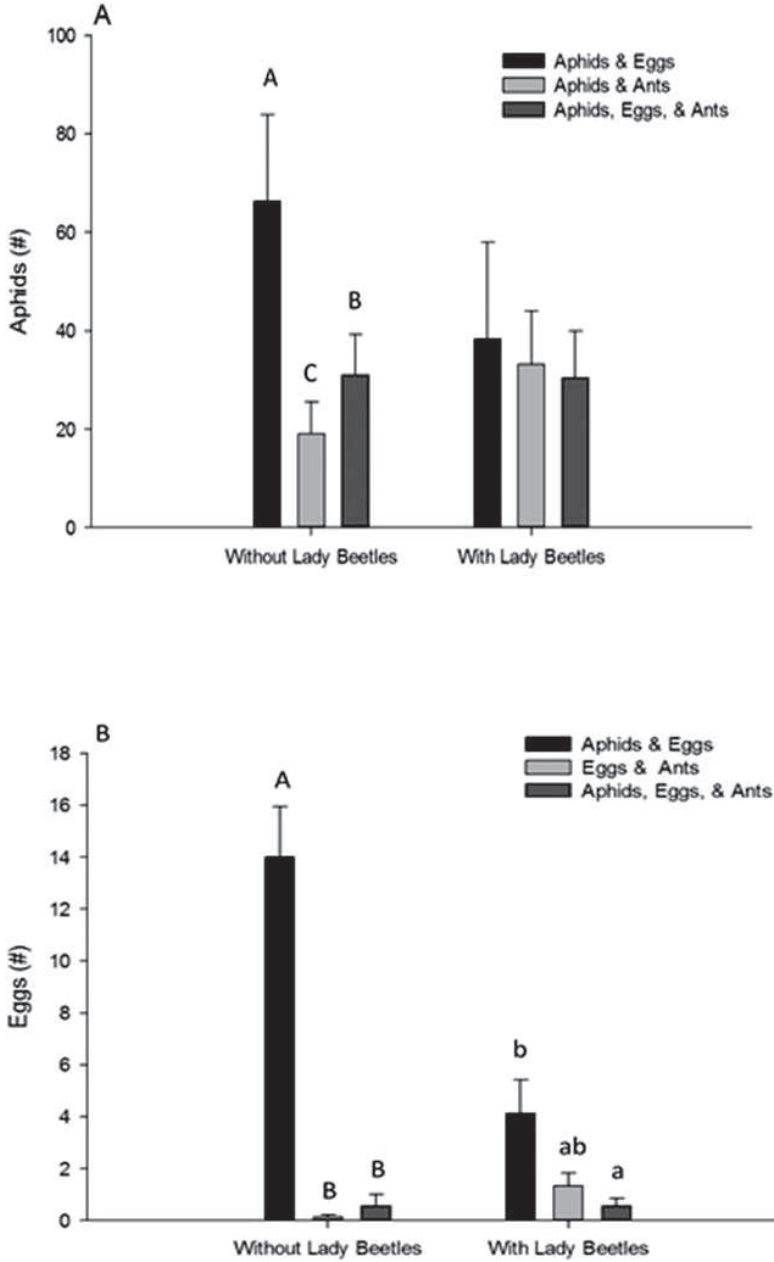


Fig. 1. Aphid and egg survival when exposed to ants and lady beetles on canola. (A) The mean (SEM) number of aphids (*Myzus persicae*) remaining at the end of the trial in comparison to the original 50 placed on the canola plant with and without lady beetles (*Coleomegilla*

treatments in which ants were present; the aphid population gained weight when no ants were present and lost weight when ants were included.

Treatments also varied significantly in the number of surviving eggs at the end of the trial (Kruskal-Wallis $\chi^2_1 = 21.06$, $P < 0.01$) (Fig. 1B). Ants substantially reduced the number of eggs in the ants and egg treatment (Dwass-Steel-Chritchlow-Fligner test statistic = 5.40, $P < 0.001$) and the ants, aphids, and egg treatment (Dwass-Steel-Chritchlow-Fligner test statistic = 5.41, $P < 0.001$) relative to the number of surviving eggs in the aphid and egg treatment.

Herbivore performance with lady beetles. The magnitude of treatment effects with the inclusion of lady beetles into these systems was substantially different from those seen in the absence of lady beetles. Ants killed 100% of lady beetles within a few days of their inclusion into the system. There was no treatment effect on the number of aphids at the end of the trial (Kruskal-Wallis $\chi^2_1 = 0.47$, $P = 0.79$) (Fig. 1A). There were not significant differences among the treatments in the aphid weights at the end of the experiment (Kruskal-Wallis $\chi^2_2 = 2.00$, $P = 0.37$). The mean (\pm SEM) aphid weight change with lady beetles was -0.008 ± 0.001 , -0.004 ± 0.001 , and -0.009 ± 0.001 for treatments aphids, eggs, and lady beetles; ants, aphids, and lady beetles; and ants, aphids, eggs, and lady beetles, respectively.

Treatments also varied marginally in the number of eggs surviving (Kruskal-Wallis $\chi^2_1 = 5.70$ $P = 0.06$) (Fig. 1B). When lady beetles were included in the system, the number of eggs surviving in the ants and eggs treatment did not differ significantly from the other two treatments (Kruskal-Wallis $\chi^2_1 = 3.627$, $P = 0.28$; Kruskal-Wallis $\chi^2_1 = 3.481$ $P = 0.47$, respectively). However, the number of eggs surviving when both herbivores, ants and lady beetles, were present was significantly lower than when just aphids, eggs, and lady beetles were present (Kruskal-Wallis $\chi^2_1 = -5.406$, $P = 0.001$).

Ant attendance. When lady beetles were omitted from the assays, there were significant differences among treatments in ant attendance on Day 1 (Kruskal-Wallis $\chi^2_2 = 7.40$, $P = 0.03$) and Day 2 (Kruskal-Wallis $\chi^2_2 = 6.87$, $P = 0.03$). Specifically, the treatment with only eggs had significantly lower ant attendance than did the treatment with only aphids. There were no differences in ant attendance among treatments on Day 3 (Kruskal-Wallis $\chi^2_2 = 4.17$, $P = 0.12$) (Fig. 2A) Using a one sample *t*-test, it was shown that only the ant, aphid, and eggs treatment had a slope that was marginally significantly different from zero ($t_8 = 2.15$, $P = 0.06$) (Fig. 3). When lady beetles were included in the assay, ant attendance was substantially lower than when lady beetles were excluded, and ant attendance when lady beetles were present was unaffected by treatment (Day 1: Kruskal-Wallis $\chi^2_2 = 0.32$, $P = 0.85$; Day 2: Kruskal-Wallis $\chi^2_2 = 0.02$, $P = 0.99$; Day 3: Kruskal-Wallis $\chi^2_2 = 0.60$, $P = 0.74$) (Fig. 2B).

←
***maculata*), and (B) the number of *Pieris rapae* eggs remaining at the end of the trial in comparison to the initial 40 placed on each canola plant for each treatment with and without lady beetles. Numbers capped with different letters are significantly different from one another ($\alpha = 0.05$). Ants were *Formica montana*.**

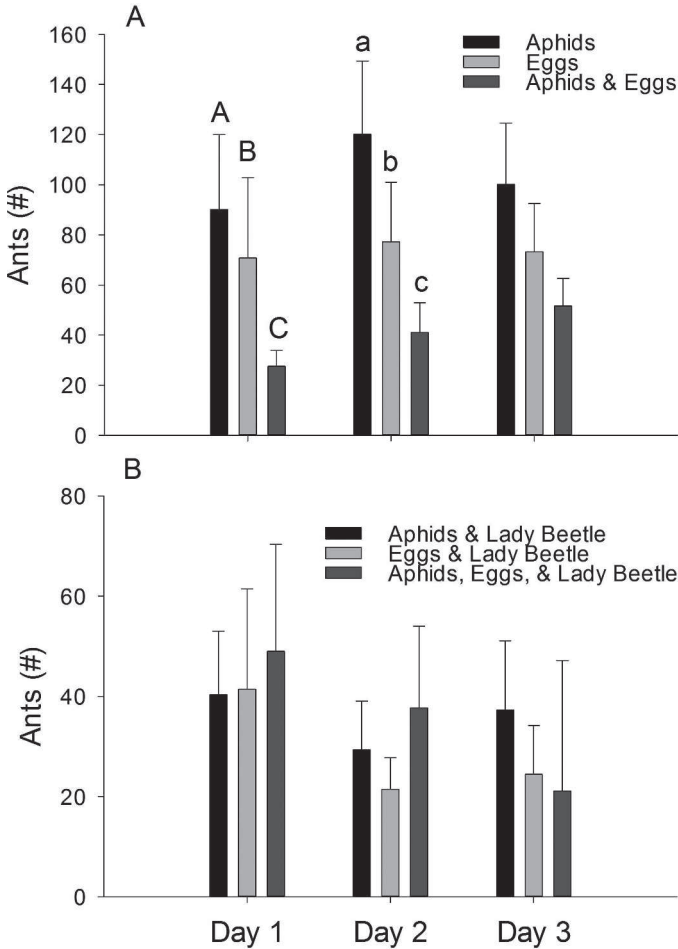


Fig. 2. The effects of herbivores and lady beetles on ant attendance on canola. Mean (SEM) summed ant (*Formica montana*) attendance are presented for Day 1, Day 2, and Day 3 in each of the three treatments (A) without lady beetles (*Coleomegilla maculata*) and (B) with lady beetles. Aphids were *Myzus persicae* and eggs were *Pieris rapae*. Bars topped with different letters are significantly different from one another ($\alpha = 0.05$).

Discussion

Egg survival. Many ants are considered as generalist predators (Hölldobler and Wilson 1990), and *F. montana* readily consumed *P. rapae* eggs in our experiment. Regardless of whether aphids or lady beetles were present in the system, few butterfly eggs survived in the presence of ants. Ants have been shown in the field to be responsible for high levels of mortality in *P. rapae* larvae and are a major factor in reducing juvenile survival (Jones 1987).

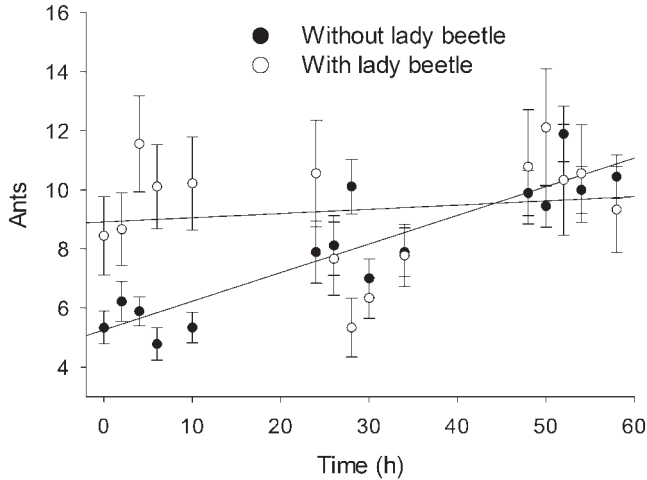


Fig. 3. Increasing ant attendance of herbivorous prey in the absence of lady beetles on canola. Mean (SEM) number of active ants (*Formica montana*) in the arena when the colony was exposed to aphids (*Myzus persicae*) and eggs (*Pieris rapae*). The two treatments varied in whether a lady beetle (*Coleomegilla maculata*) was present in the arena. Without lady beetle: ($t_8 = 2.15$, $P = 0.06$), equation for line: $y = 5.93 + 0.087x$; $r^2 = 0.75$. With lady beetle: ($t_8 = 0.16$, $P = 0.89$), equation for line: $y = 8.91 + 0.014x$, $r^2 = 0.023$ ($\alpha = 0.05$).

Schmaedick and Shelton (1999) found relatively low mortality rates (10.2 and 7.3% in two different fields) due to predation, while Dempster (1967) and Ashby (1974) found arthropod predators primarily responsible for the decline in early instars of *P. rapae*. Egg survival in our experiment, however, never exceeded 10% of the original 40 eggs when the eggs were exposed to one or both predators. This leads us to believe that eggs are considered as an important and acceptable source of protein for the ants, and that any possible defenses (Eisner et al. 2000; Gross 1993) within the eggs had little to no effect on the ants, based on the consumption rate in this closed system. Prey-associated proteins and lipids are crucial for colony reproduction (Beattie 1985), and it is common to see the accumulation of protein reserves in a colony for the future queen and other adult castes during the larval stage when protein is readily available (Vinson 1968). It was not uncommon to find ant eggs and larvae present within the soil of the experimental plant at the end of the two trials for each of the ant colonies, which indicates the availability of the necessary proteins and lipids found in the system needed for maintaining the colony. Because of their relatively low water content, insect eggs are a concentrated form of nutrients relative to other insect life stages (Lundgren 2009; McNeill 1971) and, thus, can constitute a high-quality prey item for the ants. The dietary need of the ant colony for high-quality nutrition and insect eggs' high nutrient content help explain the almost complete consumption of eggs by the ants.

Lady beetles also consumed butterfly eggs but not as efficiently as did the predatory ants. Ants on their own consumed nearly 99% of eggs in the system whereas when lady beetles were the only predator, nearly 10% of eggs survived (Fig. 1B). This egg survival was significantly reduced when ants (and aphids) were added to the system. This observed lower predation efficiency could possibly be due to a preference by lady beetles for aphids as a food over the eggs (Evans 2009), the renewability of aphids as a food source (Carroll and Janzen 1973), a possible preference for certain-aged eggs (Hippen 1982), or even possible low levels of glucosinolate as a defense within the eggs. This research substantiates previous studies that show that eggs and young larvae of *P. rapae* are readily consumed in the field (Dempster 1967; Jones 1987; Schmaedick and Shelton 1999), and the role of predation in curtailing *P. rapae* populations in the field should be examined in further detail.

Aphid survival. Ants balance their behavior between consuming aphids and tending them based on the relative resources within a habitat and the physiological status of the colony. We found that ants readily consumed aphids but always tended a population (at least 30 aphids) regardless of the availability of other prey and predators (Fig. 1A). Because aphids secrete a sugar-rich honeydew substance and are often stationary, they are considered as a renewable food source for ants (Carroll and Janzen 1973). Ants rely heavily on sugar as an energy source for all of their activities (Carroll and Janzen 1973; Dussutour and Simpson 2008; Lach et al. 2010; Markin 1970; Tschinkel 2006) and access to sucrose can cause a rapid increase in activity patterns, foraging intensity, and aggressive behavior in ant colonies (Mgockeki and Addison 2009; Ness et al. 2009; Stadler and Dixon 2008). Carbohydrates are necessary as a food source for maintaining the colony, but reproduction and production of new workers fundamentally relies on proteins and sugars (Cassill and Tschinkel 1999; Lach et al. 2010; Pontin 1958; Sorensen and Vinson 1981; Stradling 1978). As such, the intensity of ant attendance on aphids and other homopterans is affected by the relative availability of sugars and other nutrient sources (e.g., protein, lipids) within a system. As sugars become more abundant in a habitat, ants switch from tending aphids to sometimes eating them under conditions of high protein or lipid needs (e.g., during reproductive phases of the colony) (Carabali-Banguero et al. 2013; Cushman 1991; Offenburg 2001; Pontin 1958; Way 1954). This behavioral switching is evident in the results of the first experiment, in that aphid consumption decreased and ant visitation increased when both the aphids and eggs were available in the absence of the lady beetle, in comparison to higher levels of aphid consumption when alternative food sources were unavailable (Fig. 1A)

Aphidophagous lady beetles are considered as important biological control agents of aphids (Obrycki and Kring 1998, Obrycki et al. 2009), and their presence in the current study significantly affected the behavior of the ants towards the aphids. *Coleomegilla maculata* feed on a variety of prey, including aphids (Coderre et al. 1987; Lundgren et al. 2004; Mack and Smilowitz 1980,) and, regardless of the presence or absence of ants in the system, aphids were consumed by the lady beetles. In the presence of other lady beetle species, *C. maculata* becomes more aggressive with increasing aphid consumption (Leppanen et al. 2012); it is possible that this effect is present with other competitive insects such as the aggressive *F. montana* ants. It is also noteworthy that aphid consumption by lady beetles

occurred despite the death and often consumption of the lady beetle by the ants in the system. The death of the lady beetle can be speculated as a direct result of competition or high interference rate of the two aggressive predators for the shared food source within the system.

When ants were in the system with the lady beetles, it was noted that the ants altered their behavior towards the aphids and stopped eating them. Regardless of whether lady beetles were present or not, aphids were maintained at a minimum threshold level. This minimal level has been observed in other systems (Breton and Addicott 1992; Bronstein 1994; Yao et al. 2000), and the numbers of aphids tended likely depends on the density of the ant colony being tested (Jung et al. 2011; Lach 2003; Morales 2000). The lowest mean aphid population for the nine ant colonies in our experiment was never below 30 individuals (Fig. 1). In the absence of ants, lady beetles lowered the aphid population to 30 individuals per plant. If ants continued to cull the aphid populations, it is possible that the ant colony could no longer obtain the necessary amount of carbohydrates needed by the colony (Addicott 1979; Breton and Addicott 1992; Cushman 1991; Jung et al. 2011; Lach 2010; Way 1963). Despite eventually killing the lady beetle, the predation of the aphids did not resume by the ants and, instead, they maintained their protective actions on the remaining aphid population. This may be due to the possibility of the presence of other predators that could create additional competition for the aphids within the system (Buckley and Gullan 1991; Lach 2003; Way 1963).

In conclusion, our work illustrates the complexities of multitrophic interactions, even within this simplified system involving four insect species. A clear conclusion is that ant–aphid mutualisms did not disrupt predation on *P. rapae* eggs and, thus, ants remain an important biological control agent of herbivores regardless of whether aphids are present in a system. Our study demonstrated that additional predators and herbivores added into the ant–aphid mutualism affected ant behavior, aphid survival, and butterfly egg survival. These additions into a simple, well-known system could potentially play important ecological roles which are often overlooked if food webs are overly simplified. It is important for both scientists and producers to understand these interactions and their possible implications for pest management within a crop production setting.

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