Herbivore density mediates the indirect effect of herbivores on plants via induced resistance and apparent competition

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Abstract.   Herbivore-induced plant resistance and apparent competition are two indirect ways herbivores interact. If a less damaging herbivore indirectly suppresses the abundance of a more damaging herbivore via these mechanisms, then plants may ultimately benefit. Changes in herbivore density, however, can dictate the intensity of species interactions and may play a critical role in determining the outcome of plant- and predator-mediated herbivore interactions. We tested the effects of herbivore density on the strength of indirect interactions among phloem-feeding aphids and herbivorous caterpillars and the outcome of these interactions for their shared host plant, cotton (*Gossypium hirsutum*). We quantified the survival of caterpillars on host plants that were infested with varying densities of aphids in the presence and absence of predators (ladybeetles). We found that aphids induced defensive proteins in cotton plants and that caterpillar survival was negatively affected by induced resistance. Likewise, we found that the presence of aphids increased predation of caterpillars by ladybeetles, but that apparent competition between aphids and caterpillars was density dependent. Ladybeetles consumed relatively high numbers of small caterpillars at low to intermediate aphid densities, but essentially became aphid specialists at high aphid densities. Aphid induced defenses and apparent competition combined such that plant damage by caterpillars was lowest when predators were present at low aphid density (induced resistance + highest level of apparent competition). This suggests that herbivores can benefit plants, but the effect on host plants is mediated by herbivore density. Indirect herbivore-plant mutualisms may increase plant quality, plant fitness, and yield of crop plants and these interactions need to be considered in ecologically based pest management plans. In addition, these interactions likely alter arthropod community structure and natural selection on anti-herbivore defense traits in plants in natural systems.

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INTRODUCTION

Insect herbivores frequently interact indirectly through interactions mediated by both plant and predators (Strauss 1991, Denno et al. 1995, Stachowicz 2001, Kaplan and Denno 2007, Eubanks and Finke 2014). Plant-mediated interactions can occur when one herbivore induces plant resistance at relatively low densities and reduces the fitness of other herbivores that share the same host (Denno et al. 2000, Bezemer et al. 2003, Poelman et al. 2008). An insect herbivore may also negatively impact another herbivore by altering the abundance or behavior of a shared predator

(apparent competition sensu lato) (Holt 1977). In both cases, an herbivore may provide a net benefit to the plant they consume if that herbivore indirectly suppresses the abundance of a more damaging herbivore (e.g., Halitschke et al. 2011, Eubanks and Finke 2014).

Plants respond to insect herbivory in a variety of ways including inducing higher levels of plant defenses (Ryan 1990, Zhu-Salzman et al. 2008). Induced plant resistance through changes in plant quality can reduce herbivore feeding, development and performance (Ryan 1990, Felton et al. 1992, Lawrence and Novak 2006). These interactions become more complex when multiple herbivore species are involved. An overwhelming number of studies, however, show that feeding from one herbivore species can negatively impact a subsequent herbivore species through these plant-mediated effects (see examples in Kaplan and Denno 2007).

There are far fewer examples of predator-mediated indirect interactions between herbivores than plant-mediated indirect interactions (Kaplan and Denno 2007, Eubanks and Finke 2014). Predator-mediated indirect interactions occur when one prey species indirectly alters the abundance of a second prey species through a shared predator (Holt and Kotler 1987). The presence of one prey species can stimulate the feeding activity of a predator on a secondary prey species or predators altogether switch their prey preference. This has been demonstrated in a small, but growing number of studies (e.g., Siddon and Witman 2004, Prasad and Snyder 2006, Messelink et al. 2010). Very few of these studies, however, have assessed the impact of predator-mediated interactions on the shared host plant (Eubanks and Finke 2014). If apparent competition results in a decrease in the abundance of more damaging herbivores, then plants could benefit by hosting the less damaging herbivore and function as a plant mutualist.

Indirect interactions among herbivores are not necessarily occurring independently of each other. Furthermore, herbivore density may play a larger role in the outcome of these interactions when plant- and predator-mediated interactions occur simultaneously. For example, if herbivory alters plant resistance and also causes an emission of plant volatiles that attract predators to the damaged plant (De Moraes et al. 1998, Bruce et al. 2008). Shiojiri et al. (2010) found a positive relationship between the densities of caterpillars of the cabbage white butterfly, *Pieris rapae*, and plant volatile production and subsequent attraction of parasitoid wasps to damaged plants. Density-dependent attraction of predators and parasitoids to prey or hosts is widespread in nature (Herrando-Perez et al. 2012, Ohgushi et al. 2012). We tested the effects of herbivore density on the strength of indirect interactions among phloem-feeding aphids and herbivorous caterpillars in the presence of predators and the outcome of these interactions for their shared host plant, cotton (*Gossypium hirsutum*). This study provides an understanding of the intensity of species interactions dictated by changes in herbivore density and how herbivore density may play a critical role in determining the outcome of herbivore species interactions mediated by plant and predators.

Study system

The phloem-feeding aphid (*Aphis gossypii*) and the beet armyworm (*Spodoptera exigua*), a chewing caterpillar, are commonly found feeding on cotton (*G. hirsutum*) (Weathersbee and Hardee 1994, Loughrin et al. 1995, Ebert and Cartwright 1997). *Aphis gossypii* often establish early, before caterpillars, and reproduce rapidly, but typically do not reach high densities until fruiting and cotton bolls open, late in the season (Ebert and Cartwright 1997). While high densities of aphids can cause plant damage, plants can tolerate low to moderate densities of aphids with little direct effect on plant fitness (e.g., Rosenheim and Wilhoit 1993, Rosenheim et al. 1995, Ragsdale et al. 2007). Although studies on cotton aphids are lacking, feeding by some species of aphids has been shown to change plant quality and induce plant defenses (Goggin 2007, Anstead et al. 2010). Many predators prey upon aphids, especially ladybeetles (*Hippodamia convergens*) (Hatano et al. 2008, Evans 2009, Outreman et al. 2010). Ladybeetles are an abundant generalist predator that feeds on aphids and many other insects including small larvae and caterpillars and have strong numerical responses to aphids (Kindlmann and Dixon 1999, Evans 2009). Armyworm caterpillars are defoliators that feed on all parts of cotton plants including leaves, stems, and bolls

(Loughrin et al. 1995). This system provides an opportunity to examine whether cotton plants can benefit from different aphid densities through both induced resistance and increased predation of caterpillars.

We tested the hypothesis that aphid density mediates the indirect effects of aphids on caterpillars via induced resistance and apparent competition sensu lato, herbivore interactions with a shared predator and not accounting for a numerical response of ladybeetles. Specifically, we tested the hypothesis that cotton aphids induce defensive proteins (chitinase, peroxidase, polyphenyloxidase, and trypsin inhibitor) in cotton plants and that the concentration of these compounds is positively correlated with herbivore density. We also tested whether changes in aphid density in the presence of ladybeetles alters the survivorship of caterpillars and the outcome for cotton plants. Finally, we tested the hypothesis that predator feeding behavior of caterpillars is dependent on aphid density.

Methods

Field experiment: effect of induced plant defenses and predators on caterpillar suppression at varying aphid densities

We conducted a 3 × 2 (three levels of aphid density × predator presence/absence) factorial experiment to determine whether changes in aphid density in the presence or absence of ladybeetles alter the survivorship of caterpillars. The field experiment was conducted in 2009 at Texas A&M Field Laboratory in Burleson County, Texas, USA. Experimental units were 38 × 25 × 71 cm cages constructed with PVC pipe frames enclosed with a mesh (black no-see-um mosquito netting) screen. Each cage housed a single transplanted eight leaf stage cotton plant (cv. DP-493) previously germinated and grown under 27 °C 16 h photoperiod in the lab.

Aphids used for the experiment were maintained on cotton plants contained in the laboratory at 27 °C and 18 h photoperiod. Plants (five to six leaf stage) received 0, 50, or 200 aphids of mixed age 1 week before being transplanted into the field. One week after transplanting in the field, aphids were counted (this represented the T0 count confirming the aphid density

treatments per leaf; Appendix Fig. A1) and the newest expanded cotton leaf was removed and stored at −80 °C for analysis of defensive proteins (see Plant defense protein bioassay common to field and greenhouse experiments). Twenty-four hours after leaf removal, 20 neonate caterpillars from our laboratory colony were added to each plant and given 4 h to establish. Predator manipulations were then made by adding five ladybeetle adults (*H. convergens*; purchased from Rincon-Vitova, Ventura, California, USA) into each of the predator only and predator + aphid treatment cages. Ladybeetles were stored in a refrigerator (3–4 °C) without food for approximately 1 week before being included in each study and no attempt was made to determine the sex of each ladybeetle that was used. There were six unique treatment combinations of aphid density and predator with five replicates each; however, some treatment cages succumbed to red imported fire ants resulting in a total $N = 22$ for trial 1 and total $N = 23$ for trial 2. Across trials only one treatment (50 aphids) was represented by five replicates and all other treatments were represented by more than five replicates. After 3 d, we recorded the number of surviving caterpillars.

Greenhouse experiment: combined effects of induced plant defenses and predators on caterpillar suppression and plant damage at varying aphid densities

A 4 × 2 (four levels of aphid density × predator presence/absence) factorial experiment was conducted to determine whether changes in aphid density in the presence or absence of ladybeetles alter the survivorship of caterpillars and feeding damage. The greenhouse experiment was conducted in 2009 at Texas A&M University's Biological Control Facility in College Station, TX, USA. Experimental units were 15 L plastic containers (Sterilite) that sat within 38 × 25 × 71 cm cages constructed with PVC pipe frames enclosed with a mesh (black no-see-um mosquito netting) screen. Each cage housed a single three to four leaf stage cotton (cv. DP-493) plant grown under 27 °C and 18 h photoperiod. Two trials were run with some replicate cages in the first trial being lost to ants. As a result, cages in the second trial were placed on top of shallow containers filled with water that excluded ants. All cages were kept

at 32 °C and 18 h photoperiod on greenhouse benches for the duration of the experiment.

Plants were infested with aphids as described in the field experiment with the addition of a 400 aphid seeding rate, creating four aphid density treatments (0, 50, 200, and 400 aphids per leaf). After allowing 11 d to establish, aphids were then counted (representing the T0 count that established the aphid density treatment; Appendix Fig. A2A). Similar to the field experiment, the newest expanded leaf was removed, and after 24 h ten neonate caterpillars were added to each plant. Caterpillars were given 24 h to establish, after which two ladybeetles (*H. convergens*) were released into each respective, predator treatment cage. For the greenhouse experiment, there were eight unique treatment combinations of aphid density and predator, four replicates each (total $N = 23$ for trial 1; total $N = 32$ for trial 2) and three control plants (no aphid, caterpillar, or ladybeetle). Across trials only one treatment (predator only) was represented by five replicates and all other treatments were represented by more than five replicates.

The experiment was terminated after 3 d (T3) and we recorded the number of surviving caterpillars and final aphids remaining per leaf. Leaves from each plant were removed and photographed and leaf consumption by caterpillars was calculated using ImageJ 1.41 software (http://rsbweb.nih.gov/ij/).

Plant defense protein bioassay common to field and greenhouse experiments

Stored leaves from each plant for both field and greenhouse experiments were analyzed for levels of chitinase, peroxidase, polyphenoloxidase, and trypsin inhibitor following methods in Ramirez and Spears (2014). Selection of these proteins was to determine whether changes caused by aphids favored a particular defense pathway as chitinase and peroxidase relate to the salicylic acid pathway, and polyphenoloxidase and trypsin inhibitor relate to the jasmonic acid pathway (Barto and Cipollini 2005). Briefly, a microplate reader (Biotek ELx808 Absorbance Microplate Reader, Winooski, Vermont, USA) was used to measure peroxidase, chitinase and polyphenoloxidase activity from standardized 0.2 g fresh weight leaf samples. Peroxidase and polyphenoloxidase activity in soluble protein extracts was determined following the oxidation of guaiacol and caffeic acid, respectively, for 1 min at 470 nm. Chitinase activity was determined by assessing the hydrolysis of *p*-nitrophenyl-β-*N*-acetylglucosaminide measured at 405 nm. Trypsin inhibitor activity was measured by examining the diffusion of protein extracts through a trypsin-containing agar followed by staining. A standard curve using soybean trypsin inhibitor was used to determine trypsin inhibitor concentration (μg trypsin inhibitor/g protein).

Statistical analysis for field and greenhouse experiments

The relationship between aphid density and production of four plant chemical responses (chitinase, peroxidase, polyphenlyoxidase, and trypsin inhibitor) were assessed by using linear regression and including both trials to examine the relationship between aphid density and chemical response. All leaf samples collected at the beginning of the study (T0), and before treatments (caterpillars and predators) were applied, were represented in the analysis for plant chemical responses.

We examined *S. exigua* survivorship and leaf consumption within a two-way ANOVA with three levels of aphid density (0, 50, 150 aphids/leaf) for the field experiment and four levels of aphid density (0, 50, 200, and 400 aphids/leaf) for the greenhouse experiment crossed with the two predator treatments and blocked by trial. As previously described, five treatments had missing replicates across both trials in the field experiment (control [4 reps], predator only [2 reps], 50 and 150 aphids [5 and 3 reps, respectively], and predator + 50 aphids [1 rep]) and six treatments had missing replicates in the greenhouse experiment (50 aphid (2 reps), predator only (3 reps), and 1 rep each for control, 200 aphid, 400 aphid, and predator + 400 aphid). We followed our initial analysis with comparisons across all treatments using Tukey's *post hoc* test. For the greenhouse experiment, we examined final aphid counts (T3) within a two-way ANOVA with three levels of aphid density (50, 200, and 400 aphids/leaf) crossed with the two predator treatments. Tests for model assumptions (e.g., Levene's test for homogeneity of variance) were performed. Data were analyzed using SAS

9.1 (SAS Institute Inc., Cary, North Carolina, USA).

Effects of aphid density on predation and foraging

We designed a subsequent laboratory experiment to quantitate the effects of aphid density on the foraging behavior of ladybeetles (*H. convergens*). Ten neonate caterpillars (*S. exigua*) were placed in 10 cm Petri dishes lined with moistened filter paper, and one of three aphid densities (50, 200, and 400 aphids) were added to the petri dish, each treatment had three replicates ($N = 9$). A single field-collected adult female ladybeetle was starved for 24 h and added to each dish. Each predator was observed for 20 min after their introduction into the dish. We recorded the number of caterpillars that were eaten and monitored the foraging distance for each ladybeetle. Foraging distance was measured by tracing ladybeetle movement with a marker on the Petri dish lid. We photographed the tracings and calculated the distance traveled (cm) using ImageJ software version 1.42q (http://rsbweb.nih.gov/iij/). Consumption of caterpillars, aphids, and travel distance were analyzed using one-way ANOVA followed by Tukey's *post hoc* test.

Results

Direct effects of aphid density on induced plant defenses

In the field experiment, peroxidase and trypsin inhibitor concentration increased with increasing aphid density ($R^2 = 0.10$, $P = 0.035$ and $R^2 = 0.12$, $P = 0.023$, respectively; Fig. 1b,d). No relationship in chitinase and polyphenyloxidase concentration was evident with greater aphid density ($R^2 = 0.03$, $P = 0.264$ and $R^2 = 0.07$, $P = 0.088$, respectively; Fig. 1a,c). In the greenhouse experiment, aphid density significantly increased chitinase and peroxidase production ($R^2 = 0.23$, $P < 0.001$ and $R^2 = 0.25$, $P < 0.001$, respectively; Fig. 1a,b). However, no relationship was present for polyphenyloxidase and trypsin inhibitor production ($R^2 = 0.01$, $P = 0.340$ and $R^2 = 0.04$, $P = 0.090$, respectively; Fig. 1c,d).

Aphid density effects on predator- caterpillar interactions

We found a significant aphid density × predator interaction on caterpillar survival in the field ($F_{2,33} = 5.01$, $P = 0.013$) (Fig. 2). The effects of ladybeetles on
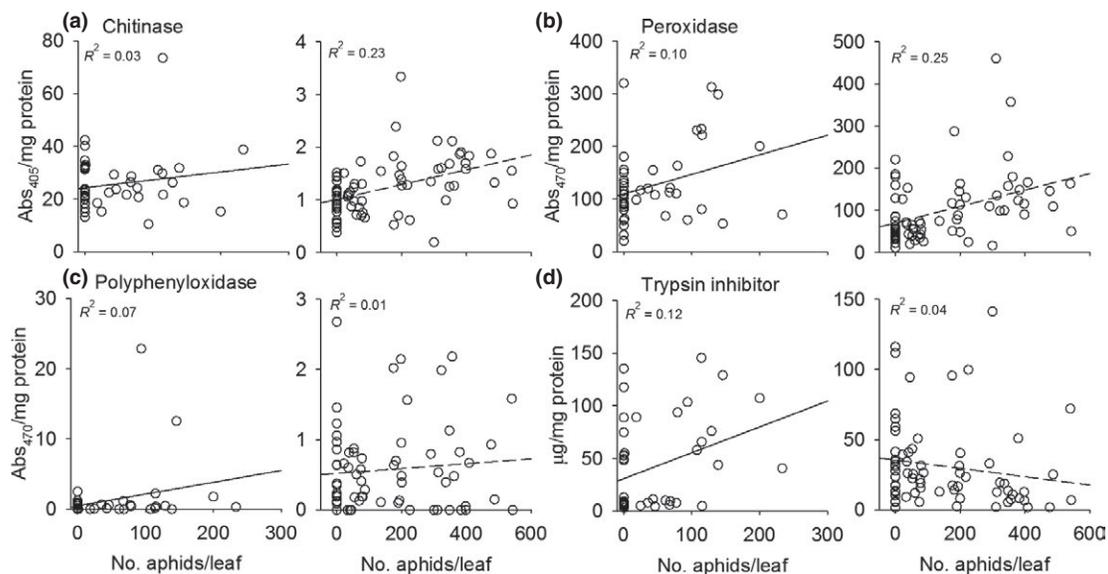

Fig. 1. At the start of the field experiment and greenhouse experiment (dashed regression lines), levels of (a) chitinase (field: $R^2 = 0.03$, $P < 0.264$; greenhouse: $R^2 = 0.23$, $P < 0.001$), (b) peroxidase (field: $R^2 = 0.10$, $P = 0.035$; greenhouse: $R^2 = 0.25$, $P < 0.001$), (c) polyphenyloxidase (field: $R^2 = 0.07$, $P = 0.088$; greenhouse: $R^2 = 0.01$, $P = 0.340$), and (d) trypsin inhibitor (field: $R^2 = 0.12$, $P = 0.023$; greenhouse: $R^2 = 0.04$, $P = 0.090$) in leaf samples plotted vs. the number of aphids per leaf for both trials.
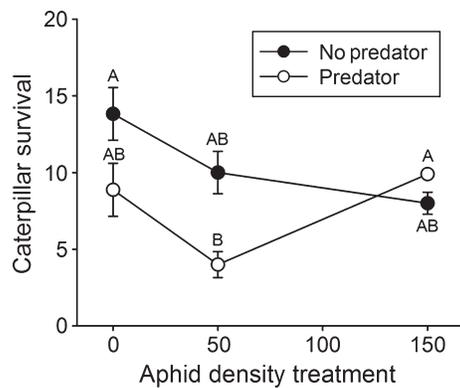
Fig. 2. Across aphid density treatments, caterpillar survivorship when ladybeetles were present (○) or absent (●). Data are means ± 1 SE. Different letters indicate significant differences across all treatments ($P < 0.05$; Tukey's HSD).

caterpillars were highly dependent on the number of aphids per leaf: The presence of aphids on plants in the absence of ladybeetles reduced caterpillar survival by 28% and 42% (50 and 150 aphids per leaf, respectively, Fig. 2). In the absence of aphids, ladybeetles reduced the survival of caterpillars by 36% (Fig. 2). When there were 50 aphids per leaf, however, the effect of ladybeetles on caterpillars dramatically increased with ladybeetles reducing the survival of caterpillars by 71% ($P < 0.001$). At 150 aphids per leaf, however, ladybeetles reduced the survival of caterpillars by only 28%.

In the greenhouse experiment, we found a significant interaction between aphid density and predator treatment ($F_{3,38} = 3.51$, $P = 0.024$; Fig. 3a), an interaction apparently driven by aphid density. In the absence of aphids, ladybeetles decreased caterpillar survival by 40% ($P = 0.016$). In the absence of ladybeetles, aphids decreased caterpillar survival by 40% and 41% from the control (200 aphids [$P = 0.01$] and 400 aphids [$P = 0.005$] per leaf, respectively; Fig. 3a). The combination of ladybeetles and 50 aphids per leaf decreased caterpillar survival by 79% ($P < 0.001$; Fig. 3a). Similar to the field experiment, the effect of ladybeetles on caterpillar survival decreased as aphid density increased.

Leaf consumption by caterpillars was decreased by the presence of aphids (main effect: $F_{3,45} = 3.63$, $P = 0.020$) and ladybeetles (main effect:
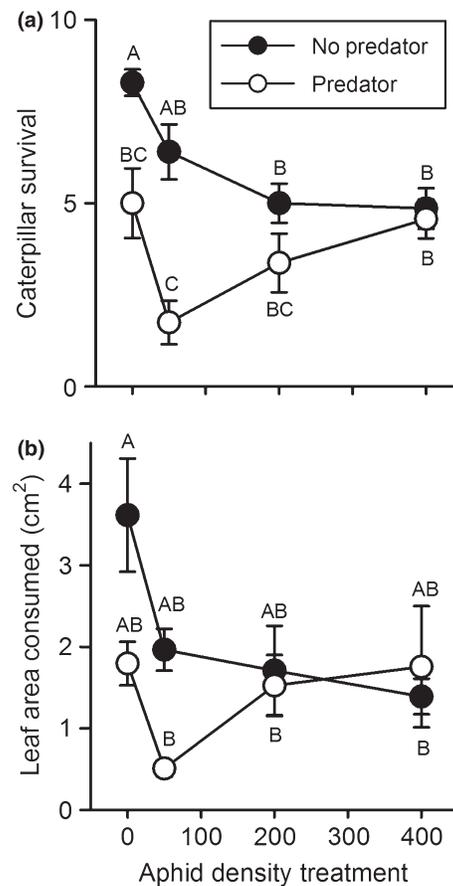

Fig. 3. Across aphid density treatments, (a) caterpillar survivorship and corresponding (b) leaf consumption when ladybeetles were present (○) or absent (●) across both greenhouse trials. Data are means ± 1 SE. Different letters indicate significant differences across all treatments ($P < 0.05$; Tukey's HSD).

$F_{1,45} = 5.79$, $P = 0.020$; Fig. 3b). In the absence of aphids, ladybeetles decreased caterpillar feeding by 50%. In the absence of ladybeetles, aphids decreased caterpillar feeding from 46% to 62% as aphid densities went from 50 aphids to 400 aphids per leaf, respectively (Fig. 3b). When there were 50 aphids per leaf and ladybeetles, caterpillar feeding drastically decreased by 86%.

The final aphid count (T3) per leaf revealed a marginally significant aphid × predator interaction ($F_{2,41} = 3.15$, $P = 0.053$), that was driven by a greater reduction in aphids at the highest aphid density (400 aphids per leaf) in the presence of ladybeetles that was not evident at the moderate

and low aphid density levels (Appendix Fig. A2B). Essentially, as aphid density increased, ladybeetles became aphid specialists.

Effects of aphid density on predation and foraging

We found evidence that aphid density strongly affected the distance foraged by individual ladybeetles and ultimately the number of caterpillars consumed by ladybeetles (Fig. 4). In the presence of 50 aphids, ladybeetles traveled 90 cm in 20 min. Ladybeetle foraging distance, however, was reduced by 65% and 60%, respectively, when aphid density increased from 200 to 400 aphids ($F_{2,8}$ = 6.12, P = 0.036; Fig. 4a). The effects of aphids on the number of
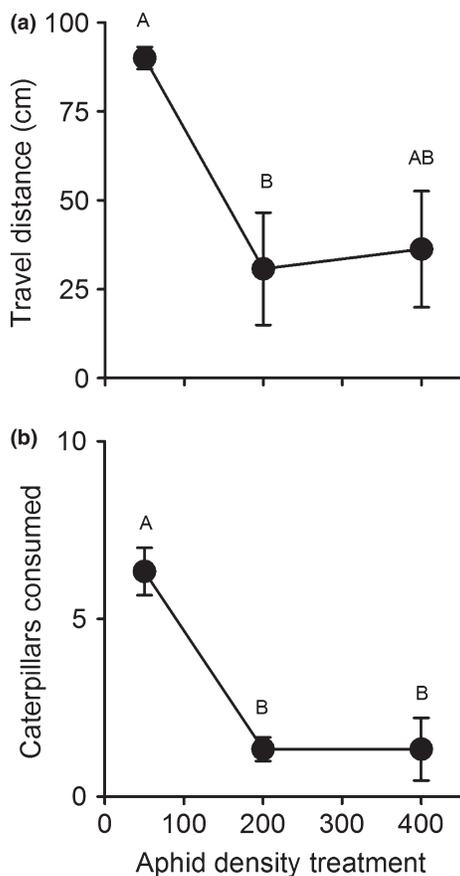

Fig. 4. Across aphid density treatments, foraging behavior of a ladybeetle in a Petri dish measured as (a) total distance traveled and (b) caterpillars eaten. Data are means ± 1 SE; letters indicate significant differences among initial aphid density ($P < 0.05$; Tukey's HSD).

caterpillars consumed by individual ladybeetles mirrored the effects of aphids on ladybeetle foraging ($F_{2,8}$ = 18.75, P = 0.003; Fig. 4b). The number of aphids consumed by ladybeetles in 20 min was not significantly different among the aphid treatments ($F_{2,6}$ = 1.27, P = 0.345).

Discussion

Our data suggest that it is possible for aphids to benefit plants by reducing caterpillar survival, although this effect was dependent on aphid density. We found that predators had their greatest effect on caterpillar survival at low aphid densities (50 aphids per leaf) (Figs. 2 and 3). Ladybeetle predation rates on caterpillars, however, was decreased as aphid density increased above 50 aphids per leaf and suggests that a density threshold may exist whereby these plant- and predator-mediated indirect interactions combine to suppress a more damaging secondary herbivore. Very few studies have documented the combined effects of induced plant resistance and apparent competition (Eubanks and Finke 2014) and no studies have examined how herbivore density alters these combined indirect interactions. We found a positive correlation between aphid density and plant defense proteins (Fig. 1) and that early establishment of aphids on plants can induce resistance. The presence of aphids can attract predators to the local environment (Hatano et al. 2008, Outreman et al. 2010) and strengthen apparent competition (Fig. 4).

Only a few studies have suggested that herbivores can function as conditional mutualists of a shared host plant (Karban et al. 1994, 1997, Halitschke et al. 2011, McArt et al. 2013). For example, wild tobacco plants attacked by mirid bugs become tolerant to caterpillars primarily because increased photosynthetic rates induced by the bug compensate for loss of leaf area from caterpillar feeding by increasing plant growth (Halitschke et al. 2011). In another case, plant defense compounds were elevated in common evening primrose when initially eaten by the invasive Japanese beetle and this lead to a reduction in seed predation by later occurring native caterpillars (McArt et al. 2013). Our study is unique in that the benefit to plants, a decrease in leaf consumption by a more damaging chewing insect, is

a result of the combination of induced resistance and apparent competition. At low to moderate densities, aphids in our system have minimal direct effects on plant fitness (Rosenheim and Wilhoit 1993, Rosenheim et al. 1995, Ragsdale et al. 2007) and chewing by caterpillars can reduce yields particularly when feeding on immature fruit (Gutierrez et al. 1975). While this may suggest a mutualism, we remain cautious because we did not test the effects of aphids on plants in our study or determine whether reduced caterpillar damage resulted in decreases in plant growth or fitness. Although the effects in our study show that a combination of induced resistance and predators suppressed caterpillars, it is possible for synergy between these two indirect factors to manifest if the secondary herbivore was monitored over its lifecycle. For instance, plant defenses have been shown to slow the development of herbivores (Karban 1988, Lawrence and Novak 2006), and smaller prey tend to be more susceptible to predator attack (Ramirez et al. 2010). "Slow-growth, high-mortality" could result in a synergistic interaction between induced resistance and apparent competition by extending the time prey are susceptible to predators and by attracting predators that are efficient at attacking smaller prey.

The outcomes of apparent competition have been modeled extensively and it is clear that the role of density, whether it be the density of the competing organisms or the shared predator, is important in mediating community structure (Chaneton and Bonsall 2000, Eubanks and Finke 2014, Hambäck et al. 2014). Furthermore, changes in herbivore density and in predator foraging behavior have been documented as major components of determining the intensity of apparent competition sensu lato (e.g., Settle and Wilson 1990 and Prasad and Snyder 2006, respectively). It has been known for decades that the density of predators is closely linked with fluctuations in prey populations as we see predator abundance follow prey abundance (Gause 1934). In our study, predator abundance was held constant and, consequently, our study may have underestimated the importance of apparent competition. The results of our study may be best explained by percolation models (Reynolds et al. 2009) that focus on patch resources and changes in resource density as key to consumer decisions about when to leave or stay in a resource patch. As the distance between prey resources decreases as prey density increases, predators travel shorter distances to locate a resource patch and are, therefore, less likely to encounter alternative prey. This scenario closely matches the results of our foraging behavior experiment (Fig. 4). It is clear that experimental studies varying density of herbivores and predators are needed to more fully understand community structure and that these density changes can influence the outcome of the interactions (Eubanks and Finke 2014, Hambäck et al. 2014).

Several studies have shown that plant defenses can follow a density-dependent dose–response (e.g., Thaler et al. 1996, Shiojiri et al. 2010, and this study) and this dose–response can affect conspecifics (e.g., Underwood 2010). We are not aware, however, of any studies demonstrating that this dose–response makes plants increasingly more resistant to subsequent herbivore species, particularly those with different feeding modes. Our study shows a dose–response of plant defense proteins to aphid density but caterpillar survival was constant as aphid density increased and when plant defense proteins would be expected to be at higher concentrations (e.g., Figs. 2 and 3). Thus, increasing aphid density and therefore plant defenses did not lead to increasingly more resistant plants. Moreover, the physical presence of aphids and competition for leaf space with increasing density was apparently not a factor affecting caterpillars in our study given caterpillar survival was constant across aphid densities and rather points to mechanisms related to induced plant resistance. This response is in line with several systems that show herbivores induce plant resistance toward subsequent herbivores by decreasing the palatability, changing the nutrition, and through other mechanisms and suggest this is wide spread in nature (Underwood 2012, McArt et al. 2013). For the plant defense proteins examined in this study, there were differences between the field and greenhouse plant responses (Fig. 1d) that suggest other factors in the field (e.g., weather, irrigation, soil) need to be considered. One constant between field and greenhouse was the increase in peroxidase with increasing aphid density, a salicylic acid pathway response and response common from general plant wound-

ing (Fig. 1b). Therefore, the strength of induced plant resistance by aphids and effects on caterpillars in our study may result from general wound responses as caterpillars are thought to be affected by jasmonic acid pathway responses (Thaler et al. 1996).

Until now much work has focused attention on the detrimental impacts of herbivorous insects on plants and strategies for herbivore suppression. Here, we emphasize that not every herbivore is ultimately damaging to plants in every environmental context and herbivores can be beneficial to plants via indirect effects. Understanding the indirect interactions among insect herbivores may allow pest management practitioners to reduce unnecessary pesticide applications and allow ecologists to determine if these "herbivore-plant mutualisms" are widespread in nature.

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Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1218/supinfo