

Dropping Behavior in the Pea Aphid (Hemiptera: Aphididae): How Does Environmental Context Affect Antipredator Responses?

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Abstract

The pea aphid *Acyrtosiphon pisum* Harris (Hemiptera: Aphididae) is a phloem-feeding insect whose antipredator defenses include kicking, walking away, and dropping from the plant. Aphid dropping, a risky and energetically costly antipredator behavior, can be increased by the release of aphid alarm pheromone; there is also evidence that insect density and plant health can affect the likelihood of aphids engaging in this behavior. We investigated whether interactions between alarm cues, insect density, and plant health can alter the dropping behavior of aphids in response to an artificial disturbance. The presence of the alarm pheromone E- β -farnesene resulted in a nearly 15-fold increase in aphid dropping behavior; the other two factors, however, did not affect dropping and none of the two- or three-way interactions were significant. This was surprising because aphids affected plant health: production of new plant biomass after 5 d of exposure to high aphid densities was 50% lower than in the control treatment. This research adds to our understanding of the factors affecting aphid antipredator behavior; the fact that neither aphid density nor feeding period impacted dropping may reflect the high energetic costs of this activity and an unwillingness to use it in any but the riskiest situations.

Key words: Antipredator behavior, nonconsumptive effect, alarm pheromone

Predators affect prey directly, via consumption (i.e., consumptive effects), and indirectly, via the induction of energetically costly antipredator responses (i.e., nonconsumptive effects). These responses can include changes in prey behavioral, morphological, or developmental traits (Petranka et al. 1987, Lima 1998, Podjasek et al. 2005, Preisser et al. 2005, Verheggen et al. 2009). Although these pathways differ in their benefit to the predator, both can negatively affect the prey: behaviors that lessen predation risk can also increase energy expenditure, reduce feeding time, or expose prey to alternative predators (Losey and Denno 1998a, Nelson 2007). Because only a small fraction of prey individuals are eaten but a much larger fraction respond to predation risk, the cumulative impact of nonconsumptive effects on prey population growth may equal or exceed that of direct consumption (Nelson et al. 2004, Preisser et al. 2005).

Because predators and prey interact in a dynamic environment, the magnitude of predator indirect effects on prey can also vary. Many mobile prey use behavioral trade-offs to balance the rewards of foraging and mating opportunities with the risk of consumption and may respond to several different types of predator cues (Lima 1998). Larvae of two-lined salamanders (*Eurceya bislineata*) and Cope's grey treefrog (*Hyla chrysoscelis*), for instance, both avoid water previously occupied by their predator, the green sunfish

(*Lepomis cyanellus*); exposure to fish cues also causes *H. chrysoscelis* tadpoles to spend more time in refuges (Petranka et al. 1987). Refuges are also important to invertebrate prey: larvae of the thrip *Frankliniella occidentalis* Pergande take refuge in spider mite webs to avoid the predatory mite *Neoseiulus cucumeris* Oudemans (Magalhaes et al. 2007). Hunger also alters prey responses to risk: although remaining hidden avoids predators, prey must forage to survive. Starved mayfly (*Baetis tricaudatus* Dodds) larvae, e.g., expose themselves to higher predation risk than less hungry individuals by spending more time in risky but resource-rich food patches (Kohler and McPeck 1989).

The family Aphididae includes many phloem-feeding species that occur in natural and managed systems and are consumed by an array of invertebrate predators. One common member of this family, the pea aphid *Acyrtosiphon pisum* (Harris), is an agricultural pest that can quickly reach high densities because of its ability to reproduce parthenogenetically during the summer growing season. Although *A. pisum* move slowly and possess few morphological defenses, they have developed antipredator behaviors to evade or deter predators. These behaviors include kicking, stylet removal, walking away, and dropping from the plant (Roitberg and Myers 1978).

Dropping behavior in *A. pisum* can be influenced by several factors, including the risk of predation, value of the plant resource, and mortality risk in the new environment (Losey and Denno 1998b). This behavior reduces feeding time and reproduction, increases the risk of desiccation, and exposes aphids to ground predators (Losey and Denno 1998a, Nelson 2007). The substantial costs of aphid dropping suggest that the frequency of this antipredator behavior should change when aphids are reared on host plants whose nutritive value has been reduced by prior herbivory (Denno et al. 1986, Awmack and Leather 2002). The generally negative relationship between plant health and aphid density, and the decrease in aphid performance (indicated by both aphid growth and the increased production of winged individuals) as plant health declines, has been extensively documented in the scientific literature (Müller et al. 2001). When food-deprived *A. pisum* are exposed to a parasitoid, e.g., they use less energetically costly behaviors (kicking) than do well-fed aphids that drop from the plant or walk away from the threat (Villagra et al. 2002). This response is not restricted to *A. pisum*; knapweed aphids, *Uroleucon jaceae* L., also reduce their dropping rate when feeding on low-quality plants (Stadler et al. 1994). Conversely, dropping rates might decrease on high-quality plants if the benefit of staying on such a host exceeds the cost of reduced dropping (Stadler et al. 2002). One study, for instance, found that *A. pisum* on high-quality diet dropped less often in response to risk cues than those reared on low-quality diet (Dill et al. 1990).

Threatened aphids also secrete an alarm pheromone, consisting of E- β -farnesene and other sesquiterpene hydrocarbons such as α - and β -pinene (Pickett and Griffiths 1980), that elicits an array of antipredator behaviors in other aphids (Roitberg and Myers 1978). Response to this pheromone can be affected by the aphids' environment. Pea aphids exposed to E- β -farnesene were more likely to drop with increasing amounts of alarm pheromone and when the pheromone cue is preceded by a vibrational cue indicative of a predator (Clegg and Barlow 1982). Pea aphids are particularly sensitive to this alarm cue, a larger portion of *A. pisum* drop in response to E- β -farnesene than any other aphid species sensitive to this alarm pheromone (Montgomery and Nault 1977). Antipredator behavior in some aphid species is also sensitive to population density. In the wheat aphid, *Schizaphis graminum* Rondani, dropping behavior in response to a coccinellid predator increases as a function of population density; whereas similar work on *A. pisum* did not find a relationship between aphid density and dropping, densities lower than 30 aphids per plant were not tested (McConnell and Kring 1990, Losey and Denno 1998b).

We report the results of research assessing how interactions between prey density, alarm cues, and food resources affect aphid responses to predation risk. Specifically, we tested how dropping behavior in response to an artificial predator differed at low versus high aphid densities, in the presence or in the absence of alarm cues, and on host plants that had been fed upon for a short or long time period. Our findings add to the results of previous research by addressing how the potential interplay between these factors can affect aphid antipredator defense.

Materials and Methods

Pea aphids were collected from pea plants (*Pisum sativum* L.) cultivated at the agronomy farm at the University of Rhode Island, Kingston, RI, in September of 2014. Aphids were reared in the laboratory on broad bean plants (*Vicia faba* L. var. "Varoma" source: Johnny's Selected Seeds, Maine). Seeds were planted in 26 g of

potting mix (Metro-mix 360; Sun Gro Horticulture, Agawam, MA). Bean plants emerged approximately 1 wk after planting. Aphids were maintained in mesh-sided insect cages containing two 2-wk-old *V. faba* plants. Two fresh plants were added to each insect cage every 5 d, and aphids were given 12 h to transfer to the new plants before the old plants were removed and composted. Plants and insects were raised in a temperature-controlled laboratory at 22°C and a photoperiod of 16:8 (L:D) h under fluorescent shop lights.

Prior to the start of an experimental trial, eight uninfested 2-wk-old *V. faba* plants were trimmed to two leaves per plant and individually potted in a 20-cm plastic pot. After 24 h, each pot-plant combination was covered with a cylindrical lid made of clear plastic transparency film and dacron chiffon netting (70 per inch mesh size, 240- μ m hole size). The netting allowed light and air to pass through while remaining impermeable to aphids. Each pot, plant, and lid combination was used as an individual experimental arena.

Experimental Design

We tested how *A. pisum* dropping behavior was affected by aphid density (low and high), length of feeding period (short and long), and exposure to aphid alarm cues (present and absent). We tested aphid density by transferring 5 (low density) or 50 (high density) aphids onto individual *V. faba* plants. These densities reflect those found during a field survey on the least- and most-infested quartiles of leaves on aphid-colonized *V. faba* plants (E.L.P., unpublished data). We tested the length of feeding period by allowing aphids to feed on a previously uninfested plant for 1 (short-feeding period) or 5 d (long feeding period). We tested exposure to alarm cues by exposing aphids to a crushed aphid (E- β -farnesene present) or neutral cue (same disturbance regime without the cue). We assessed the positive and control cue in each of the four main experimental groups listed above for a total of eight experimental groups. All three factors were crossed for a total of eight (2*2*2) experimental treatments, and each treatment was replicated eight times (=64 total replicates).

We started each replicate by using a damp fine-point paintbrush to gently transfer an appropriate number of aphid nymphs from the source population onto an experimental plant. Although immature aphids are more conservative in their dropping behavior than adult aphids (Roitberg and Myers 1978, Gish et al. 2012), we chose to use third-instar nymphs in order to maintain the initial aphid densities (i.e., prevent the aphid population from growing) throughout the experiment. Immediately following aphid transfer, we re-counted aphids on each experimental plant to ensure that none had fallen off during the transfer process. Replicates from the eight experimental treatments were randomly interspersed on a metal shelf underneath fluorescent shop lights (light and temperature conditions as in 'Materials and Methods') to prevent spatial bias. In order to measure the impact of aphid feeding on plant growth, we also included five control plants that were treated similarly (i.e., removal of all but two leaves, mimicking of disturbance caused by aphid inoculation) but did not have aphids added to them.

We added the first-instar nymphs in the 5-d treatments in order to prevent aphid reproduction; the third-instar nymphs were used for the 1-d treatment. Because most of the first-instar nymphs matured into the third-instar nymphs by the end of the 5-d treatments, our tests of dropping behavior primarily assessed the third-instar nymphs in both the short- and long-feeding-period treatments.

Aphid dropping behavior in a given replicate was tested either 1 or 5 d after the experiment began. Our testing protocol consisted of exposing aphids for 2 min to a paintbrush with or without a crushed aphid cue. During the 2 min, the paintbrush was firmly brushed

onto parts of the leaf immediately adjacent to aphids for 10–15 s to simulate predator presence, but did not physically contact any aphids. At the end of the 2-min testing period, both dropped and remaining aphids were counted. The above-ground biomass of plants in the 5-d and control treatment was then divided up into old growth and new growth (i.e., biomass added between the start and the end of the 5-d period). The old- and new-growth biomass of each plant was then weighed in order to determine the percentage of plant growth after 5 d in the control (zero aphids/plant), low-density (5 aphids/plant), and high-density (50 aphids/plant) treatments.

Statistical Analysis

We tested whether our aphid densities affected plant resources using a one-way ANOVA to compare the amount of new growth per plant after 5 d in the control, low-density, and high-density treatments. We tested the impact of risk cues, aphid density, and feeding period on the arcsine square-root transformed proportion of aphids dropping using a full-factorial three-way ANOVA. Data were checked prior to analysis to ensure that it was normally distributed and that variances were homogeneous. Although the data from the cue present treatments met both criteria, the data from the cue absent treatments did not (because so few aphids dropped); ANOVA is, however, highly robust to departures from both assumptions when per-treatment sample sizes are large (Underwood 1997). JMP 9.0.0 (SAS 2010) was used for all analyses.

Results

Aphid feeding over a 5-d period did not impact the weight of existing plant tissue ($F_{2,21}=0.06$, $P=0.95$), but substantially reduced the amount of new plant growth ($F_{2,21}=6.48$, $P=0.006$). As a result, new growth made up 13.4% and 27.6% of total above-ground biomass in the 50-aphid and aphid-free treatments, respectively (Tukey's test with $P=0.05$, Fig. 1).

Aphid alarm cues increased the percentage of aphids dropping, from 1.5% in cue-absent treatments to 22.4% in cue-present treatments ($F_{1,40}=44.9$, $P<0.01$; Fig. 2). Despite the decrease in new plant growth associated with aphid feeding (Fig. 1), neither feeding period nor aphid density affected dropping behavior ($F_{1,40}=1.27$

and 0.21, respectively, both $P>0.20$). There were also no significant two- or three-way interactions between any of the main effects.

Discussion

The presence of aphid alarm cues (in this case, a crushed prereproductive aphid) increased dropping rates 15-fold, with 22.4% of aphids dropping in the cue-present treatments versus 1.5% in the cue-absent treatments. E- β -farnesene, the substance previously identified as the predominant component of aphid alarm pheromone (Pickett and Griffiths 1980), is an active volatile terpenoid in aphid cornicle droplets; the results of our experiment are consistent with previous research finding that even prereproductive aphids reared in the absence of predators produce E- β -farnesene (Bowers et al. 1972, Mondor et al. 2000, Francis et al. 2005). Previous work has shown that the volatiles emitted from crushed aphids are nearly identical to those produced by cornicle droplets, and that aphids show similar defensive responses to both substances (Pickett and Griffiths 1980).

Although we anticipated that aphid alarm cues increased dropping rates, we were surprised at how few aphids dropped in their absence. In all of the treatments, we used a fine-point paintbrush to disturb the leaves and approach (although not touch) each individual aphid. Although the aphids would sometimes respond to the paintbrush by moving away or kicking, they apparently did not view the combined visual and vibrational stimulus presented by the paintbrush as an immediate threat (as per Ben-Ari and Inbar 2014). Although vibration in combination with E- β -farnesene can increase dropping behavior in younger instars, the act of dropping is costly and can reduce fecundity or increase mortality (Roitberg and Myers 1978, Nelson et al. 2004, Nelson 2007). In addition, cornicle droplets are high in triglycerides and are costly for aphids to synthesize; the secretion of a single cornicle drop can delay offspring production (Callow et al. 1973, Mondor and Roitberg 2003). The high cost of E- β -farnesene should limit its use to the riskiest of situations and make it a highly reliable indication of imminent danger (Orrock et al. 2015). In the absence of an alarm pheromone, however, the vibrational and visual cues presented by the paintbrush alone were insufficient to induce dropping in our experiment.

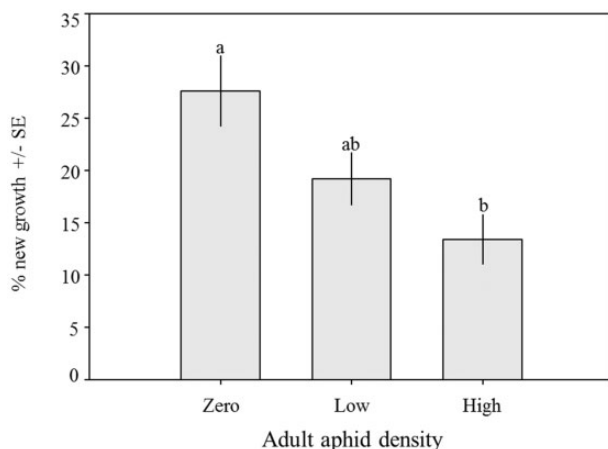


Fig. 1. Effect of aphid density on percent new plant growth (mean \pm SE) over a 5-d feeding period. The percentage of aboveground plant biomass made up of new growth was significantly lower in the high-aphid density treatment than in the zero-aphid control (13 and 27%, respectively; $F_{2,21}=6.48$, $P=0.006$). Bars with different letters represent significant differences (Tukey's test, $\alpha=0.05$).

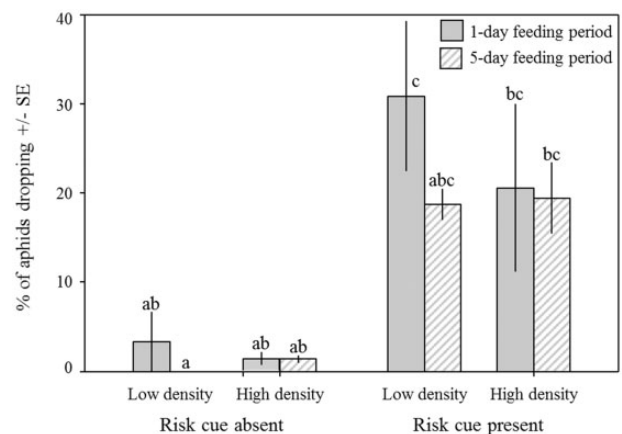


Fig. 2. Effect of alarm cue, aphid density, and feeding period on aphid dropping (mean \pm SE) in response to disturbance. The presence of an alarm cue significantly increased the percentage of aphids dropping across all treatments ($F_{1,40}=36.5$; $P<0.01$). There was no significant effect of aphid density or length of feeding period (both $P>0.05$), and there were no significant interactions between any of the main effects. Bars with different letters represent significant differences (Tukey's test, $\alpha=0.05$).

Although increased aphid densities reduced plant growth (Fig. 1), neither aphid density nor feeding period affected aphid dropping behavior (Fig. 2). In addition, neither factor interacted with the presence/absence of aphid alarm cues. The lack of a density effect may be explained by the fact that our numbers were too low to affect aphid behavior. Although previous work on the greenbug *Schizaphis graminum* found that dislodgement increased with increasing aphid density, aphid numbers in their low-density treatment were similar to those of our high-density treatment (McConnell and Kring 1990). In contrast, Losey and Denno (1998b) used densities similar to ours and found no relationship between pea aphid density and dropping response. Aphid numbers in our high-density treatment were chosen to increase the likelihood that their host plants, which we trimmed to two leaves to aid in aphid detection, would survive 5 d of aphid feeding. Although plant growth in the high-density treatment was significantly lower than in the no-aphid treatment, the impact of this reduction in growth on plant quality appeared insufficient to alter aphid dropping behavior. It is possible, however, that less energetically costly aphid defensive behaviors such as kicking or walking away may be more sensitive to changes in density than aphid dropping (Villagra et al. 2002). Because immature aphids are less likely than adults to drop in response to risk (Roitberg and Myers 1978, Gish et al. 2012), it is also worth noting that adult aphids may exhibit different responses.

Our results suggest a number of ways to modify our experimental design in order to better study *A. pisum* dropping behavior as a function of density and plant health. First, increasing aphid numbers in the high-density treatment above 50 insects per plant should magnify the insects' effect on plant health and increase our ability to detect differences between the low- and high-density treatments. Second, our 1- versus 5-d feeding period treatments, which were intended to assess the importance of plant quality, could be replaced with treatments in which plants were either protected from or exposed to feeding prior to their experimental use as seen in Tokunaga and Suzuki (2007). The length of aphid feeding period could also be extended in order to include the next generation of aphids and observe any transgenerational effects of E- β -farnesene exposure, aphid density, and/or plant health. The alarm pheromone E- β -farnesene has been shown to modulate transgenerational wing induction in *A. pisum*, and solitary aphids produce less E- β -farnesene than do aphids with conspecifics (Podjasek et al. 2005, Verheggen et al. 2009). Nymphs of maternal aphids exposed to alarm pheromone also select less risky feeding sites, a decision that alters colony structure (Keiser and Mondor 2013). As a result, it is possible that the impacts of our treatments can only be observed across a longer time period than the one we chose.

Although we did not see an impact of aphid density or plant health on dropping behavior, our results still contribute to our understanding of aphid antipredator responses, and the conditions in which they do (or do not) manifest. The fact that aphids responded strongly to alarm pheromone demonstrated that they can alter their dropping behavior; the fact that the other factors did not induce similar variation may reflect the energetic and fitness cost of this behavior. By demonstrating how reluctant aphids can be to increase dropping in response to all but the most serious threat (i.e., alarm pheromone), our findings thus add to the results of previous research addressing into the factors affecting aphid antipredator defense.

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