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Dropping Behavior in the Pea Aphid: How Does Environmental Context Affect Anti-Predator Responses?

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DROPPING BEHAVIOR IN THE PEA APHID:
HOW DOES ENVIRONMENTAL CONTEXT AFFECT
ANTI-PREDATOR RESPONSES?

BY

KATHARINE VAN NOSTRAND HARRISON

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
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OF

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ABSTRACT

The relationship between predator and prey has traditionally focused on the act of consumption. More recently, interest has shifted to the intimidation of prey through predator presence and the resulting alterations in prey morphology, behavior, or development. These prey responses, broadly called non-consumptive effects, are energetically costly to prey and can result in changes in prey populations. The study of non-consumptive effects can help further explain complicated trophic level relationships and food webs. The pea aphid, *Acyrtosiphon pisum* (Hemiptera: Aphididae), is a small, phloem-feeding insect that has developed an array of anti-predator responses when threatened. Dropping from a host plant, the costliest of the pea aphid's behaviors, has been subject to a wealth of previous literature. This thesis work explored the relationship between dropping behavior, alarm cues (an indication of predator threat), aphid density, and plant health. Pea aphids were added to broad bean plants (*Vicia faba*) at a low (five aphids) and high (fifty aphids) density and also for a short (one day) and long (five day) feeding period. The treatments were then exposed to a simulated predator via a present or absent alarm cue. The number of aphids that dropped were counted for all treatments. The presence of an alarm cue resulted in significantly more dropping than with alarm cue absent. The density of aphids and length of feeding time did not affect dropping behavior despite a reduction of new plant growth in the high density/long feeding period treatment from control.

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PREFACE

The following thesis has been organized in manuscript format.

TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGMENTS	iii
PREFACE.....	iv
TABLE OF CONTENTS.....	v
LIST OF FIGURES	vi
MANUSCRIPT 1.....	1
TITLE PAGE.....	1
ABSTRACT	2
INTRODUCTION	3
MATERIALS AND METHODS.....	7
RESULTS	10
DISCUSSION	11
ACNOWLEDGEMENTS	14
REFERENCES CITED.....	15

LIST OF FIGURES

FIGURE	PAGE
Figure 1. Effect of aphid density on percent new plant growth over a five-day feeding period.....	18
Figure 2. Effect of alarm cue, aphid density, and feeding period on aphid dropping in response to disturbance	19

TITLE PAGE

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RUNNING TITLE: Factors affecting aphid antipredator responses

TITLE: Dropping behavior in the pea aphid: how does environmental context affect anti-predator responses?

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ABSTRACT

The pea aphid *Acyrtosiphon pisum* Harris (Hemiptera: Aphididae) is a phloem-feeding insect whose anti-predator 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, since aphids affected plant health: production of new plant biomass after five days 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 anti-predator 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.

KEYWORDS

Pea aphid, *Acyrtosiphon pisum*, non-consumptive effect, anti-predator behavior, alarm pheromone, predator, prey, dropping, E- β -farnesene, density

INTRODUCTION

Predators affect prey directly, via consumption (i.e., consumptive effects, ‘CEs’), and indirectly, via the induction of energetically costly anti-predator responses (i.e., non-consumptive effects, ‘NCEs’). 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). While 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, and/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 NCEs 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 tradeoffs 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* take refuge in spider mite webs to avoid the predatory mite *Neoseiulus cucumeris*

(Magalhaes et al. 2007). Hunger also alters prey responses to risk: while remaining hidden avoids predators, prey must forage to survive. Starved mayfly (*Baetis caudatus*) larvae, for example, expose themselves to higher predation risk than less hungry individuals by spending more time in risky but resource-rich food patches (Kohler 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 parthogenetically during the summer growing season. While *A. pisum* move slowly and possess few morphological defenses, they have developed anti-predator 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 low-quality host plants. When food-deprived *A. pisum* are exposed to a parasitoid predator, for example, they use less energetically-costly antipredator 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*, 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. 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, E- β -farnesene, that elicits an array of anti-predator 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*, dropping behavior in response to a coccinellid predator increases as a function of population density; while 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 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*) cultivated at the agronomy farm at the University of Rhode Island, Kingston, RI, USA 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, USA). Seeds were planted in 26 grams of potting mix (Metro-mix 360; Sun Gro Horticulture, Agawam, MA, USA). Bean plants emerged approximately one week after planting. Aphids were maintained in mesh-sided insect cages containing two two-week-old *V. faba* plants. Two fresh plants were added to each insect cage every five days, and aphids were given twelve hours 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 16L:8D photoperiod under fluorescent shop lights.

Prior to the start of an experimental trial, eight uninfested two-week-old *V. faba* plants were trimmed to two leaves per plant and individually potted in a 20 cm plastic pot. After 24 hours, 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-micron 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, high), length of feeding period (short, long), and exposure to aphid alarm cues (present, absent). We tested aphid density by transferring five (low density) or 50 (high density) aphids onto individual *V. faba* plants. We tested the length of feeding

period by allowing aphids to feed on a previously-uninfested plant for one (short feeding period) or five days (long feeding period). We tested exposure to alarm cues by exposing aphids to a crushed aphid (E- β -farnesene present) or neutral cue (absent). 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. We used pre-reproductive aphids to prevent aphid population growth over the experiment and maintain the selected densities 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 above) 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 first-instar nymphs in the five-day treatments in order to prevent aphid reproduction; third-instar nymphs were used for the one-day treatments. Because most of the first-instar nymphs matured into third-instar nymphs by the end

of the five-day treatment, our tests of dropping behavior primarily assessed third-instar nymphs in both the short- and long-feeding-period treatments.

Aphid dropping behavior in a given replicate was tested either one or five days after the experiment began. Our testing protocol consisted of exposing aphids for two minutes to a paintbrush with or without a positive crushed aphid cue. The paintbrush was moved equally among the leaves, gently touching the leaves to simulate predator presence but without contacting any aphids. At the end of the two-minute testing period, both dropped and remaining aphids were counted. The above-ground biomass of plants in the five-day and control treatment was then divided up into old growth and new growth (i.e., biomass added between the start and end of the five-day period). The old- and new-growth biomass of each plant was then weighed in order to determine the percentage of plant growth after five days in the control (zero aphids/ plant), low density (five 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 five days in the control, low density and high density treatments. We tested the impact of risk cues, aphid density, and feeding period on dropping behavior using a fully-factorial three-way ANOVA. Data was checked prior to analysis to ensure that it was normally distributed and that variances were homogeneous; JMP 9.0.0 (SAS 2010) was used for all analyses.

RESULTS

Aphid feeding over a five-day 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 aboveground biomass in the 50-aphid and aphid-free treatments, respectively (Tukey's HSD 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 ($F_{1,40} = 36.5$, $p < 0.01$; Fig. 2). Despite the decrease in new plant growth associated with aphid feeding (Fig. 1), neither feeding period nor aphid density affected dropping behavior ($F_{1,40} = 0.87$ and 1.00 respectively, both $p > 0.10$). 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 ‘alarm pheromone’, is the 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). While we anticipated that aphid alarm cues increased dropping rates, we were surprised at how few aphids dropped in their absence considering the importance of leaf vibration and APBs in previous work (Roitberg and Myers 1978, Clegg and Barlow 1982). 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, the combined visual and vibrational stimulus presented by the paintbrush appeared to evoke minimal ‘fear’ in third-instar pea aphids. While 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). Additionally, 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). Vibrational and visual

cues, in contrast, are not necessarily indicative of risk; in the absence of an alarm pheromone, these cues appear insufficient to induce dropping in our experiment.

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. While 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 five days of aphid feeding. While 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. Less energetically-costly aphid defensive behaviors such as kicking or walking away may be more sensitive to changes in density than aphid dropping; the frequency of these responses might differ according to density in the five-day treatments (Villagra et al. 2002).

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/plant should increase our ability to detect differences between the low- and high-density treatments. Second, our one- versus five-day 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 which 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 anti-predator 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 the factors affecting aphid antipredator defense.

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Figure Legends

Figure 1. Effect of aphid density on percent new plant growth over a five-day 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 HSD, $\alpha = 0.05$).

Figure 2. Effect of alarm cue, aphid density, and feeding period on aphid dropping 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 HSD, $\alpha = 0.05$).

Figure 1.

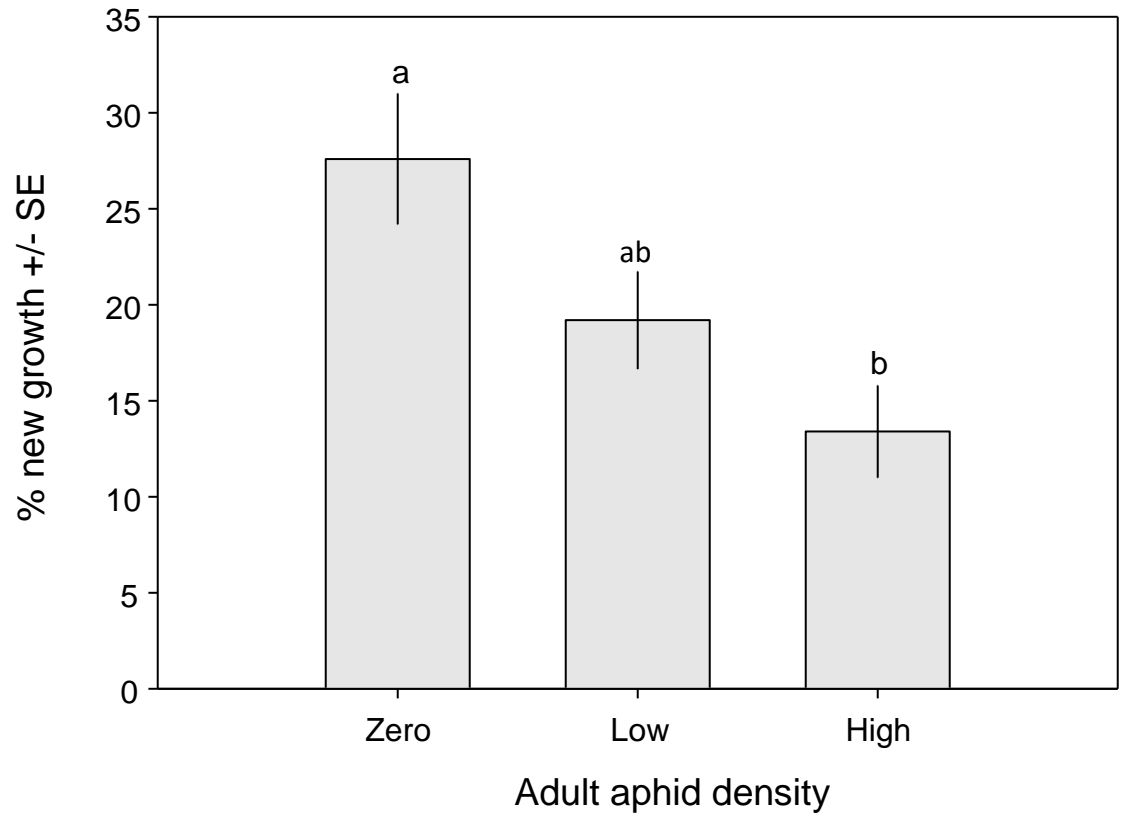


Figure 2.

