

NON-CONSUMPTIVE EFFECTS OF PREDATORY SPINED SOLDIER BUG ON  
COLORADO POTATO BEETLE PREY

A Thesis

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Master of Science

by

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## ABSTRACT

Although the lethal effect of predators on their prey is well established, we know little about how prey in terrestrial insect systems perceive and respond to non-lethal predation threat (non-consumptive effects). By investigating the relationship between the Colorado potato beetle, *Leptinotarsa decemlineata* and its predator, the spined soldier bug, *Podisus maculiventris*, we examined prey behavioral responses when there was a risk of predation as well as the mechanism of prey perception of predators. When exposed to predation risk, beetle adults and larvae reduce their feeding. This effect was still present when larvae were exposed predator volatiles alone. Further, adult beetles reduced oviposition in the presence of non-lethal predators. These results are particularly interesting in attempting to understand the full effect of predators, like the spined soldier bug, as biological control agents in agricultural systems.

## BIOGRAPHICAL SKETCH

Sara began college at Polk County State College in Winter Haven Florida in 2008. After one year, in 2009, she received her Associates of Arts degree from Polk State focused in Liberal Arts. In 2009, Sara enrolled in the Environmental Science and Policy program at the University of South Florida in Tampa, Florida. She earned her Bachelors of Science from USF in 2011, graduating with honors. Then, Sara continued school at Cornell University where she completed her Masters of Science degree in the Department of Entomology with Dr. Jennifer Thaler.

To my beautiful and inspirational son who's warm heart and happy hiccups guided me through the winter. And to J, who will always make up the other half of my whole.

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CHAPTER 1

PREY PERCEPTION OF PREDATION RISK: VOLATILE CHEMICAL CUES  
MEDIATE NON-CONSUMPTIVE EFFECTS OF A PREDATOR ON A  
HERBIVOROUS INSECT

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## ***Abstract***

Predators can affect prey in two ways—by reducing their density (consumptive effects) or by changing their behavior, physiology or other phenotypic traits (non-consumptive effects). Understanding the cues and sensory modalities prey use to detect predators is critical for predicting the strength of non-consumptive effects and the outcome of predator–prey encounters. We evaluated how Colorado potato beetle, *Leptinotarsa decemlineata*, larvae perceive predation risk by isolating cues from its stink bug predator, the spined soldier bug, *Podisus maculiventris*. When exposed to male “risk” predators that were surgically manipulated so they could hunt but not kill, beetles reduced feeding 29 % compared to controls. Exposure to risk females caused an intermediate response. Beetles ate 24 % less on leaves pre-exposed to predators compared to leaves never exposed to predators, indicating that tactile and visual cues are not required for the prey’s response. Volatile odor cues from predators reduced beetle feeding by 10 % overall, although male predators caused a stronger reduction than females. Finally, visual cues from the predator had a weak effect on beetle feeding. Because multiple cues appear to be involved in prey perception of risk, and because male and female predators have differential effects, beetle larvae likely experience tremendous variation in the information about risk from their local environment.

## ***Introduction***

Predator–prey interactions have been classically considered in terms of the direct reduction in prey density caused by predation. However, in many animal systems, more than half of the effect of predators on prey is due to the risk of predation alone, also known as non-consumptive effects (Preisser et al. 2005; Werner and Peacor 2003). Non-consumptive effects are demonstrated through the predator’s ability to impact prey behavior, physiology, development and morphological traits (Werner and Peacor 2003). These changes have the potential to alter prey host choice (Schmitz et al. 1997), survival (McCauley et al. 2011), reproduction (Sheriff et al. 2009, Zanette et al. 2011), and population growth (Nelson et al. 2004), as well as indirect effects on community members and dynamics (Halaj and Wise 2001; Beckerman et al. 1997; Schmitz 1998; Lima 1998; Bernot and Turner 2001). Because non-consumptive effects rely on the ability of prey to perceive predators before being eaten, determining the cues used by prey is essential in predicting the ecological consequences of predator–prey encounters.

Prey can detect predators using visual, tactile, vibrational, chemical or other cues. Multiple cues are typically used by prey independently or simultaneously to detect predation risk (Rosier and Langkilde 2011). For example, in large vertebrate systems, prey use combinations of chemical, visual and auditory cues to detect predators (Webster and Webster 1971; Swihart et al. 1991; Thuppil and Coss 2013).

The perception of predators has also been well studied in aquatic systems in which olfactory cues from predator pheromones, prey alarm chemicals, or chemicals which result from predation of conspecifics drive non-consumptive effects (Kiesecker et al. 2002; Ferrari et al. 2010). Because the diffusion of chemicals in water versus air is quite different, the cues employed and the extent of multi-modal signals in aquatic versus terrestrial systems may also differ. Nonetheless, despite the suggestion that smaller terrestrial organisms such as insects and mice share some facets of perception with large vertebrates, relatively little is known about their olfactory and other sensory abilities (Ache and Young 2005).

The effect of predator cues on prey behavior has been investigated in a few terrestrial insect systems. For example, chemical contact cues left by ladybirds, *Coccinella septempunctata*, have been shown to alter cherry-oat aphid, *Rhopalosiphum padi*, colonization behavior (Ninkovic et al. 2013). Striped cucumber beetles, *Acalymma vittatum*, detect a combination of tactile and visual cues from wolf spiders, *Rabidosa rabida* (Williams and Wise 2003). Most research on predator cue perception in insect systems has focused on adults, which were originally thought to have a stronger perceptive ability than other life stages. However, it has become evident that larval and adult olfactory and chemosensory abilities are similar and are important for larval host choice (Boer and Hanson 1987; Nordenhem and Nordlander 1994; Gerber and Stocker 2007), as well as responses to predation risk by larvae in aquatic systems (Ferrari et al. 2007). Although the visual ability of larval insects is limited, predaceous tiger beetle larvae are able to identify their prey (Mizutani and Toh 1998), and some herbivorous larvae can find host plants at close range (Saxena and Khattar 1977; Harris et al. 1995). Understanding the sensory mechanisms that underlie non-consumptive effects in larval insects could lead to a better understanding of larval insect perception in general, as well as provide insight into possible manipulation of predator-prey dynamics in managed systems.

In this study, we investigated which sensory cues play a role in the detection of a predator by a larval herbivorous insect. The prey, the Colorado potato beetle, *Leptinotarsa decemlineata*, is a major agricultural pest, which can consume up to 100 % of potato foliage and reduce potato yield by >90 % (Ferro 1983; Hare 1990). The spined soldier bug, *Podisus maculiventris*, an omnivorous stink bug predator feeds on Colorado potato beetles naturally and is also augmented for biological control in agricultural crops (Hough-Goldstein and McPherson 1996). Previous work has shown that the threat of predation by this stink bug predator reduces the amount of leaf area consumed by Colorado potato beetle larvae and their mass (Kaplan et al. 2014). The spined soldier bug has the potential to provide tactile, visual, chemical or other cues that the beetle larvae could respond to. In addition, male and female stink bugs each produce a distinct volatile pheromone blend (Aldrich et al. 1984b).

The goal of our study was to tease apart various cues used by Colorado potato beetle larva to perceive predation risk, and to breakdown sources of variation among predators that might contribute to differential outcomes following a predator-prey encounter. We asked four specific questions. First, what is the net non-consumptive

effect of male and female predators on prey feeding? Second, are beetles able to detect the previous presence of predators? Third, do beetles use volatile cues from predators and do they respond differently to the volatiles emitted by male and female predators? Fourth, do beetles use visual cues to detect predators?

## ***Materials and Methods***

### **Study system**

Colorado potato beetle, *Leptinotarsa decemlineata* (Chrysomelidae: Coleoptera), used in this research were obtained from a colony originally established with insects collected from Tompkins County, NY, USA, and reared on potato plants. Wild-collected beetles were introduced every season. Beetles were kept in eleven 3/4"-cube (19-mm<sup>3</sup>) BugDorm rearing cages (BioQuip Products) in the laboratory under an 18:6, L:D cycle. Egg clutches laid by individual females vary in size with an average between 20 and 60 eggs (Hare 1990). Since our experiments required the use of many eggs, clutches were collected from multiple females (one clutch per female) and each clutch was divided evenly between treatments in experiments to account for possible variation in responsiveness.

Predatory spined soldier bugs, *Podisus maculiventris* (Pentatomidae: Hemiptera), used in this study were lab reared offspring obtained from a wild-collected colony from Tompkins County, NY, USA, and reared on a diet of mealworms and potato plants in the laboratory under an 18:6, L:D cycle. Wild-collected stink bugs were introduced to the colony each season. We manipulated predation by the stink bugs in our experiments by creating a predation risk treatment. These "risk" predators were created by removing the final segment of the stink bug's rostrum with a sharp razor blade. Previous studies using this technique have shown that this is effective in preventing the spined soldier bug from consuming prey while still allowing it to forage, survive, mate, and plant-feed normally (Griffin and Thaler 2006; Kaplan and Thaler 2010; Thaler et al. 2012).

*Solanum tuberosum* (cv Yukon Gold potatoes) were planted in 4" (c.100-cm) plastic pots with commercial potting soil. Plants were watered daily and fertilized weekly (21:5:20 N:P:K) in a greenhouse with an 18:6 light cycle. We used leaves from 2- to 3-week-old plants in the experiments.

### **Non-consumptive effects of male and female risk predators**

In order to evaluate the net non-consumptive effects of risk predators and whether the beetle prey responded differently to male versus female risk predators, we compared beetle feeding with three different treatments: male risk predator present, female risk predator present, and a no predator control. Risk predators were placed in a Petri dish (90 × 15 mm; Fisherbrand®) that contained a potato leaflet, moistened cotton and a single 1-day-old beetle larva. Control dishes lacked a risk predator. The experiment was repeated on five separate dates with a total of 30 male risk predator, 28 female

risk predator, and 58 control replicates.

After 3 days, the leaflet was scanned using a CanoScan LiDE 700F Scanner at 600 dpi and the amount of leaf area consumed by larval feeding in each replicate was measured using Image J. A line following the natural curvature of each leaflet edge that was eaten by larva was drawn on the image manually, then the image was changed into black and white so each space eaten registered as white and the undamaged leaflet registered as black. Treatments were assigned numbers and selected randomly for blind analysis when drawing lines. Then Image J calculated total area by filling in all the leaf area, including damaged space, and then calculating the area of undamaged black space by not considering the damaged space. Both measurements were given as an output from the program, the difference was calculated to get total leaf area removed. The scale of each image was standardized.

All experimental data were analyzed with JMP 10 (SAS Institute, Cary, NC, USA, 2012). A two-way ANOVA with predator treatment (control, male, female) and trial as main factors was used to analyze differences in beetle feeding. A square root-transformation of the data was necessary in order to correct for non-normal distribution of the data. An experimental trial was included as a fixed effect in the model. Fixed effects were used in analyses due to unreliability of random effects with less than six groups (Bolker et al. 2008). Tukey's HSD post hoc comparison was used to separate significant differences between means.

#### Previous presence of the predator

To examine the potential influence of residual predator cues on prey feeding behavior, we developed an assay that eliminated the direct effects associated with contact, visual and vibrational cues. Petri dishes were lined with filter paper (90 mm; Whatman™) and a potato leaflet moistened with a cotton ball was placed on top of the filter paper. Then, a predator pre-treatment was conducted that consisted of two adult stink bugs—one male and one female—placed together in treatment dishes and allowed to move freely for 24 h prior to beetle introduction. The stink bugs were able to plant-feed, defecate, and potentially disperse any pheromones/chemical cues. Control dishes were set up the same way but lacked the predators during the 24-h predator pre-treatment. Twenty replicates of each treatment were used in the experiment with beetle larvae from three clutches divided equally between the control and predator pre-treatment.

After 24 h, the stink bugs were removed and a single 1-day-old beetle larva was placed on each leaflet and allowed to feed for 3 days before removal. Leaf area consumed by larval feeding was assessed as described in the above visual experiments. Two-way ANOVA was used to examine the effects of treatment (predator pre-treatment and control) as a fixed effect and beetle clutch (as a fixed effect) on the amount of leaf area removed by each beetle.

#### Volatile cues from the predator

Volatile odors from predators were isolated by blowing air over a group of stink bug predators and into an enclosed arena containing beetle larvae. A 500 mm × 200 mm closed glass volatile collection chamber (Analytical Research Systems, Gainseville, FL, USA) containing stink bugs (mixed sexes, male only, or female only; described below) and one 50-mL beaker with moistened cotton balls (as a water source for the stink bugs) was set up 24 h before each experiment to allow a buildup of chemical odors within the chamber. There was no plant material or beetle larvae within the chamber, eliminating cues from either source. The control glass chamber contained just one 50-mL beaker with moistened cotton balls.

After this initial 24 h, the feeding response of beetle larvae to air blown from the predator or control chambers was assayed. Two wooden 57 cm × 60 cm × 46 cm framed boxes (one for control air and one for predator odor air) with fine mesh sides were attached to the glass chamber using 1/4" × 0.125" (c. 6.3 mm × 3.18 mm) Teflon tubing (Alltech, Deerfield, IL, USA). Each box contained the bottom portion of 30 Petri dishes which were each closed with a piece of 700-μm mesh fixed with a rubber band to prevent beetle escape. Each dish had a single 1-day-old beetle larva and one potato leaflet with moistened cotton. Charcoal filtered air was blown at approximately 100 L/ min through the glass chambers containing the predator treatment or control, and into the boxes containing Petri dishes and larvae. Larvae were allowed to feed for 3 days after which they were removed and leaf area consumed was measured as described above.

We repeated the above described experimental design in nine paired trials with one of three predator sex treatments. Three trials used mixed-sex predators as the odor source (10 adult males and 10 adult females), three trials used male predators only (15 adults), and three trials used female predators only (15 adults). In all trials, there was a control odor and an odor from the predator treatment. Trials with different predator sex treatments were temporally interspersed. Each trial assayed 30 beetle larvae per odor treatment. Beetle larvae from six different clutches were divided evenly between control and predator odor in each trial.

To determine whether there was an effect of predator odor on beetle feeding, our first level of analysis considered odor treatment (control or predator odor), odor treatment pair nested with predator sex treatment, and predator sex treatment (mixed sex, male, or female) in a nested ANOVA. This is a conservative analysis because it treats each trial as an independent replicate ( $n = 18$  independent odor sources). Because we found significant effects of both odor treatment and predator sex treatment on beetle feeding damage, we subsequently analyzed each predator sex treatment separately using ANOVA. In this case, we used the individual beetle as the unit of replication, with our primary goal being to determine the magnitude of effect of male versus female predators on beetle feeding. Data were square-root transformed to correct normality.

Visual effect of the predator

To test whether visual cues from the predator affect beetle feeding, three treatments were established: visual cue only, no-cue control, and predation risk. The predation risk treatment was a male or female non-lethal predator, manipulated as described above, used as a positive control generating the full non-consumptive effect of the predator. Each replicate consisted of a stack of two Petri dishes. The top Petri dishes were sealed using Parafilm®. This design, with the separate Petri dish providing the visual cue, ensured that the prey could not utilize any tactile cue or chemical cues.

The visual treatment received a single stink bug predator in the top Petri dish with moistened cotton to ensure its survival during the experiment while the control lacked visual stimuli of the stink bug in the top dish but included the moistened cotton. The bottom dish of both the visual and control contained a potato leaflet with moistened cotton and a 1-day-old beetle larva. The predation risk treatment consisted of the manipulated risk predator in the bottom dish along with the potato leaflet, moistened cotton and beetle larva while the top dish contained moistened cotton alone. All experiments were conducted in a 18:6 L:D growth chamber at 20 °C. The experiment was repeated in two experimental trials, trial one included 10 replicates of each treatment and trial two included 20 replicates of each treatment. After 3 days, the leaflets were analyzed as above for leaf area consumed as described above.

To determine the effects of visual cues from predators on beetle larvae, ANOVA was used to compare the amount of leaf area removed by beetle larvae among the three treatments. Square root-transformation of the data was necessary in the visual experiment to correct for a lack of normality. Experimental trial was included in the analysis as a fixed effect. Tukey's HSD post hoc comparison was used to separate significant differences between means.

## **Results**

### **Non-consumptive effects of male and female risk predators**

Risk predators changed beetle feeding when compared to controls ( $F_{2,101} = 4.57, p = 0.013$ ). Specifically, the presence of male risk predators resulted in a 29 % decrease in prey feeding when compared to controls ( $p = 0.012$ ). Although, there was also a 20 % decrease in beetle feeding caused by female risk predators, this effect was not significantly different from either controls or the male predator treatment ( $p = 0.20$ ; Fig. 1). The amount of leaf material that larvae consumed differed by experimental trial ( $F_{4,101} = 2.70, p = 0.035$ ), but the experimental trial  $\times$  treatment interaction was not significant ( $F_{8,101} = 1.19, p = 0.31$ ).

### **Previous presence of the predator**

Pre-exposure of leaves to predators caused a 24.4 % reduction in leaf area removed by beetle larvae over 3 days ( $F_{1,34} = 4.73, p = 0.037$ ) when compared to controls

without predator exposure (Fig. 2). The amount of leaf material that larvae consumed differed marginally between clutches ( $F_{2,34} = 2.72$ ,  $p = 0.08$ ), but the clutch  $\times$  treatment interaction was not significant ( $F_{2,34} = 1.03$ ,  $p = 0.39$ ).

#### Volatile cues from the predator

Across our nine experimental trials, volatile odors from predators reduced larval feeding by 10 % compared to larvae receiving the control volatile treatment (volatile treatment:  $F_{1,8} = 5.63$ ,  $p = 0.045$ ; pair nested within predator sex treatment:  $F_{6,8} = 29.83$ ,  $p \leq 0.001$ , predator sex treatment:  $F_{2,8} = 6.41$ ,  $p = 0.022$ ). Compared to controls, volatiles from the mixed-sex predator treatment reduced larval feeding 16 % ( $F_{1,158} = 7.87$ ,  $p = 0.0057$ ), male volatiles reduced beetle feeding 10.6 % ( $F_{1,141} = 4.25$ ,  $p = 0.041$ ; Fig. 3b), and female volatiles had no effect on prey feeding ( $F_{1,155} = 0.008$ ,  $p = 0.93$ ; Fig. 3c).

#### Visual effect of the predator

Visual cues from the predator had an intermediate effect on prey feeding ( $F_{2,81} = 3.71$ ,  $p = 0.029$ ). The full-cue risk treatment decreased feeding by 26 % ( $p = 0.019$ ) compared to the no-cue control. However, the effect of the visual cue alone did not differ from either the no-cue control ( $p = 0.68$ ) or the full-cue risk treatment ( $p = 0.14$ ; Fig. 4) even with a decrease in feeding of 8 and 19 %, respectively. Although the amount the beetle larvae consumed differed by experimental trial ( $F_{1,81} = 39.5$ ,  $p \leq 0.001$ ), there was no experimental trial  $\times$  treatment interaction ( $F_{2,81} = 0.49$ ,  $p = 0.62$ ).

### Discussion

Our results demonstrate the importance of chemical cues in detecting predators for a terrestrial, larval insect. The presence of a predator in the arena prior to the introduction of the herbivore was enough to reduce feeding. Exposure to volatile cues from the predator reduced beetle feeding with stronger effects of odors from male predators compared to female predators. The net non-consumptive effect of the predator appears to result from a combination of olfactory and other cues, the latter of which had weak effects on their own. Our results are consistent with research in large vertebrate and aquatic systems that show prey respond to predator odors. For example, the presence of odors from predators of Eurasian beavers, *Castor fiber*, especially from the river otter, *Lutra lutra*, caused reduced foraging rates and damage by the beaver (Rosell and Czech 2000).

The sensory mechanisms that drive non-consumptive effects have not been well explored in terrestrial insect systems. Although adult and larval insects are known to use olfactory cues to locate their host plants (Landolt et al. 1999; Boiteau et al. 2003; Tanaka et al. 2009), the only study to evaluate olfactory sensory perception in Colorado potato beetle larvae was conducted over half of a century ago (Wilde 1958).

What we do know about how insect larvae use olfactory information from predators suggests that this will be an exciting area of research. For example, aquatic mayfly larvae are able to use chemical cues to distinguish between water with risky and non-risky fish, perhaps through the detection of cues from consumed mayflies (McIntosh and Peckarsky 2004).

Chemical cues from predators can be general, resulting from consumption of prey conspecifics, or species-specific, resulting from unique chemical compounds or blends that individual predator species exhibit (Ferrari et al. 2010). In our experiments, the stronger feeding reduction in response to male predator volatile cues shows sex-specificity in the predator cue. Since our stink bug predators had never consumed Colorado potato beetles as a part of their diet, we can attribute any chemical cue to the predator rather than an odor from beetle conspecifics within predator frass. Known differences in chemical blends produced by male versus female spined soldier bugs could explain the lack of prey response to female odors (Aldrich et al. 1984b, c). Only 1 of 12 identified major dorsal abdominal gland chemicals, (*E*)-2-hexenal, is shared between male and female spined soldier bugs (Aldrich et al. 1984b). At least three pheromone blends have been identified in adult stink bug males, one of which acts as an aggregation pheromone that has been successfully used to attract beneficial predators to Colorado potato beetle infested fields (Aldrich et al. 1984a).

The chemical and visual showiness of male animals may increase detection by their prey (Sakaluk 1990). Because female insects are typically larger and require different quality and quantity of nutrients than males (Telang et al. 2006), it is possible that males are driven by their need to be chemically apparent to females, while females are selected to be stealthy and efficient hunters. A study using beet armyworm, *Spodoptera exigua*, as prey showed that female spined soldier bugs kill, on average, 3.5 times the number of eggs and 1.8 times the number of larvae per day than males (Clerc and Degheele 1994). This suggests the possibility that the potato beetles could be eavesdropping on a sexually selected signal.

Our study shows that specific cues from male predators are important. However, prey likely also respond to non-specific cues and general disturbance and many studies find that prey use multiple cues to detect predators (Williams and Wise 2003; Hlivko and Rypstra 2003). Future studies that distinguish between general disturbance responses and specific responses to predators will be valuable. Ramirez et al. (2010) found that Colorado potato beetle larvae responded differently to being prodded by a wooden dowel than to a damsel bug or a ladybug glued onto a wooden dowel, suggesting that larvae differentiate predator probing from just any disturbance. Although we did not find strong evidence for tactile, visual or vibrational cues being essential in prey perception and response to predators, these and other cues such as predator-induced plant responses could be important, especially for detecting female predators. In our study, volatile cues from the female do not appear to be strong on their own. However, our experiments measuring prey responses to experimentally manipulated, non-lethal male and female stink bug predators, show an intermediate response of prey



to females (Fig. 1). Our previous research also demonstrates that Colorado potato beetle larvae can respond to non-lethal female predators (Kaplan et al. 2014), which suggests that other cues, perhaps tactile or disturbance cues, are important for prey detection of female predators.

Greater knowledge about non-consumptive effects in plant-feeding insect systems could lead to an increased understanding of biological control in agroecosystems. The difference in levels of consumption and risk cues from male and female predators may be a mechanism decoupling the consumptive and non-consumptive effects of predators on prey (Peckarsky et al. 2002). In addition, the cues beetle larvae use to detect male and female predators may operate at different spatial scales, with long-range volatile cues from males and short-distance contact and probing cues from females. The decoupling of consumptive and non-consumptive effects of predators means that, by studying consumption alone, we can miss an important component of predation and that these patterns may be different for male and female predators. A blend of the aggregation pheromone chemicals from the spined soldier bug predator has been successfully used to attract and maintain higher numbers of predators in the field, which aids in suppressing Colorado potato beetle populations (Aldrich and Cantelo 1999). Our results suggest that the application of volatile predator chemicals could have an alternative beneficial effect by directly altering prey feeding behavior in an agricultural field. The spatial pattern of predation, measured as consumption of prey, is directly related to proximity of predators and prey. Although more research must be done to evaluate the ability of these cues to affect other life stages of the prey as well as possibility for cues to change over time, adding the volatile cue from a predator has the potential to decrease the density of predators needed for successful biological control. Predator odors have been used as biological control in several other systems. For example, urine of a number of predators reduces feeding damage by snowshoe hare, *Lepus americanus*, on coniferous tree seedlings (Sullivan and Crump 1984). In another system, Rypstra and Buddle (2013) show that non-volatile cues such as the physical presence of silk collected from spider predator *Tetragnatha elongata* reduces plant damage by Japanese beetle, *Popillia japonica*, and Mexican bean beetle, *Epilachna varivestis* prey. Our unpublished results indicate that non-consumptive effects of stink bug predators account for 50 % of the reduction in leaf damage by Colorado potato beetle caused by a lethal predator (Thaler, unpublished), suggesting that enhancing this effect could result in more effective biological control. Our understanding of larval herbivore olfaction in an ecological context is in its infancy, yet may be important for using predator cues to our advantage, such as in biological control systems in which we attempt to maximize the consumptive and non-consumptive effects of predators.

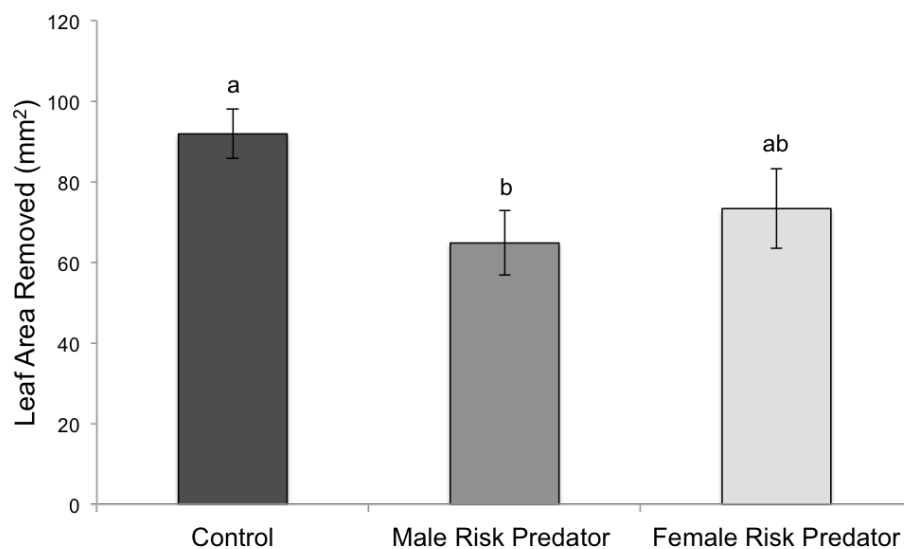
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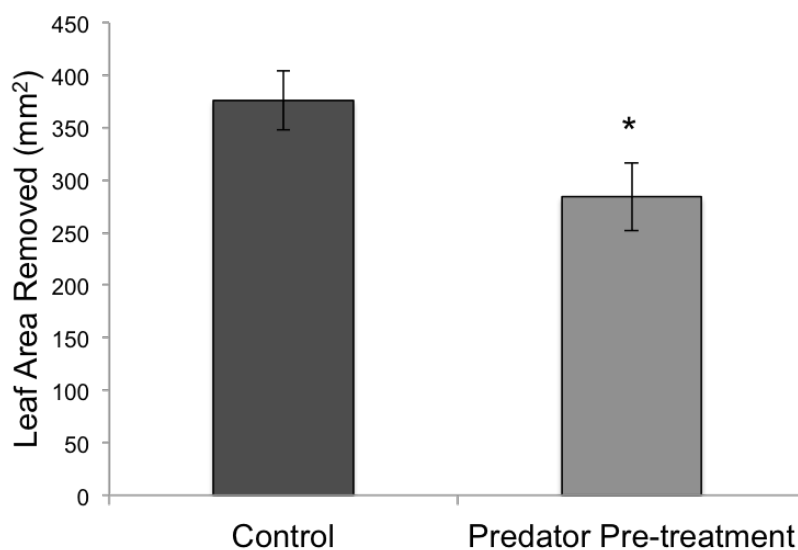
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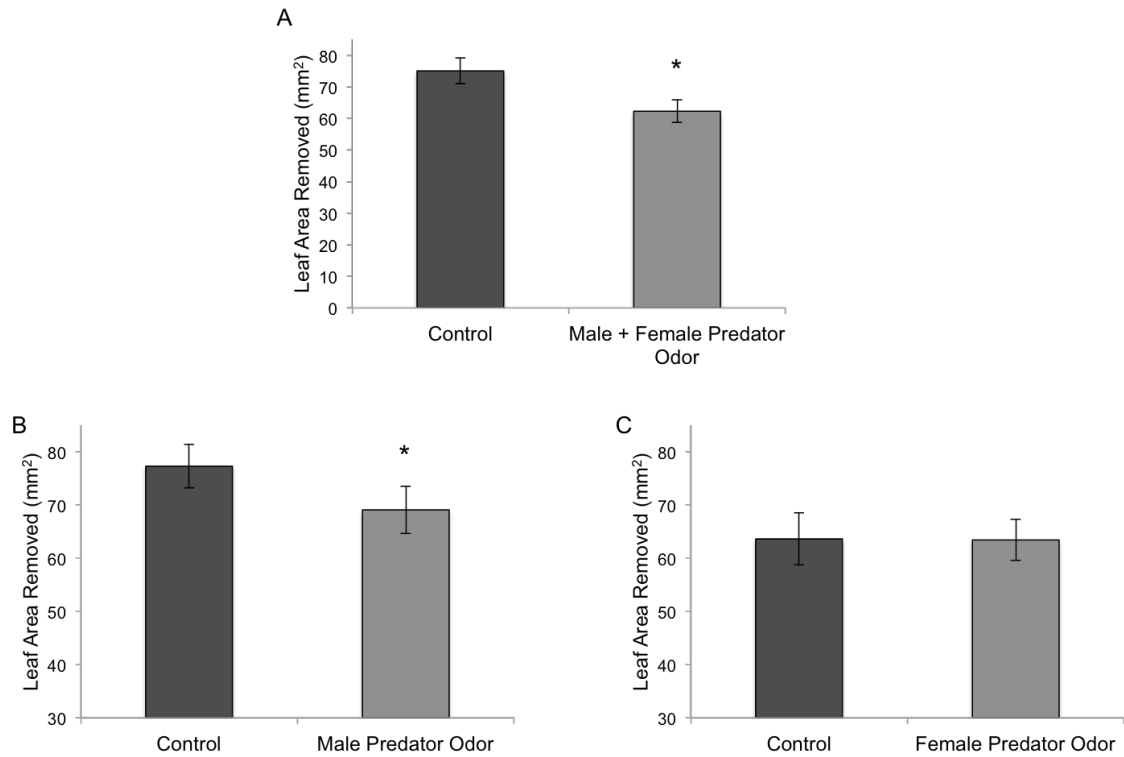
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**FIGURES**

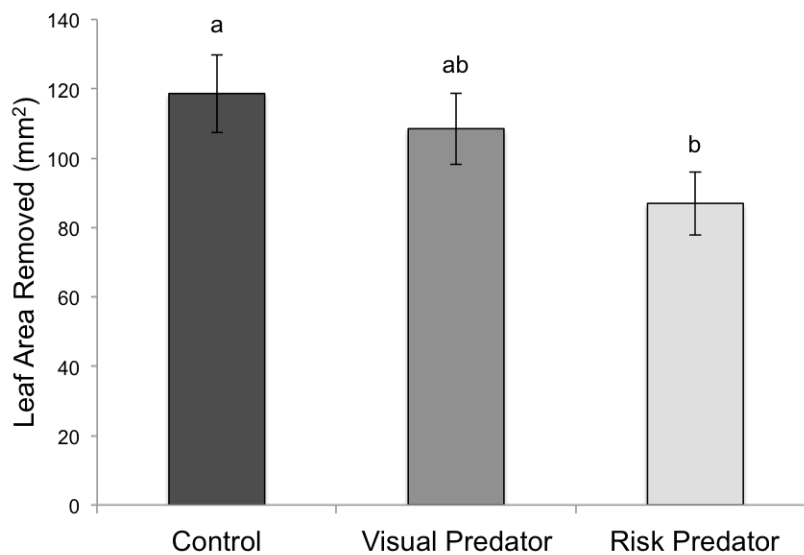
**Figure 1** – Leaf area removed by Colorado potato beetle larvae in response to exposure to control (no predator), a male risk predator or a female risk predator. Bars = means ( $\pm 1$  SE). Letters above bars indicate significant differences at  $P < 0.05$  following Tukey's post-hoc test.



**Figure 2** – Leaf area removed by Colorado potato beetle larvae in response to a control and an arena where a predator was previously present but subsequently removed (predator pre-treatment). Bars = means ( $\pm 1$  SE).



**Figure 3** – Leaf area removed by Colorado potato beetle larvae in response to volatile chemical odors from the predator versus control (blank) air. A) Effect of control air versus mixed sex stink bugs on beetle feeding. B) Effect of control air versus male stink bugs on beetle feeding. C) Effect of control air versus female stink bugs on beetle feeding. Bars = means ( $\pm 1$  SE).



**Figure 4** – Leaf area removed by Colorado potato beetle larvae in the control, visual predator, and risk predator treatments. Bars = means ( $\pm 1$  SE). Letters above bars indicate significant differences at  $P < 0.05$  following Tukey's post-hoc test.



## CHAPTER TWO

### PREDATOR DRIVEN BEHAVIORAL SHIFTS IN AN AGRICULTURAL PEST SYSTEM

## ***Abstract***

Much of the impact of predators on prey occurs via non-consumptive effects, where the prey changes its behavior, development or habitat to avoid being eaten by the predator. Most research on non-consumptive effects in insects has been conducted on the prey stages that are vulnerable to predators, but we know less about how adults are affected by predator presence. Adults may change their feeding and oviposition behavior to protect their offspring from predation. To evaluate this potential effect we used a common and devastating agricultural pest as prey, the Colorado potato beetle, *Leptinotarsa decemlineata*, as well as the commonly used generalist stink bug predator, Spined Soldier Bug, *Podisus maculiventris*. Using laboratory and field experiments Colorado potato beetle oviposition and feeding behavior was measured either with or without risk stink bug predators. Risk predators, could not feed on Colorado potato beetle but left other cues intact. The laboratory results show a significant reduction in feeding in the presence of the predator as well as a reduction in number of egg clutches laid. Field results show a significant reduction in feeding in response to stink bug predators but no change in beetle colonization. Pinpointing which cue(s) drive these behavioral changes could lead to potential manipulation of the relationship, which could increase the overall efficiency of the predator effect. This is of increasing interest in agricultural systems attempting to use biological control due to the rapid insecticide resistance that is seen in many insect pests as well as the overall negative effect that pesticides have on the environment and human health.

## ***Introduction***

Interactions between predators and prey are complex; more than just the simple interaction of predator catching and consuming, prey also respond to the threat of predators before they are eaten. Prey respond to predators by altering feeding behavior (Swihart et al. 1991; Schmitz et al. 1997; Griffin and Thaler, 2006), changes in reproduction and life history (Sheriff et al. 2009; Zanette et al. 2011), and even by reduced survival (McCauley et al. 2011). These behavioral changes vary across taxa as well as between life stages within a particular species. Altered behavior can affect the individual or its offspring. For example, adult insects are often less vulnerable to predators than the early stages of their offspring; therefore adult behavior may change to protect themselves from predators or to ensure their offspring begin life in a safe environment. For example, in Kenya, adult *Anopheles gambiae* mosquitos lay fewer eggs in rainwater containing the scent of backswimmer (*Notonecta* sp.) predators even though these predators solely threaten larval stages (Munga et al. 2006). This behavioral shift is also found in the mayfly, *Baetis bicaudatus*, where oviposition sites are chosen based upon lower risk of egg predation (Peckarsky et al. 2000). The previous two examples are extreme because the adult is not even present in the larval environment and so completely escapes predation, in other examples, both the adult

and its offspring are potentially vulnerable to predation. In this case, the adult may try to reduce both its own risk of predation and that of its offspring.

Though we know that non-consumptive effects occur in many natural systems, less is known about how pest species in an agricultural context will respond to predation threat. Prey behaviors such as colonization, oviposition, and feeding are especially important because they lead to the negative impact of pest species and can be affected by predators. And, because adults make decisions based on the safety of their offspring, predators may affect life-stages of pests that are typically not eaten by the predator, which opens up new avenues for using predators in biological control. Understanding the relationship between pest and predator has the opportunity to increase the efficiency of applied manipulation of predation in integrated pest management.

Recently, there has been evidence that larvae of the Colorado potato beetle, an important agricultural pest species, respond to odors from adult stink bug predators, by feeding less (Hermann and Thaler, 2014; Kaplan et al. 2014). But, we don't know if the adult beetles in this system also change their behavior. Adult insects have a need to protect themselves and their offspring in order to increase fitness and may therefore utilize enemy free-space. Without a choice, we expect the threat of predation will cause the beetle to alter its behavior to be less risky or obvious to the predator.

This study was designed to explore the effect of predation risk on adult Colorado potato beetles. Three specific questions were considered. First, do beetles alter oviposition in presence of risk predators? Second, does the presence of predators in the field alter natural beetle colonization and host plant consumption? Lastly, do beetles alter consumption in the presence of predators in a no-choice field setting?

## **Methods**

### *Study System*

Colorado potato beetle, *Leptinotarsa decemlineata* (Chrysomelidae: Coleoptera) used in this research were lab reared offspring obtained from a wild-collected colony from Tompkins County, NY, USA and reared on a diet of potato plants. Wild-collected beetles were introduced to the colony every season. Beetles were kept in 11-3/4" cube BugDorm rearing cages (BioQuip Products Inc.) in the lab under an 18:6, L:D cycle. Adult beetles were collected as they emerged, sexed and placed in a small solo cup as male-female pairs for 24 hours to mate. Mated females were used about 5-7 days post emergence in laboratory experiments.

Predatory spined soldier bugs, *Podisus maculiventris* (Pentatomidae: Hemiptera) used in this study were lab reared offspring obtained from a wild-collected colony from Tompkins County, NY, USA and reared on a diet of mealworms and potato plants under an 18:6, L:D cycle. Wild-collected stink bugs were introduced to the colony

each season. We manipulated predation risk in our experiments by creating stink bug predators that could hunt but not kill Colorado potato beetles. These “risk” predators were created by removing the final segment of the stink bug’s rostrum with a sharp razor blade. Previous studies using this technique have shown that this is effective in preventing the spined soldier bug from consuming prey while still allowing it to survive, mate, and plant-feed normally (Griffin and Thaler 2006; Kaplan and Thaler 2010; Thaler et al. 2012).

*Solanum tuberosum* (cv Yukon Gold potatoes) were planted in 4” plastic pots with commercial potting soil. Plants were watered daily and fertilized weekly (21:5:20 N:P:K) in a greenhouse with an 18:6 light cycle. We used two to three-week-old plants in the experiments.

#### Oviposition by Beetles *Greenhouse Oviposition Assay*

To examine the potential effects of predator presence on Colorado potato beetle oviposition we set up a ‘no-choice’ experiment using intact potato plants. Individual plants were centered on top of a clear plastic deli lid (Solo<sup>®</sup>, product LGC88B). Each plant was then covered with a 12” tall and 5” wide clear cylinder made from plastic film (Grafix<sup>®</sup>, 0.005 Dura-Lar Film) that fit inside the lip of the plastic lid for a sealed arena. The clear plastic cylinder was closed on the top with a 700-μm mesh fabric to eliminate test insects from escaping, provide sufficient exchange of air, and reduce the formation of mold and fungi.

Each arena was randomly assigned one of two treatments: risk predator or control. Risk predator treatments consisted of a single mated adult female Colorado potato beetle as well as 2 stinkbug adults (one male and one female). Control treatments were predator-free, receiving only one adult female Colorado potato beetle. A total of 68 replicates were completed, 34 from each treatment.

Experiments ran for four days in the greenhouse under an 18:6 light cycle. After four days, each plant arena was monitored for the number of egg clutches as well as the total number of eggs laid by each Colorado potato beetle. The beetles are polyandrous and will lay many egg clutches throughout their lifetime (Hare, 1990). When the beetle died or no eggs were found within an arena, the entire replicate was removed from the study to eliminate error resulting from misidentification of individual beetle sex. In total, 13 risk predator treatments and 15 control treatments were removed.

All experimental data were analyzed with JMP 10 (SAS Institute, Cary, NC, USA, 2012). A Student’s t-test was conducted to compare the mean number of eggs as well as the mean number of clutches laid by beetles in risk predator or control settings.

#### *Presence of Beetles in the Field/Colonization*

The effect of predator presence on beetle colonization was measured in the field by monitoring where adult beetles chose to settle on potato plants. Yukon Gold potatoes were planted from tubers on May 16<sup>th</sup>, 2013 in a field plot in Freeville, New York. Using a potato planter, potatoes were spaced 0.3m between plants within a row and 1m between plants across rows. They were left to grow undisturbed until breaking through the ground at which point they were covered (June 9<sup>th</sup>, 2013) with landscaping fabric (Agrifabrics PRO17, Alpharetta, Georgia) to reduce colonization by insects. When the plants were approximately 0.5m tall (June 19<sup>th</sup>, 2013) the landscaping fabric was removed and two treatments were randomly assigned: risk predator (5 male and 5 female stink bug adults bagged on the plant using PRO-17 fabric (Agrofabrics, Alpharetta, Georgia)) and control (bagged plant). The risk predator treatment in this experiment utilized bagged predators rather than manipulated free-range predators (as used in the previous experiment) to ensure predator location within the field was confined to our treatment plots and to allow for open plots where beetles could colonize naturally. There were a total of 43 replicate plots, each plot consisting of 5 consecutive plants: 1 treatment plant at one end and 4 subsequent surveyed plants. The plots were separated by at least 1m within rows. The treatment plant was randomly selected to be on the right or the left of the plot and the treatments were randomly assigned to plots throughout the field.

Plants adjacent to the bagged treatment plant were monitored for adult Colorado potato beetle presence on 6 occasions starting on day 2 and every 3 days after that up to day 18. On each occasion beetles were removed along with any egg clutches and at the end of the 18 day period the total number of adults found in each replicate was tallied and pooled. The time between monitoring is not long enough for eggs to hatch, therefore reducing unwanted damage from the larval stage.

At the end of the 18 day period, we removed all damaged leaves from the plant directly adjacent to the treatment or control plant to evaluate whether risk predator treatment altered beetle feeding in the field. Only Colorado potato beetles and flea beetles (*Epitrix cucurmeris*) were found in significant numbers within the field; these two beetles have distinctly different damage that was easily distinguishable using our methods. We analyzed amount of leaf area consumed by adult beetle feeding in each replicate using methods from Hermann and Thaler (2014). Two plots where larvae were found were removed from the study so we could ensure the damage was from adult beetles only. There were a total of 41 replicates, 20 predator treatment and 21 control.

A Fisher's exact test was used to determine whether the number of eggs or egg clutches differed between predator present plots or control plots. To compare the amount of leaf area removed by beetles in the field in predator present or control replicates the data were log transformed to correct for normality and a Student's t-test was done.

## **Results**

### *No-choice Oviposition Assay*

The number of eggs laid by beetles decreased 36.6% in the presence of risk predators when compared to total eggs laid in controls ( $t = -2.94$ ,  $p = 0.005$ ; Figure 1a). Similarly, there was a 26.4% reduction in the total number of clutches laid by beetles in predator present treatments compared to controls ( $t = -2.40$ ,  $p = 0.019$ ; Figure 1b).

#### *Presence of Beetles in the Field/Colonization*

While the total number of beetles found in the field did not differ between treatments (Control – 21 individuals, predator treatment – 14 individuals;  $p = 0.543$ ), risk predators in a field plot did result in a 63.9% reduction in beetle feeding when compared to control plots with no stink bugs ( $t = -2.23$ ,  $p = 0.032$ ; Figure 2).

#### **Discussion**

Colorado potato beetle adults responded to non-consumptive effects from their stink bug predator, the spined soldier bug. Changes in Colorado potato beetle oviposition in response to stink bug predators were demonstrated in our study by a 35% reduction in total eggs laid and a 26% reduction in the number of clutches. Although we have observed adult Colorado potato beetles being consumed by the stink bugs in the laboratory and field, past studies only consider the egg and larval stages to be affected by predators (Hough-Goldstein and McPherson, 1996; Alyokhin, 2009). This research brings to light that even a relatively invulnerable life stage can be affected by predator presence.

Current research on oviposition preference changes considers enemy-free space and food quality in sites, mostly for the benefit of the offspring. For example, tree frogs *Hyla femoralis* have been shown to avoid oviposition in water containing their fish predators (*Umbra pygmaea*), choosing to lay in pools that may not have sufficient algae, which correlated to poor larval development (Rieger et al. 2004). Changes in oviposition location and amount have also been shown to later affect development, body and wing size and ultimately survival of the progeny in *Anopheles pseudopunctipennis* mosquitos (Bond et al. 2005). Furthermore, choosing to oviposit in a “safer” place may better your chances for survival; in the case of *Manduca quinquemaculata*, adults will choose to oviposit on lower leaves, even though they have a higher level of harmful nicotine substances, because of the risk of predation attack is 40% lower (Kessler and Baldwin, 2002). These kinds of tradeoffs between safe sites and sites with good quality food may also occur for Colorado potato beetle as the stink bugs prefer low resistance plants (Thaler et al. 2014). This observed spatial change in oviposition preference in response to predation threat may lead to a change in host choice preference all together (Ballabeni, 2001).

Oviposition in a predator-free space is only one reason that an animal may alter host choice preference. There exists a general need for self-protection to preserve fitness and promote optimal nutrition, which will eventually lead to successful mate finding and reproduction. In our study, capture rate of adult Colorado potato beetles was low

in the field and may not have accurately demonstrated where they would settle in the field. However, in other systems, colonization is affected by predation risk. For example, white tailed deer, *Odocoileus virginianus*, have been shown to reduce foraging on their preferred host plant in response to bobcat (*Canis latrans*) and coyote (*Lynx rufus*) predator odors (Swihart, 1991). In another system, colonization of bird cherry-oat phid, *Rhopalosiphum padi*, is reduced upon detection of cues from seven spot ladybird (*Coccinella septempunctata*) predators.

Altered host-choice preference in a system can affect quantity of food source for the prey as well as the quality of the food source. Because of this, there is a constant need to balance feeding choices with anti-predator responses (Lima and Dill, 1990). If a prey item chooses to stay on the optimal host, there may be a reduction in the amount of food eaten to avoid being seen. Reduction in feeding amount is a common response to predation risk (Pressier et al. 2005; Hassell and Southwood, 1978). Some organisms can reduce feeding while at the same time compensating for the lack of consumed food with increased assimilation efficiency (Thaler et al. 2012). But, changes in feeding amount typically come at a cost to the prey in the long term (Metcalf and Monaghan, 2001; Auer et al. 2010). However, reduced feeding as a consequence of predation risk can be a useful trait to manipulate in systems where it is undesirable for the prey item to consume its host.

Many studies now acknowledge the importance of non-consumptive effects when attempting to understand predator-prey dynamics in their entirety (Schmitz et al. 2004; Preisser et al. 2005; Griffin and Thaler 2006), though only a few recognize the potential to manipulate non-consumptive predator effects (Blaustein et al. 2004; Thaler and Griffin, 2008). Finding significant changes in behaviors that would benefit the crop in question, such as oviposition and leaf consumption of a major pest highlight the importance of investigating what types of changes occur in response to predators, especially by predators used as biological control agents in agricultural or applied systems.

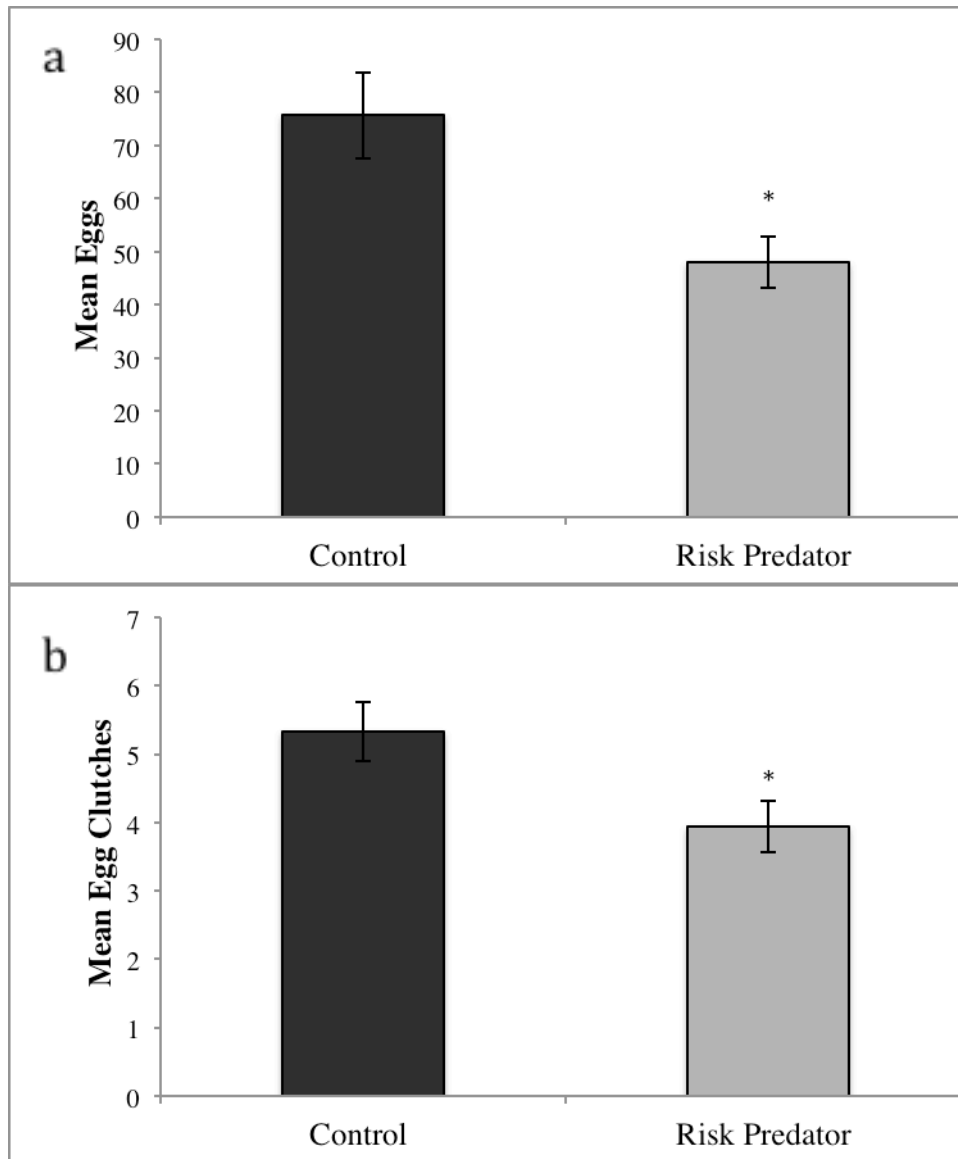
This study provides insight into the potential for agricultural systems to be a model for new integrated pest management strategies, where the threat of predation causes changes in the prey pest species that would have a beneficial outcome to the crop. Risk of predation, or the non-consumptive effect, can lead to increased understanding of biological control systems where predators are augmentatively released to control specific life stages. In this system Colorado potato beetles are consumed by the stink bug biological control agent, which can be used to effectively control the larval stages (Hough-Goldstein and McPherson, 1996). However, the fate of the field is determined, in part, by initial field colonization by overwintering adult beetles (Baker et al. 2001). Our study shows that life stages that are important to control but are typically considered uncontrollable by predators are affected in non-lethal ways by the predators. This can lead to more pinpointed and sustainable control methods within crop systems to control pests that have been increasingly difficult to manage due to developed resistance and increased regulations on chemical control.

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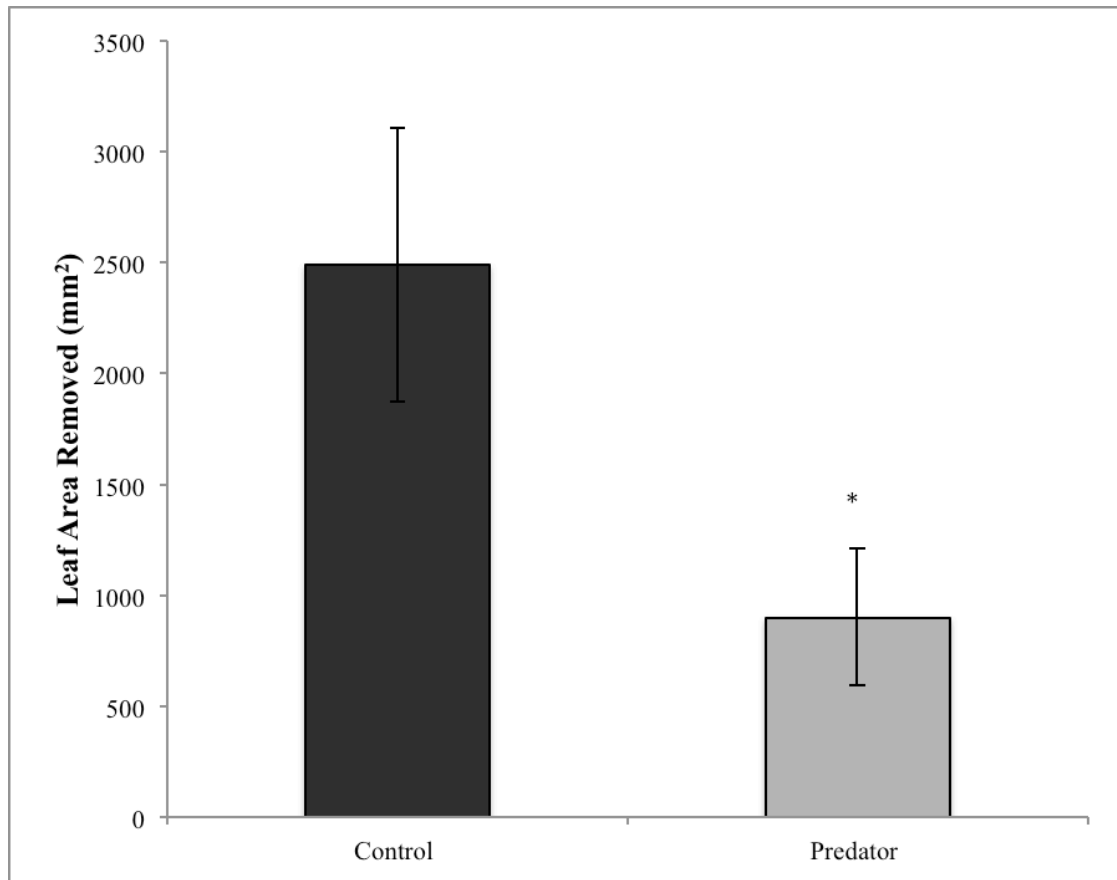
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**FIGURES**

**Figure 1a & 1b** – Effect of stink bug risk predator on Colorado potato beetle oviposition in a greenhouse study. Each experimental arena consisted of a single potato plant enclosed by a small plastic cylinder and one female Colorado potato beetle adult. Treatments within the arenas included either no predators (control) or risk predators. **a** Effect of predator presence on total number of eggs laid by Colorado potato beetle. **b** Effect of predator presence on the total clutches laid by Colorado potato beetle. Bars mean ( $\pm$ SE).



**Figure 2** – Leaf area removed by Colorado potato beetle adults in the field in either control (predator-free) or predator treatments. *Bars* mean ( $\pm$ SE).