IMPACT OF PREDATION RISK ON THE BEHAVIOR AND PHYSIOLOGY OF INSECTS IN AGRICULTURAL SYSTEMS

By

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A DISSERTATION

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

Entomology–Doctor of Philosophy Ecology, Evolutionary Biology and Behavior–Dual Major

ABSTRACT

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Non-consumptive effects are impacts on prey survival and performance that are related to investment in anti-predator defenses. Without considering direct consumption by predators, non-consumptive effects contribute a large portion of the net effects of predatory insects have on their prey. Because the central tenant of agricultural systems is to produce the highest profitable yield; understanding how we can harness, manipulate, and foster predator non-consumptive effects will be helpful in development of pest management techniques. While non-consumptive effects are now known to occur quite commonly, both the spatial and temporal scale of studies are limited (demonstrated in Chapter 1). It is therefore necessary to expand our work to better understand these interactions in natural systems over longer time periods. Here I examined the potential for non-consumptive effects in an important agricultural predator-prev system between a common herbivore prey (*Pieris rapae*) and ubiquitous predator (*Harmonia axyridis*) in both the laboratory (Chapter 2) and field (Chapter 3). Interestingly, even though these species overlap in spatial distribution and phenology, little to no effect of predation risk on prey behavior was found. Further, when examining their interactions in an open-field environment, no significant non-consumptive effects on Pieris rapae were found. However, manipulatively increasing predator cues in the open-field experiment did have significant impacts on a secondary insect pest (Aphidae spp). This work emphasizes the importance of examining community interactions at the field level. In addition, nonconsumptive effects resulting from *H. axyridis* on aphids (*Myzus persicae*) were found to be strong, resulting from changes in both aphid behavior and physiology (Chapter 4). Overall, these studies demonstrate that predator non-consumptive effects are prey species dependent and that studies in natural settings, over larger spatial and temporal scales, will allow us to better understand these complex interactions.

To my house full of beautiful boys: the one who has held my hand and heart for the past 12 years (JGA), the one who warms my soul, gives me hope and made me a mother (AEA) and to the newest addition who kicked me from the inside, keeping me motivated and so incredibly grateful through the final year of this process (JKA).

ACKNOWLEDGEMENTS

I thank the members of my Ph.D. committee: Drs. Douglas Landis, Tom Baker, Rufus Isaacs and Scott Peacor for their flexibility, guidance, encouragement and feedback during this process. I would also like to thank Christie Bahlai for statistical consulting, mentorship and friendship throughout my degree. There were many people that played a role in execution of this work including Julia Perrone, Carissa Blackledge, Lane Proctor, Danielle Ellis, Sydney Bird, Aubrey McElrath, Marguerite Bolt, and JP Kole. Funding for this research was made available in part by Project GREEEN, the MSU Plant Science Fellowship, NSF LTER and USDA NIFA AFRI Predoctoral Fellowship (2017-07208). I also thank MSU and the Department of Entomology for providing several travel grants for domestic and international travel which allowed me to present this work in a variety

of venues.

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CHAPTER 1: SCALING UP OUR UNDERSTANDING OF NON-CONSUMPTIVE EFFECTS IN INSECT SYSTEMS

INTRODUCTION

Predator-prey interactions are among the most important topics in insect ecology and have significant implications for pest management. Understanding how arthropod predators alter prey abundance through direct consumption is critical to understanding population structure and community dynamics. However, prey are not defenseless and constantly make adjustments in behavior and physiology to avoid predation (Sih 1986). Increasingly, it is apparent that prey also detect and utilize a variety of cues to avoid encounters with predators (Turner et al. 2000). Predator-mediated changes in prey biology that do not involve direct consumption are termed non-consumptive effects (hereafter, NCEs). Further understanding of how NCEs alter predator-prey dynamics is particularly needed in managed ecosystems where biological control seeks to manipulate insect natural enemies for pest management (Hajek 2004).

Non-consumptive effects — also referred to as non-lethal effects, risk effects or trait-mediated interactions — are changes in prey biology driven by predation threat. Predator induced NCEs are well studied in aquatic and terrestrial vertebrate systems and can manifest as changes in prey behavior, life history, reproduction, physiology, as well as other phenotypic traits (Werner and Peacor 2003b). These diverse predator effects can alter species interactions, and are critical for understanding community and ecosystem dynamics. In fact, NCEs have been shown to be equally or more important in altering prey population dynamics than consumptive effects (Werner and Peacor 2003b, Preisser

et al. 2005a). Far less is known about NCEs in terrestrial insects, although the field is rapidly expanding.

Most of our current knowledge of how insect predators affect insect prey comes from small-scale laboratory or mesocosm experiments over relatively short time scales. In small-scale experiments, NCEs have been shown to have impacts that cascade to nonprey organisms and even further into the ecosystem processes and functions themselves (Werner and Peacor 2003b). For example, Hawlena et al. (2012) described an increase in the body C:N ratio of grasshoppers (*Melanoplus femurrubrum*) in response to hunting spider (*Pisuarina mira*) presence compared to predator-free controls (Hawlena et al. 2012). The resulting physiologically stressed body carcasses altered soil community function by slowing decomposition of leaf litter in small mesocosms. The indirect effects of insect NCEs could have wide-reaching impacts at the community and ecosystem level, further investigation of this understudied aspect of insect science is critically needed at more realistic scales.

Here we review studies of terrestrial insect NCEs to identify recent advances in the field and knowledge gaps. Given the relative youth of this field, we reviewed studies published within the last five years (n = 34) that examined the NCEs of terrestrial arthropod predators on prey in the absence of lethal encounters. Authors established predator risk to prey in the absence of a lethal encounter by; 1) manipulation of mouthparts (physical removal or gluing them shut), 2) physically isolating predators from prey with a barrier, or 3) by using isolated predator cues (visual models or predator

odors). Such studies allow isolation of the impact of fear of predation on prey behavior and biology. Below we discuss ways that insect prey may respond to predation risk, the mechanisms that allow for risk detection, the ecological consequences of these interactions, and implications for using this knowledge to increase pest suppression.

Non-Consumptive Effects of Predators

Insect Responses to Predation Risk

Shifts in insect prey traits in response to predators include behavioral changes (Ballantyne and Willmer 2012, Gonzálvez and Rodriguez-Gironés 2013, Tan et al. 2013, Li et al. 2014, Siepielski et al. 2014), life history adjustments (Sitvarin et al. 2015, Xiong et al. 2015, Elliott et al. 2016), and physiological changes (Kaplan and Thaler 2012, Thaler et al. 2012, Janssens and Stoks 2014, Wineland et al. 2015, Rendon et al. 2016) (See Table 1.1 & 1.2 for behavioral and physiological changes, respectively). The majority of studies to date link NCEs to changes in behavior, including changes in feeding (Rypstra and Buddle 2013a, Kaplan et al. 2014, Thaler et al. 2014), oviposition (Wasserberg et al. 2013, Sendoya et al. 2015), colonization or dispersal (Ninkovic et al. 2013a, Bucher et al. 2015, Kersch-Becker and Thaler 2015b), host-plant preference or habitat use (Wilson and Leather 2012, Lagrue et al. 2015, Sidhu and Wilson Rankin 2016) and increased predator avoidance (Hoefler et al. 2012, Lee et al. 2014). As a whole, prey tend to respond to predators by modifying their behavior to become less apparent and reduce predator encounters (i.e. less risky). Understanding the mechanisms by which prey detect and respond to predation risk is key to the ability to manipulate NCEs for pest suppression.

Mechanisms of Predator Detection

Mechanisms for detecting predators with sufficient time/space to avoid predation is essential for prey. This could result in selective pressure favoring eavesdropping behaviors that allow them to respond before a predation event, and conversely for predators to become stealthier. In studies where the mechanism of NCEs is considered, the vast majority focus on chemical cues whereas other mechanisms including visual or tactile cues are currently understudied. Moreover, the potential for multiple mechanisms of predator detection has hardly been considered in insect systems, with only a few studies directly investigating a combination of cues (Janssens and Stoks 2013a, 2013b, Bray and Nieh 2014, Hermann and Thaler 2014).

Visual Detection of Predators: Insect visual ability is often underestimated, but has been shown to contribute to predator detection (Freitas and Oliveira 1996). For example, predator models made to look like crab spiders (Thomisidae) decreased visitation by pollinators to floral resources (Antiqueira and Romero 2016). In addition, dead ants (*Camponotus and Cephalotes*) - dried and pinned to plants - to serve as a visual predator proxy, decreased oviposition by butterflies (*Eunica bechina*) (Sendoya et al. 2015). Other evidence of visual cues driving prey perception of risk has been found by isolating a dragonfly predator (*Anax*) behind a clear barrier, which led to slower growth, reduced escape speed and increased oxidative damage to its damselfly prey (*Enallagma cyathigerum*) (Janssens and Stoks 2013a, 2013b). The visual ability of insects is not considered to be exceptional and may underestimate its potential to play a role in NCEs,

especially for juvenile insects. However, visual cues could provide important information in conjunction with other predator cues and should not be discounted.

Chemical Detection of Predators: It is well established that insect predators use chemical cues to locate prey (Hatano et al. 2008) and more recently it has been shown that prey have the ability to eavesdrop on predator chemical cues (Gonthier 2012, Hoefler et al. 2012, Gonzálvez and Rodriguez-Gironés 2013, Hermann and Thaler 2014). Studies examining the role of chemical cues in NCEs have investigated isolated semiochemicals (Li et al. 2014, Sidhu and Wilson Rankin 2016), chemicals associated with consuming conspecifics (Janssens and Stoks 2013a), footprints or previous foraging of predators (Cembrowski et al. 2014), and crushed predators applied to plants as a slurry (Gonthier 2012). Using an experimental arena where predators had previously foraged and were subsequently removed, researchers can isolate specific contact chemical cues as a source of information for prey. For example, one experiment revealed significantly fewer bird cherry-oat aphids (*Rhopalosiphum padi*) colonize plants where the ladybeetle predators (Coccinella septempunctata) had previously foraged relative to control plants (Ninkovic et al. 2013a). Furthermore, the potential for volatile cues to affect prey behavior has been demonstrated by observing Colorado potato beetle (Leptinotarsa decemlineata) feeding behavior while under a constant airflow containing volatiles from their stink bug predator (Podisus maculiventris). Here, beetle feeding was significantly reduced compared to beetles feeding under a blank airflow which suggests that beetles are capable of detecting and responding to specific volatile chemical cues just as they would to an actual predator (Hermann and Thaler 2014).

Interestingly, even predator byproducts have been shown as a source of predator chemical information for prey. A series of small-scale field experiments examined whether spider silk could affect the feeding behaviors of two agricultural pests, the Japanese beetle (*Popillia japonica*) and the Mexican bean beetle (*Epilachna varivestisi*). Herbivory was measured in response to strands of silk from the spider (*Tetragnatha elongate*) as a proxy for predation pressure, compared to no silk and silkworm (*Bombyx mori*) silk controls (controlling for potential tactile or visual cues) in bush style snap-bean (*Phaseolus vulgaris*) fields. A significant reduction in plant damage was found when spider silk was present, suggesting that manipulating NCEs can have implications for crop protection (Rypstra and Buddle 2013a). NCEs have diverse consequences on focal prey but the cascading effects that may occur as a result of NCEs influence target prey, their hosts and other key players within the community. While early evidence is promising, larger field-scale studies that span a growing season are necessary to understand long-term effects and potential benefits to crop yield.

Ecological Consequences of Non-Consumptive Effects

Trophic Interactions

Although NCEs are typically evaluated in a manner that emphasizes prey responses, the ecological consequences of NCEs can cascade through trophic systems. Predators can influence food web dynamics by direct consumption of prey (Hairston et al. 1960) (**Figure 1.1**). As a result, there can be indirect changes mediated by prey removal, which are beneficial to the prey's host plant (depicted by the double lined arrows, **Figure**

1.1a). Trophic dynamics resulting from NCEs increase the complexity of interactions, as there can be either positive or negative effects of the predator on prey, the prey's host plant and possibly even from the prey on the predator itself (depicted by the double lined arrows, Figure 1.1b). These indirect interactions may have particularly important effects that can add to our understanding of how biological control agents impacts pests. For example, the presence of the convergent ladybeetle (*Hippodamia convergens*) risk predators, which are commonly used biological control agents, reduced the population growth of potato aphids (*Macrosiphum euphorbiae*) without direct consumption. Further, the presence of the predators also lead to an increase in the formation of winged prey, indicating preparation for dispersal away from the host plant. Increased dispersal in response to predator risk can have an indirect benefit on the host plant by reducing the probability of negative pest impacts. The indirect impact on the host plant was not measured in the aforementioned study, however, there are an increasing number of studies that attribute trophic cascades to NCEs. For example, Antiqueira & Romero (2016) examined the possibility for indirect effects driven by behavioral changes in pollinator response to models of predatory crab spiders (after Thomisidae). Visual models alone resulted in decreased visitation by pollinators which led to a 33% reduction in fruit biomass and a 28% reduction in seed production (Antiqueira and Romero 2016). Clearly, predators can play a large role in regulating prey host seeking behaviors and thus have a large effect on lower trophic levels (Hairston et al. 1960).

In addition to affecting pollinator visitation, predation risk can also alter prey host plant choice in ways that affect plant communities (Schmitz 1998). The ability to sense

predator cues on plants where potential threat level is the highest allows prey to modify their choice of host plant, which may lead to a selection tradeoff between safe and nutritious host plants. For example, grasshopper prey (*Melanoplus femurrubrum*) were shown to switch from preferred grasses to less nutritive forbs in the presence of risk spider predators (*Pisaurina mira*) in field mesocosms (Schmitz 1998). The observed habitat shifts have strong impacts on both the preferred host (increased biomass from lack of grasshopper damage) as well as the less-suitable host (decreased biomass). These community level changes may increase competition for insects that seek refuge in new habitats or even change nutrient dynamics through altered plant abundance and composition (Hawlena et al. 2012).

Landscape Level Considerations

Landscape structure has a well-known influence on communities of beneficial, natural enemies and their impact on prey populations. Habitats with a high level of plant diversity harbor an increased abundance and diversity of predatory and parasitic natural enemies (Tscharntke et al. 2005). Increased habitat diversity at the local landscape scale also leads to a decrease in prey abundance (Gardiner et al. 2009). What remains unclear is the role of NCEs in these relationships. Since we know predator communities change with landscape structure, it is important to understand how associated NCEs may also change and how prey may respond. If key mechanisms of NCEs are understood, it may be possible to manipulate cues to create a landscape of fear for pest species.

The landscape of fear is defined as varying levels of predation risk that prey perceive across space, often depicted as peaks and valleys of threat versus safety (Laundré et al. 2010). As prey seek enemy free space, i.e. safe places to feed, oviposit, and rest (Jeffries and Lawton 1984), their distribution in the landscape is altered to facilitate predator avoidance. For vertebrates, it has been demonstrated that spatial and temporal land use by prey is driven by fear of predation (Laundré et al. 2001). This can result in parts of the landscape where resources may be over or under exploited. To date, there has been no study that has attempted to understand or manipulate similar landscape of fear in an insect system.

In order to understand the impact of habitat and landscape factors on insect NCEs, studies done in open field settings are necessary. Behavioral observations of pests, coupled with yield measurements may help to illustrate the impact of landscape on pest management. Since NCEs do not necessarily lead to reduced pest populations but can reduce pest pressure, surveys of pest abundance across landscape may not capture the net effect of predators on prey and the impact of those interactions on crops. In addition, some landscape factors may also influence the strength of the response a given prey has to its predator. For example, if more refuges or alternative habitats are available in a complex landscape, the strength of NCEs on prey may be reduced since the refuge provides added safety. Understanding the impact of landscape factors on trophic interactions will allow for better predictions of the strength and outcome of insect predator-prey interactions in managed and natural systems.

Arguments for Scaling-Up

Current studies of insect NCEs largely focus on short time scales in laboratory settings. Of the studies we reviewed, 41% were completed solely in a laboratory setting, 35% were performed in the field, less than 1% in a greenhouse and 20% used a combination of settings (see **Table 1.3** for a table indicating both the experimental setting and the length of experiments within each reviewed study). In order to investigate potential cascading effects as a result of NCEs, there is a need to increase studies that combine meaningful laboratory/greenhouse results and investigate them in open field settings. By investigating the spatial and temporal scale of predator risk effects on prey populations, as well as the resulting effects on plants, we can gather essential insights into important community dynamics as well as possible non-target effects. Additionally, the consumptive effect of predators has a relatively short range effect on prey populations since it is limited to the area within a habitat that predators physically reach. NCEs of predators, however, have the potential to be farther reaching if predator cues can be detected by prey at a distance. Yet, the effective spatial scale of predator cues and NCEs in terrestrial insect systems has not yet been investigated.

In addition to spatial scale, the temporal scale of predator cues is understudied. Only 32% of papers reviewed were on a time scale that lasted longer than one week (see **Table 1.3**). Furthermore, over 50% of the reviewed studies performed experiments that lasted 24 h or less. By limiting experiments in both space and time, our current understanding of cue persistence, and potential prey habituation are nearly absent. Understanding the temporal dynamics of these interactions in a field setting is essential if NCEs are to be useful as an alternative pest management strategy. Further, by scaling up

our understanding of NCEs to larger spatial and temporal scales, we can then ask how habitat and landscape diversity may influence these interactions.

CONCLUSIONS

Evidence for influences of NCEs in insect systems is growing. However, spatial and temporal scales of previous work is limited. More work in terrestrial insect systems where insects are both predators and prey is needed to elucidate effects of scale. Studies in agroecological systems would be particularly crucial, as farmers are increasingly manipulating habitats to increase predator abundance and improve pest suppression through consumptive effects of natural enemies (Gurr et al. 2017). However, there has been no attempt to manipulate pest communities and alter species interactions through the landscape of fear by taking advantage of NCEs.

We know insects use visual and chemical cues to assess habitat suitability, and pest taxa likely have similar mechanisms to detect habitats/landscapes with increased predation risk. Identifying specific cues that elicit prey fear will allow us to better understand systems and begin manipulating them. There is also the potential to utilize chemical cues, predator mimics, or crop planting patterns that signal increased predator risk and reduce pest damage. In push-pull pest management, plant defensive chemistry or pest alarm pheromones can be manipulated to repel, or push, pests away from a crop while plant-derived attractants or alternative attractive hosts act to pull pests away from the crop plants. Predator cues have yet to be used in these systems to alter behavior or physiology of pests but could be applied as an alternative tool for pest management in push-pull systems (Cook et al. 2007).

In addition, multiple predator cues (visual, chemical, vibrational) could have additive effects on prey and should be investigated further. While visual cues that provide information about habitat suitability could be recognized at larger scales, visual information about predator presence is likely more localized. The distance over which chemical cues play a role is also unclear but has the potential provide information from a distance. More research on the mechanism that drives NCEs across terrestrial insect systems as well as their temporal and spatial effectiveness is needed to determine if the use of predator cues that influence the behavior and physiology of pests, coupled with habitats that maintain or enhance consumptive effects could provide the infrastructure for sustainably pest-suppressive landscapes (Landis 2016).

TABLES

Table 1.1. Behavioral responses to predation risk in studies of non-consumptive effects of arthropod predators on prey in studies from2012-16. Only significant results were included for simplicity.

Predator spp.	Prey spp.	Measured Prey Response to Predation Risk	Reference
Tasmanicosa leuckartii	Helicoverpa armigera	Altered habitat use	Rendon et al. 2016
Camponotus & Cephalotes spp.	Eunica bechina	Reduced oviposition	Sendoya et al. 2015
modeled after Thomisidae <i>spp.</i>	Various Lepidopterans, Dipterans & Hymeonpterans	Decreased visitation, Increased avoidance	Antiqueira & Romero 2016
Hippodamia convergens	Macrosiphum euphorbiae	Increased dispersal, increased number of nymphs	Kersch-Becker & Thaler 2015
Linepithema humile	Apis mellifera	Reduced acceptance of inflorescences	Sidhu & Rankin 2016
Tenodera sinensis	Apis mellifera	Increased avoidance, reduced recruitment dancing	Bray & Nieh 2014
Vespa velutina Vespa tropica	Apis crana	Reduced individual foraging, reduced individual feeding duration, reduced colony foraging allocation	Tan et al. 2013

Table 1.1. (cont'd)

Oecophylla smaragdina	Apis dorsata	Reduced landings, altered host choice location	Li et al. 2014
Myrmica rubra	Bombus impatiens	Decreased pollen removal	Cembrowski et al. 2014
Podisus maculiventris	Leptinotarsa decemlineata	Reduced feeding	Hermann & Thaler 2014
Podisus maculiventris	Manduca sexta	Reduced feeding	Thaler et al. 2012
Podisus maculiventris	Manduca sexta, Leptinotarsa decemlineata & Trichoplusia ni	Reduced feeding	Kaplan et al. 2014
Coccinella septempunctata	Rhopalosiphum padi	Reduced colonization and visitation	Ninkovic et al. 2013
Tetragnatha elongata	Popillia japonica & Epilachna varivestis	Reduced feeding	Rypstra & Buddle 2012
Podisus maculiventris	Manduca sexta	Reduced feeding, increased assimilation efficiency, increased resting metabolic rate	Thaler et al. 2014
Lasius niger	Bombus terrestris	Reduced visitation (post-training in the lab)	Ballantyne & Wilmer 2012
Azteca instabilis	Margaridisa spp	Reduced feeding, reduced visitation	Gonthier 2012

Table 1.1. (cont'd)

Pisaura mirabilis	Various Ants/Parasitoids/Aphids	Reduced herbivory (leaf damage)	Bucher et al. 2015
Hogna frondicola	Melanoplus borealis	Reduced feeding	Wineland et al. 2015
Hogna frondicola	Melanoplus dawsoni	Reduced feeding, increased avoidance (more time spent in enemy-free space)	Wineland et al. 2015
Harmonia axyridis	Sitobion avenae	Altered habitat use	Wilson & Leather 2012
Oecophylla smaragdina	Nomia strigata	Spent more time examining flowers	Gonzálvez & Rodriguez- Gironés 2013
Cordulegaster boltonii	Potamophylax sp.	Increased feeding, altered habitat use	Lagrue et al. 2015
Anax spp.	lschnura cervula	Increased activity level	Siepielski et al. 2013
Erythemis simplicicollis, Plathemis Lydia, or Pachydiplax longipennis	Aedes albopictus	Reduced oviposition	Wasserberg et al. 2013
centipede	Acheta domesticus	Increased avoidance (less time spent near predator cue)	Hoefler et al. 2012
Delphastus catalinae	Bemisia argentifolii	Increased avoidance (more time spent in enemy- free space) within a single plant	Lee et al. 2014

Table 1.2. Physiological responses to predation risk in studies of non-consumptive effects of arthropod predators on prey in studies from 2012-16. Only significant results were included for simplicity.

Response category	Predator spp.	Prey spp.	Measured Prey Response to Predation Risk	Reference
	Hippodamia convergens	Macrosiphum euphorbiae	Increased alate production	Kaplan & Thaler 2012
	Anax spp.	Enallagma cyathigerum	Reduced growth rate, increased oxidative damage	Janssens & Stoks 2013a
opment	Anax spp.	Enallagma cyathigerum	Reduced growth rate, reduced oxygen intake	Janssens & Stoks 2013b
th and Devel	Harmonia axyridis	Helicoverpa armigera	Increased development, increased weight (pupae), reduced survival (3rd gen), increased wing deformity (1st gen), increased fecundity (3rd gen)	Xiong et al. 201
Growi	Tasmanicosa leuckartii	Helicoverpa armigera	Increased time spent in pupal stage	Rendon et al. 2016
	Tenodera sinensis	Drosophila melanogaster	Breeding: offspring developed faster and weighed less. Non-breeding: Increased mortality, decreased body mass, surviving flies had lower fecundity and fewer, lighter offspring.	Elliot et al. 2016

Table	1.2.	(cont'	d)
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ation	Pisuarina mira	Melanoplus femurrubrum	Increased body C:N ratio	Hawlena et al. 2012
) and Assimi	Podisus maculiventris	Manduca sexta	Increased assimilation efficiency (short term), decreased assimilation efficiency (long term), decreased glycogen levels	Thaler et al. 2012
mpositio	Podisus maculiventris	Manduca sexta	increased assimilation efficiency	Kaplan et al. 2014
Body Cor	Anax spp.	Enallagma cyathigerum	Increased oxidative damage, decreased escape performance (reduced escape speed)	Janssens & Stoks 2014
tality	Anax spp.	lschnura cervula	Increased mortality and size	Siepielski et al. 2013
al and Mor	Tigrosa helluo	Pardosa milvina	Reduced lifespan of females (compared to ground beetle predator cues but not to control)	Sitarvin et al. 2015
Surviv	Hogna frondicola	Melanoplus dawsoni & Melanoplus borealis	Reduced survival	Wineland et al. 2015

Table 1.3. Color coded indication of experimental length within the reviewed articles (published between 2012-2016) investigating the non-consumptive effects of arthropod predators on prey. Studies in green were 24 h or shorter, gray indicates a study over 24 h yet under 7 d in length, shaded studies had at least one portion of their study under 24 h, yellow indicates that at least one portion of the experiment was 7 d or longer, and blue studies were conducted for 7 d or longer.

Predator	Prey	Setting	Article Information
Tenodera sinensis	Apis mellifera	Field	Bray & Nieh 2014
Lasius niger	Bombus terrestris	Field	Ballantyne & Wilmer 2012
Azteca instabilis	Margaridisa spp	Field	Gonthier 2012
modeled after <i>Thomisidae</i> sp.	Various Lepidopterans, Dipterans & Hymeonpterans	Field	Antiqueira & Romero 2016
Linepithema humile	Apis mellifera	Field	Sidhu & Rankin 2016
Vespa velutina & tropica	Apis crana	Field	Tan et al. 2013
Oecophylla smaragdina	Apis dorsata	Field	Li et al. 2014
Camponotus & Cephalotes spp.	Eunica bechina	Field	Sendoya et al. 2015
Oecophylla smaragdina	Nomia strigata	Field	Gonzálvez & Rodriguez- Gironés 2013
Coccinella septempunctata	Rhopalosiphum padi	Laboratory	Ninkovic et al. 2013
Podisus maculiventris	Manduca sexta	Laboratory	Thaler et al. 2014
Myrmica rubra	Bombus impatiens	Laboratory	Cembrowski et al. 2014
Anax sp.	Ischnura cervula	Laboratory	Siepielski et al. 2013
centipede	Acheta domesticus	Laboratory	Hoefler et al. 2012
Erythemis simplicicollis, Plathemis lydia, orPachydiplax longipennis	Aedes albopictus	Field	Wasserberg et al. 2013
Pisaura mirabilis	Various Ants/Parasitoids/Aphids	Field	Bucher et al. 2015

Table 1.3. (cont'd)

Hippodamia convergens	Macrosiphum euphorbiae	Laboratory	Kersch-Becker & Thaler 2015
Podisus maculiventris	Leptinotarsa decemlineata	Laboratory	Hermann & Thaler 2014
Harmonia axyridis	Sitobion avenae	Laboratory	Wilson & Leather 2012
Podisus maculiventris	Manduca sexta, Leptinotarsa decemlineata & Trichoplusia ni	Greenhouse	Kaplan et al. 2014
Delphastus catalinae	Bemisia argentifolii	Laboratory/Greenhouse	Lee et al. 2014
Tasmanicosa leuckartii	Helicoverpa armigera	Laboratory/Greenhouse	Rendon et al. 2016
Tetragnatha elongata	Popillia japonica & Epilachna varivestis	Laboratory/Greenhouse	Rypstra & Buddle 2012
Hogna frondicola	Melanoplus borealis & dawsoni	Field/Laboratory	Wineland et al. 2015
Podisus maculiventris	Manduca sexta	Field/Greenhouse	Thaler et al. 2012
Hippodamia convergens	Macrosiphum euphorbiae	Field	Kaplan & Thaler 2012
Tenodera sinensis	Drosophila melanogaster	Laboratory	Elliot et al. 2016
Anax sp.	Enallagma cyathigerum	Laboratory	Janssens & Stoks 2014
Anax sp.	Enallagma cyathigerum	Laboratory	Janssens & Stoks 2013a
Anax sp.	Enallagma cyathigerum	Laboratory	Janssens & Stoks 2013b
Harmonia axyridis	Helicoverpa armigera	Laboratory	Xiong et al. 2015
Tigrosa helluo	Pardosa milvina	Laboratory	Sitarvin et al. 2015
Pisuarina mira	Melanoplus femurrubrum	Field/Laboratory	Hawlena et al. 2012
Cordulegaster boltonii	Potamophylax sp.	Field/Laboratory	Lagrue et al. 2015

FIGURES



Figure 1.1. Food web dynamics considering consumptive effects of predators (a.) and non-consumptive effects of predators (b.). Solid lines indicate consumptive effects while dashed lines indicate behavioral/trait changes and double lines indicate indirect effects. Modified from Schmitz et al. 199

CHAPTER 2: PREDATOR CUES HAVE LIMITED IMPACT ON PIERIS RAPAE OVIPOSITION AND LARVAL GROWTH

INTRODUCTION

Insect predator-prey interactions tend to focus on consumptive effects such as the efficiency at which predatory insects locate and consume their prey. However, even in the absence of direct consumption, perception of risk by prey can alter prey behavior and physiology with the potential to impact population growth (Werner and Peacor 2003). Such changes are termed non-consumptive effects (NCEs). While the effects of predation risk and subsequent NCEs in vertebrate and aquatic systems are well-known (Turner 1996, Stankowich and Blumstein 2005, Thuppil and Coss 2013, MacLeod et al. 2017), terrestrial insect systems have received relatively less attention (Hermann and Landis 2017). Importantly, a broader understanding of predation risk in highly diverse terrestrial insect systems could allow us to better predict population patterns in natural systems, inform conservation efforts or aid in management of insect pests in agroecosystems.

Various anti-predator behavioral responses have evolved to help prey insects avoid predator attack. For example, pea aphids (*Acyrthosiphon pisum* L.) drop from host plants upon encounters with predators (Hoki et al. 2014) and Colorado potato beetles (*Leptinotarsa decemlineata* Say) regurgitate toxins when disturbed (Ramirez et al. 2010). Since these behaviors can come at a significant cost (Persons et al. 2002), their initiation relies on the ability of prey to reliably detect the risk of predation prior to a potentially lethal attack. However, such so-called fear responses may also not occur for any given predator-prey pair. Lack of response could occur when predators represent a low or variable risk to the prey, or prey lack the ability to detect predator cues, both of which

could be affected by the degree of co-evolutionary history or the general spatial or temporal overlap of a predator and prey (Sih et al. 2010).

The response to predation risk by prey can also vary by life stage (Ramirez et al. 2010) as juvenile and adult forms of prey can vary dramatically in size, defense and mobility. Therefore, we might expect larval stages of insects to respond differently to predators than their adult counterparts. In some cases, even the invulnerable stage of an insect might respond to predation risk (Hermann & Thaler, 2018). For example, if adult life stages are not susceptible to a predator that can consume their offspring, the adult insects might still alter their behavior to reduce risk to their offspring by adjusting the amount or location of oviposition (Stav *et al.*, 1999; Wasserberg *et al.*, 2013; Sendoya *et al.*, 2015; Morse, 2017; Hermann & Thaler, 2018) or via altered physiology which influences the growth rate of their offspring (Xiong et al. 2015, Elliott et al. 2016, Freinschlag and Schausberger 2016). Determining what life-stage(s) of insect prey respond to predation risk is crucial in understanding the ecology and outcome of predator-prey interactions.

The imported cabbageworm (*Pieris rapae* L.) once native to Europe, Asia and North Africa is now a common crop pest across the globe (Shelton 1999). As a specialist on Brassicaceae, *P. rapae* feeds broadly on plants within that family. The damage caused by *P. rapae* larvae is often severe, and if left untreated can lead to extensive defoliation (Oatman and Platner 1969). Within crucifer agroecosystems, there are many predators and parasitoids that attack both the eggs and larval instars of *P. rapae* (Schmaedick and

Shelton 2000). Moreover, adult *P. rapae* use various host odor cues to determine satisfactory oviposition sites (Renwick and Radke 1988, Ômura et al. 1999). Evidence suggests that the presence of predators or competitors can influence those choices (Ohsaki and Sato 1994, Layman and Lundgren 2015). For example, the co-occurrence of competitors (*Myzus persicae*, Sulzer) and predators (*Coleomegilla maculata*, DeGeer) significantly reduced *P. rapae* oviposition on *Brassica oleracea*, while occurrence of competitors alone had no effect (Layman and Lundgren 2015).

The multicolored Asian lady beetle (*Harmonia axyridis* Pallas) is a generalist predator, native to Eastern Asia (Koch 2003). Following releases for biological control in the United States, *H. axyridis* spread, with the first established North American population detected in 1988 (Koch 2003). *H. axyridis* can now be found in many agroecosystems across the United States. In Michigan, *H. axyridis* is one of the most abundant coccinellids in agricultural landscapes, where its voracity can lead to competition with native species (Bahlai et al. 2013). Due to its tendency to overwinter within homes and other structures, and strong odor when disturbed or crushed (Cai et al. 2007, Sloggett et al. 2011, Fischer et al. 2012), *H. axyridis* is considered a household nuisance (Huelsman et al. 2002). It is also a pest of some soft-bodied fruits, like grape, where its presence can cause off tastes in wine, detectable with as little as 4 ladybeetles per kilogram of grapes (Koch and Galvan 2008, Botezatu et al. 2013).

H. axyridis is found in crucifer agroecosystems alongside *P. rapae* and although not considered a major predator of *P. rapae*, will readily consume the egg and early larval stages. In preliminary no-choice assays, we found that individual adult *H. axyridis*

can consume 30 P. rapae eggs in 72 h, 30 1st instars in 48 h, and 10 2nd to 3rd instars in 48 h (Hermann, unpublished data). Therefore, since these are two of the most ubiquitous insects found in agroecosystems, our aim was to understand the potential of *P. rapae* to detect predation risk and respond to this generalist predator by altering larval and adult behaviors. In addition, *H. axvridis* has a distinct chemical odor blend that has the potential to act as an indicator of predator presence for their prey (Sloggett et al. 2011). In other coccinellid species, chemical signatures of these predators led to reduced colonization and increased wing formation in aphids (Dixon and Agarwala 1999, Ninkovic et al. 2013b). For example, bird cherry-oat aphids (*Rhopalosiphum padi* L.) avoid plants where seven-spotted ladybeetles (Coccinella semtempunctata L.) had previously walked, suggesting that chemical cues from coccinellids can act as deterrents (Ninkovic et al. 2013b). Since insect predator cues have also been shown to influence oviposition patterns (Lee et al. 2014, Layman and Lundgren 2015) and larval feeding behaviors (Hermann and Thaler 2014, Thaler et al. 2014), understanding which prey life stages respond to predation risk, and if predator cues alone are sufficient to elicit a response, will deepen our understanding of these complex interactions.

In this study, we asked 1) do *P. rapae* larvae alter their feeding and/or growth in the presence of *H. axyridis*, 2) do *P. rapae* adults change their oviposition behaviors in the presence of *H. axyridis*, and 3) do *H. axyridis* chemical cues mediate the interaction between this predator and its prey? We predicted that the presence of *H. axyridis* or its chemical cues on the host plant would result in fewer eggs laid by *P. rapae* adults. We

also predicted that *P. rapae* larval consumption of its host plant, and thus overall growth, would be reduced when raised in the presence of *H. axyridis*.

METHODS

Plants and Insects

Collard plants (var. Georgia, *Brassica oleracea* L., Brassicales: Brassicaceae) were sprouted from seed in commercial potting soil (SUREMIX Perlite, Michigan Grower Products, INC.[™], Galesburg, MI). Once plants developed their first set of true leaves (approx. 2 weeks), seedlings were transplanted into 10.2 cm square pots where they remained until use in experiments. Plants were watered daily and fertilized weekly (20-20-20, Peters Professional® Water Soluble Fertilizer, Brantford, Ontario) in a greenhouse under 16:8 L:D at approximately 27 °C. All experiments used plants that were 4-6 weeks old. All experiments were performed during the summer of 2016.

A colony of *P. rapae* was established with eggs purchased from Carolina Biological Supply (Item # 144100, Burlington, NC) and supplemented with eggs laid from field-collected adults. All larvae were reared together on collard plants throughout the larval stage, with new plant material presented daily until pupation in W60 x D60 x H60 cm tent cages (BugDorm-2120, Taiwan) in greenhouse conditions (described above). Upon pupation, all plant material was removed from the cage and a cotton wick submerged in a 10% sugar water solution was added. Adults were presented with fresh sugar solution every three days and held without access to plants for oviposition prior to experiments.
Multicolored Asian ladybeetles were field collected in Ingham County, Michigan; all stages were reared on a diet of *R. padi*. These aphids were reared in colony on barley (*Hordeum vulgare* L.) plants in a climate-controlled insectary, 16:8 L:D and approximately 27 °C. Aphid infested barley was added to tent cages (as stated above) that housed adult *H. axyridis* every two days and checked for presence of *H. axyridis* eggs three times per week. All eggs were removed and placed in a new cage to keep lady beetle life stages separated. Only the adult stage of *H. axyridis* was used in our experiments.

Pieris rapae Larval Consumption and Development

We assessed the impact of *H. axyridis* adults on larval growth and feeding behavior of *P. rapae* in 90mm plastic petri dish arenas (Fisher Scientific, Hampton, NH). Control arenas consisted of a leaf disc from a 6-week-old collard plant cut to fit the petri dish and placed atop a moistened sheet of filter paper (WhatmanTM, Cat. No. 1004 090, United Kingdom). Predator treatments were identical, but also included an individual *H. axyrids* with its mouthparts glued with a drop of superglue (Loctite, Düsseldorf, Germany) to render it non-lethal. Mouthpart manipulation has been used on ladybeetles in the past to obtain a non-lethal predator (Kersch-Becker and Thaler 2015b). Experiments were conducted in a climate-controlled growth chamber with 16:8 L:D cycle and held at 27 C. A single *P. rapae* neonate was placed on each leaf disc at the start of the experiment and monitored daily until pupation. Each day the individual larval instar was assessed by measuring the head capsule width using an electronic caliper (Richards 1940). Leaf discs were changed daily; leaves with herbivory were scanned (Epson Perfection V39) and the cumulative leaf area removed by larvae was analyzed using Image J (O'Neal et al. 2002). Upon pupation, the final leaf disc was analyzed and the pupa was weighed. Ten replicates of each treatment were performed.

Pieris rapae Oviposition

Physical Presence of Predators

To examine the effect that the presence of the predator has on oviposition behavior, two treatments were used: control or predator. Control treatments were a single potted plant with a 30x20cm mesh bag (BugDorm, DC3220) fixed to a central leaf, containing only a moistened cotton ball. Predator treatments consisted of a single potted collard plant with a mesh bag enclosing a central leaf, containing five unmanipulated *H. axyridis* adults and a moistened cotton ball as a water source for the predators. Oviposition tests were performed in tent cages (as above). In each case, a single sevenday-old female was placed in each replicate cage and given 24 h to oviposit in a nochoice setting. After 24 h, the female was removed and all eggs deposited on the plant were counted. Each treatment was replicated 10 times, over two days resulting in n=20 per treatment.

Predator Chemical Cues

To examine the effect of predator semiochemicals on oviposition behavior, two treatments were used: semiochemical blend or control. The characteristic odor of H. axyridis was formulated into a semiochemical blend based on Cai et al. (2007), which identified four major components. Our blend consisted of three commercially available methoxypyrazines: 1.4 mL of 2-isopropyl-3-methoxypyrazine (IPMP), 567 µl of 2-secbutyl-3-methoxypyrazine (SBMP), and 11.3 µl of 2-isobutyl-3-methoxypyrazine (IBMP), from 1 ng/ μ l stocks that were diluted in methanol solvent (Sigma-Aldrich[®], ST. Louis, MO). Stock solutions of 1 ng/ µl of each methoxypyrazine were diluted in MeOH. Our control consisted of pure methanol solvent. The predator odor mixture or the control solvent were placed in individual 5/8 dram amber glass vials (DVA-5/8D-OR, Premium Vials, Tullytown, PA) at a total volume of 4 mL with diffuser caps to allow for slow release of volatiles. Vials were then placed directly into the soil below the plant foliage throughout the experiment. As in the above experiment, oviposition by a single P. rapae female was counted after 24 h in semiochemical and solvent control no-choice tent cages. We completed a total of n=23 per treatment over two consecutive days.

Statistical Analyses

All data were analyzed using JMP (JMP Pro[®], Version 12. SAS Institute Inc., Cary, NC, 1989-2007). To analyze the amount of larval consumption, a two-sample Student's t-test was used to compare the amount of feeding by larvae in the predator treatment to that of the control treatment. Here, we predicted the amount of feeding would differ between treatments and the null hypothesis was equal feeding. Similarly, we

expected the weight of pupae to differ between treatments and thus the null hypothesis was equal pupal weight. A two-sample Student's t-test was used to compare the mean weight between treatments.

We used the non-parametric Wilcoxon signed-rank test to analyze whether oviposition by *P. rapae* differed from the null hypothesis of equal oviposition between treatments for both the physical predator presence experiment and the semiochemical blend experiment. These data were not normally distributed due to a high presence of zeroes that could not be easily remedied by transformation, precluding parametric tests.

RESULTS

Pieris rapae Larval Consumption and Development

P. rapae larvae raised in the presence of non-lethal *H. axyridis* adults tended to consume slightly less leaf area compared to control larvae (**Figure 2.1A**). However, the difference was not statistically significant (p = 0.132). In both treatments, pupation occurred on either the 11th or 12th day post-eclosion and did not differ between treatments (for both treatments: pupation on day 11, n=3 and pupation on day 12, n=7). Pupal weight also tended to be lower and more variable for larvae raised in the presence of a predator compared to control larvae (**Figure 2.1B**), but again, the difference was not statistically significant (p = 0.33).

Pieris rapae Oviposition

Oviposition was unaffected by the presence of ladybeetle predators on plants in the no-choice greenhouse trials (p = 0.86; Figure 2.2A). Oviposition tended to increase in the presence of the ladybeetle semiochemical blend (Figure 2.2B), but was not significantly different (p = 0.2)

DISCUSSION

We hypothesized that the potential for NCEs is high in our study system due to the fact that *H. axyridis* readily consume *P. rapae* eggs and young larva, have highly apparent volatile semiochemicals, and commonly co-occur with *P. rapae* in crop fields. Contrary to our expectations, we found little evidence that *P. rapae* altered its behavior in the presence of *H. axyridis*. Here we discuss these results in relation to larval growth, adult behavior, and the possible role of predator consumptive rate and evolutionary history.

There are several examples of altered feeding rate by immature arthropods in the presence of predators (Kaplan & Thaler, 2012; Thaler et al., 2014, 2015; Hermann & Thaler, 2014; 2015; Wineland et al., 2015). Changes in feeding likely occur because the act of feeding typically leaves prey species exposed and more visually apparent (Bernays 1997) and potentially induces plant defenses that attract natural enemies (Turlings et al. 1990, De Moraes et al. 1998). Therefore, we predicted that larval *P. rapae* would reduce their feeding in the presence of *H. axyridis* predators and that a reduction in feeding would result in smaller pupae. However, when *P. rapae* larvae were raised in the

presence of a single *H. axyridis* predator, the amount of feeding was unchanged compared to larvae raised without predator presence and the final weight upon pupation did not differ between treatments. While *H. axyridis* readily consumed *P. rape* eggs and larvae in preliminary no-choice trials, in the field lady beetles frequently prefer consuming aphid species or insect prey with limited mobility (Provost et al. 2006), suggesting that *H. axyridis* may not be significant predators of *P. rapae* in nature, and thus would not elicit a response in their larvae. In addition, *P. rapae* feed solitarily which may help them to avoid predator encounters and have coloration that matches their host plants, providing them camouflage to predators (Müller et al. 2003). Since the major reported predators of *P. rapae* larvae are vespid wasps, spiders and other ground predators (Richards, 1940), perhaps the selective force to respond to ladybeetle predators is too weak to elicit behavioral anti-predator responses.

Prior studies suggest that adult insects should seek to find enemy-free space for themselves and their progeny (Jaenike 1978, Jeffries and Lawton 1984, Bernays and Graham 1988, Thompson 1988, Morse 2017). However, we did not find an effect of predator presence on adult oviposition. We evaluated the number of eggs laid by *P. rapae* adults in the presence of non-lethal predators in a no-choice setting. Here, *P. rapae* did not significantly alter their egg-laying behavior in response to predator presence. Oviposition by female insects has been shown to be significantly reduced in the presence of other predators on host plants (Wasserberg et al. 2013, Sendoya et al. 2015). However, there are relatively few studies that directly examine this aspect of adult behavior since adult insects are more mobile and thus relatively invulnerable compared to their juvenile

counterparts. The adult stage of this butterfly is reportedly most at risk by avian predators (Hooks et al. 2003) and perhaps explains their lack of response to *H. axyridis*. Since our experiment ran for 24 h in a no-choice setting, it is also possible that *P. rapae* adults make shorter term changes in oviposition, or that they would have preferentially laid eggs on a predator-free plant if it was presented as a choice in the same arena.

Certain organismal characteristics might allow for more efficient or reliable detection of predators. For example, the presence of predator semiochemicals can drive NCEs and elicit fear-based responses in prey resulting in altered feeding rates and colonization (Hermann and Thaler 2014, Hermann and Landis 2017). In our system, we used a semiochemical blend to mimic the threat of predation by *H. axyridis*. We observed a non-significant trend towards increased oviposition on plants with *H. axyridis* semiochemicals present. Chemical cues of predators seem to be a major factor in the detection of predation risk in other insect systems (Gonthier 2012, Hoefler et al. 2012, Ninkovic et al. 2013b, Hermann and Thaler 2014, Ebrahim et al. 2015, Sidhu and Wilson Rankin 2016). Much of the chemical ecology of *H. axyridis* has been examined by viticulturists in direct relation to wine taint (Botezatu et al. 2013), therefore it is possible that our semiochemical blend was not adequate to induce behavioral changes in the adult butterfly. Indeed, *H. axyridis* has been shown to release additional volatile components that were not commercially available to include in our blend (Fischer et al. 2012). Butterflies also use a variety of visual cues to assess host plant suitability (Renwick and Radke 1988) and thus, the physical presence of a foraging lady beetle in addition to the semiochemical blend might lead to stronger effects. Yet, in our experiments with

ladybeetles confined to a mesh bag, both volatile cues and limited visual cues were present and our results do not suggest the combination of cues lead to stronger effects. It is also possible that since *H. axyridis* poses no risk to the adults themselves, that *P. rapae* does not identify the presence of this predator as a threat.

In our system, *H. axyridis* and *P. rapae* coexist in nature; the predator will consume the prey, and predator cues are likely to be present. However, we did not find any influence of *H. axyridis* on *P. rapae* behavior in any of our experiments conducted in this study. One potential explanation is since these two insects have only lived in association since the 1980's in the United States, the predator might not yet have cues that are associated with this specific predator which enable detection. A shared evolutionary history could also play a role in the ability to detect and use predator chemical cues (Sih et al. 2010). For example, if the prey and predator are a new association but the prey is closely related to another predator that has a shared evolutionary history with the prey, then certain conserved cues might be detectable. Since the adult form of *P. rapae* is not at risk of predation by *H. axyridis* (or other Coccinellids), it makes sense that it might not detect the chemical cues. Yet, in other insect systems, adults alter oviposition behavior when they themselves are not at risk (Hermann & Thaler, 2018). Future work is justified examining the role of predator cues in mediating NCEs, especially ones that examine predator-prey pairs that vary in consumption rate/threat level and coevolutionary history.

FIGURES



Figure 2.1 A. Mean (\pm SEM) leaf area consumed by *Pieris rapae* larvae raised in control (no predator) arenas or in the presence of a single non-lethal adult *Harmonia axyridis* (predator). B. Mean (\pm SEM) final weight of *P. rapae* pupae raised from larvae in control (no predator) arenas compared to larvae raised in the presence of a non-lethal *H. axyridis* predator throughout larval development.



Figure 2.2. A. Mean (\pm SEM) eggs laid by *Pieris rapae* adults on control (no predator) host plants compared to plants with five adult *Harmonia axyridis* predators bagged to a leaf on the plant. B. Mean (\pm SEM) eggs laid by *P. rapae* adults on control (no predator) host plants or on plants in arenas that contained *H. axyridis* chemical cues (semiochemical blend).

CHAPTER 3: FIELD EVALUATION OF THE IMPACT OF PREDATORS AND PREDATOR CUES ON COLONIZATION AND HOST USE BY HERBIVOROUS PREY

INTRODUCTION

Humans have harnessed the ability of arthropod natural enemies to control crop pests in agricultural landscapes for hundreds of years; through importation, augmentation or conservation of predators and parasitoids in (Gurr et al. 2000, van Lenteren 2000, Jonsson et al. 2008). The importance of these efforts is highlighted by the monetary value associated with the ecosystem services provided by natural enemies, which equates to around 4.5 billion USD annually to US agriculture (Losey and Vaughan 2006). However, direct removal of individual herbivores through consumption is not the only way predators can impact prey. When threatened, prey can initiate potentially costly antipredator defensive strategies that lead to altered behavior, morphology, physiology and life-history (Lima 1998a, Werner and Peacor 2003a, Nelson et al. 2004). The resulting impacts of these trait changes on prey survival and performance are termed nonconsumptive effects of predators, and they have the potential to influence prey populations as dramatically as the consumptive effect (Peacor and Werner 2001). In particular, the risk of predation alone has been shown to reduce prey feeding (Thaler et al. 2012, Hermann and Thaler 2014, Bucher et al. 2015), alter habitat and resource use (Wilson and Leather 2012, Rendon et al. 2016), increase dispersal (Kersch-Becker and Thaler 2015a), and reduce colonization (Ninkovic et al. 2013a). All of these documented changes in prey (herbivore) behavior can be tied to outcomes in agroecosystems, yet few studies exist at that scale or context (Hermann & Landis 2017). Understanding how nonconsumptive effects might contribute to the net effect of predators on prey could be of

particular importance in agriculture where manipulation of insect natural enemies for pest control is a major pest management strategy.

Non-consumptive effects have been shown in several agriculturally relevant insect systems (Kaplan and Thaler 2010, Rypstra and Buddle 2013b, Xiong et al. 2015, Hermann and Thaler 2018). Yet, the majority of examples come from studies performed dominantly in the laboratory setting, and over small spatial and temporal scales (reviewed in Hermann and Landis 2017). Without data collected at realistic scales across space and time, it will remain exceedingly difficult to predict how predator effects function across managed landscapes. It is widely known that predator-prey interactions act in a cyclical fashion and that populations can fluctuate, to a large degree, both within and across years (Krebs et al. 2001, Peckarsky et al. 2008). In agricultural cropping systems, the plant community is managed, but the community of predators, prey and other interacting members of the food web are constantly fluctuating (Southwood and Comins 1976). Therefore, examining the importance of non-consumptive effects in the field, is increasingly important if we aim to eventually harness these dynamics to improve pest suppression and ultimately crop success in agroecosystems.

Trophic cascades resulting from top-down pressure by predators can reduce the overall impact of herbivorous prey on plants and thus benefit the overall biomass of primary producers (Hairston et al. 1960). Traditionally, the ability for predators to initiate trophic cascades was attributed solely to the consumptive effect. However, in addition to the role of consumption, predator non-consumptive effects can lead to 'trait-mediated

indirect interactions' stemming from altered behavior, morphology or life-history of prey that can also dramatically impact the success of primary consumers (Schmitz et al. 2000, 2004, Halaj and Wise 2001, Peacor and Werner 2001). In addition to impacts on the primary producers, non-consumptive effects could also play a role in affecting other key herbivores or additional natural enemies within a system. Therefore, indirect effects on other community members, especially ones that provide ecosystem services, would be crucial to understand. Importantly, identifying which organisms in the community are influenced and which pest species non-consumptive effects act on is relevant to understanding trophic cascades in agricultural systems. The goal of our study was to investigate the potential for an important agricultural predator to elicit non-consumptive effects in a major herbivorous pest while ultimately the considering the possibility for cascading impacts on another major group of insect pests, the abundance of other predators and parasitoids in the system and ultimately the cumulative effect on crop yield.

The multi-colored Asian ladybeetle, *Harmonia axyridis* Pallas, is a generalist predator native to Eastern Asia which was brought to the United States as a biological control agent as early as 1916 with the first established population found in 1988 (Chapin & Brou, 1991). *Harmonia axyridis* is arguably one of the most abundant predators across agricultural crops and is an efficient predator of many aphid species (Gardiner & Landis, 2007; Xue et al 2009), various Tetranichidae, Psyllidae, immature Chrysomelidae, Curculionidae, and Lepidoptera (Koch, 2003). Because this is a highly voracious, generalist predator there is a strong potential for a consumptive effect across many prey species and thus predator avoidance and subsequent non-consumptive effects would be

predicted to be prevalent. In addition, *H. axyridis* has a well-studied suite of semiochemicals associated with its biology (Cai, 2007) that could act as a cue for prey in detecting predation risk. *Harmonia axyridis* ' generalist nature, unique chemistry, ubiquity, abundance and overall voracity makes it a model predator to study non-consumptive effects.

For this study we selected various focal organisms to track over a growing season to evaluate the impact of non-consumptive across the community of herbivores. First, the imported cabbage worm, *Pieris rapae* (Linnaeus), is a ubiquitous butterfly found throughout North America after its accidental introduction into Canada in the later 1800's. As an adult, *P. rapae* use a combination of visual and olfactory cues to find nectar sources for food and oviposition sites (Renwick & Radke, 1988; Ômura et al. 1999). Once oviposition sites are selected, the larvae of this pest feed voraciously and are capable of effectively removing all plant tissue, leaving only stems and veins. Because of this, *P. rapae* are among the most important pests of Brassicaceae, on which they are specialists. The adults are mainly at risk of predation by avian predators while the larvae are attacked by a wide variety of arthropod predators including wasps, ground beetles, spiders, ladybeetles and parasitoids (Shelton 1999, Schmaedick and Shelton 2000).

While *P. rapae* are often considered the main threat to Brassica crops, there are other herbivores commonly found on these plants that are also capable of causing economic harm. In particular, a suite of generalist and specialist species of aphids have been found on a variety of Brassicacae (Blackman and Eastop 2000). Aphid pests have a very different life-history, behavioral ecology and feeding mechanism, compared to *P. rapae* and thus might be influenced by predator manipulations differently. Importantly, while aphids are consumed by a variety of arthropod predators, they have a very tight association with ladybeetle predators, and have historically been utilized as successful biological control agents (Obrycki and Kring 1998, Dixon 2000, Snyder et al. 2004). For these reasons, we chose to compare and contrast the impact of predator manipulations on these two distinct pests.

By designing experiments that consider multiple levels of the arthropod community which can be influenced by predator non-consumptive effects, we can better evaluate implications for agroecology. Using this framework and an open-field experimental design, we hypothesized that (1) colonization by naturally occurring herbivores would be lower in plots where increased the presence of predators or predator chemical cues, 2) that the arthropod natural enemy communities on plants with predators and/or predator cues would differ from plants without predators or predator cues and 3) that non-consumptive effects resulting from predator presence will ultimately influence plant yield.

METHODS

Plants & Insects

A colony of *H. axyridis* was established from larval and adult beetles field collected in Ingham County, Michigan. All stages of *H. axyridis* in the colony were fed bird cherry-oat aphids (*Rhopalosiphum padi* Linnaeus) which were reared on barley

(*Hordeum vulgare*) plants, grown in 10 cm round pots. Colony cages were stored in a climate-controlled insectary (25 C; 16:8 L:D photoperiod). Three times per week senescing barley plants that no longer harbored aphids were removed from ladybeetle cages and replaced with new aphid infested plants to ensure a constant food supply was available. At this time, ladybeetle eggs were also removed and placed into a new colony cage in order to separate life stages. Only adult male and female *H. axydris* were used in experiments.

Collard plants (*Brassica oleracea*, cv. Georgia) were obtained from a commercial supplier for use in the field experiment (Garden Harvest Supply, Berne, IN). Plants were grown from seed in 2.54 x 3.81 cm cell plug trays by the supplier and were approximately 5 cm tall when we received and re-potted them. Out of 4,800 plants purchased, half were transplanted directly into the ground using a commercial transplanter. The other 2,400 plants were transplanted into 2.37 L pots, measuring 16.5 cm diameter x 16.5 cm height, for use as sentinel plants.

Predator Chemical Cues

We developed a predator chemical cue blend for use in the field experiment with a mixture of semiochemicals produced by *H. axyridis*. The three dominant predatorproduced chemicals, all methoxypyrazines (detailed below), found in the volatile chemical profile of *H. axyridis* adults were used to compose the predator chemical cue (Cai et al. 2007). First, 0.001 μ g/ μ l stock solutions of 2-isopropyl-3-methoxypyrazine (IPMP), 2-sec-butyl-3-methoxypyrazine, (SBMP) and 2-isobutyl-3-methoxypyrazine

(IBMP) were diluted from pure compounds (Sigma Aldrich, St. Louis, MO) in methanol (MeOH) solvent. Specifically, the final blend consisted of 1,449 µl IPMP, 567 µl SBMP and 11.34 µl IBMP which was placed in 5/8 dram, brown glass vials with diffuser tops and sealed with a cap and parafilm prior to use in the field. (DVA-5/8D-OR, Premium Vials, Tullytown, PA). The chemical blend was prepared each week on the day of experimental deployment to avoid degradation.

Field Experiment

Our experiment was conducted on a 135 x 60 m plot of land at the Michigan State University Entomology Research Farm in Ingham County, East Lansing, MI, USA (42.691600, -84.490270). The land was sub-divided into 21 experimental plots, measuring 10 x 10 m, each separated from other plots as well as the field edge by 10 m (**Figure 3.1 A**). All experimental plots were planted on 27 June 2016 following commercial standards with 1 m spacing between collard transplants within a row and 1 m spacing between rows. Each plot consisted of approximately 100 plants which served to attract and sustain naturally occurring insect populations.

Among the planted collards in each experimental plot, 17 - 17 x 17 cm holes were dug into the ground with a shovel. One hole was placed in the center of the plot and then 4 holes were dug in each cardinal direction from the center at 0.5, 1, 2 and 4 m away from the central hole (**Figure 3.1 B**). The central, sentinel collard plant in each plot was assigned one of three treatments: 1) control (an empty mesh bag fastened to the uppermost fully extended leaf on the collard plant), 2) chemical cue (chemical blend from *H. axyridis*, as described above, placed in the soil at the base of the potted plant and an

empty mesh bag fastened to the uppermost fully extended leaf on the collard plant) or 3) predator (five *H. axyridis* adults placed in a mesh bag fastened to the uppermost fully extended leaf on the collard plant). Treatments were applied directly after sentinel collard plants were placed in the field each week. Since *H. axyridis* were bagged to a plant without prey for a period of 5 d, a wet cotton ball was provided (and properly controlled for in the control and chemical cue treatments) as a water source.

Herbivore Abundance

The remaining 16 holes in each plot were used to place sentinel, potted collard plants at each radial distance from the central plant (**Figure 3.1 B**). These sentinel plants were used to assess the abundance of *P. rapae* eggs and winged aphid colonists in response to the three treatments after 3 and 5 dof plant exposure in the field. At the end of each week, sentinel plants were removed from the plots and discarded. The experiment ran for 5 consecutive weeks, with a new set of 357 sentinel plants (17 per plot x 21 plots) placed in the field each week for the 5 d exposures.

Natural Enemy Abundance

In addition to herbivore assessment, natural enemy diversity and abundance was measured each week with yellow sticky cards. Cards were placed adjacent to the central, sentinel collard plant in each experimental plot on step in fence posts, approximately 1 m above the ground, on Monday of each week and removed with the sentinel plants on Friday of each week after the 5 d exposure. Yellow sticky traps were taken back to the

lab and placed in the freezer until predator identification was completed using a dissecting microscope in the laboratory.

Impact of Predators and Predator Cues on Yield

At the end of the five-week experiment, a minimum of eight collard plants were randomly selected from collards that were planted in the ground within each experimental plot and harvested to assess the impact of the three treatments on yield (**Figure 3.1 C**). Plant material was placed in a commercial drying oven for 48 h and then weighed. Weights were then standardized by number of plant samples taken in each experimental plot for analysis.

Statistical Analysis

To test the effect of the three treatments on *P. rapae* oviposition and winged aphid colonists, we used a GLM analysis of deviance with treatment, distance and week as predictor variables and egg or aphid abundance as a response variable. The interaction between treatment*distance*week was removed from the model when non-significant, followed by non-significant two-way interactions. Because these data represent populations, poisson and negative binomial models were both tested to assess best fit. Model selection was then performed by examining the residual deviance and AIC, which showed negative binomial models best fit our data. The effect of the three treatments on overall natural enemy abundance was analyzed using ANOVA. We also used ANOVA to assess the effect of treatments on plant yield. Analyses were carried out in R version 3.2.2 and RStudio version 1.0.143 (R Core Team (2015)).

RESULTS

Herbivore Abundance

Pieris rapae

Throughout the experiment, sentinel collards were sampled twice per week providing us with 3,198 plant observations of 1480 eggs (averaging 0.46 eggs per plant). For the first sampling of each week, 663 eggs were found with 208 eggs found in control plots, 231 in the chemical treatment and 224 in the predator treatment plots on 1518 plants. This low number equates to an average of 0.44 eggs found per plant over the first 3 days of plant deployment. We found that treatment was not a significant predictor of egg abundance (Rao (2, 1670) = 0.586, p = 0.746; **Figure 3.2A**). However, we observed an effect of both week and distance: egg counts varied dramatically from week to week (Rao (4, 1666) = 93.5, p<0.001; **Figure 3.6**), and there was a slight positive relationship between distance from plot center and number of eggs (slope = 0.167 ± 0.035).

Overall egg numbers were also low during the second sampling of each week (day 5). In total, 817 eggs were found, equating to an average of 0.48 eggs per plant across the 1680 plants sampled. Specifically, there were 293, 255, and 269 eggs found in the control, chemical and predator treatments, respectively. Again, treatment was not a strong predictor of egg abundance in this experiment (Rao (2, 1677) = 2.525, p = 0.283; **Figure 3.2B**). However, egg abundance did vary dramatically by week (Rao (4, 1673) = 146.86, p < 0.001; **Figure 3.6**) and there was again a slight positive relationship between the number of eggs and distance from the central treatment plant (slope = 0.167 ± 0.030).

Aphid Colonization

Throughout the field experiment, aphid abundance was greater than that of *P*. *rape*. During the first 3 days of sampling each week, aphid numbers reached 4751, across the sentinel plants in all treatments. The most aphids were found in the control treatment (1854), followed by the chemical treatment (1613) and finally, the fewest aphids were found in the predator treatment (1284). In this case, treatment was a significant predictor of aphid abundance (Rao (2, 1667) = 22.99, p < 0.001; **Figure 3.2A**). Aphid abundance also varied dramatically by week (Rao (4, 1663) = 354.81, p < 0.001; **Figure 3.7**), with numbers reaching the highest in weeks 1, 2 and 3 and very few aphid colonists found in weeks 4 and 5 across treatments.

During the second sampling of each week, 6473 aphids were counted across treatments, this time with the highest aphid abundance found in the predator treatment (2285), followed closely by the control treatment (2233) and the fewest aphids found in the chemical treatment (1955). Unlike the first sampling of each week, treatment was not a strong predictor of aphid colonization on plants (Rao (2, 1677) = 4.63, p = 0.099; **Figure 3.2B**). Again, aphid abundance varied by week (Rao (4, 1673) = 2209.41, p < 0.001; **Figure 3.7**) driven mostly by a strong peak of aphid abundance in week 2. We collected and identified sub-samples of aphids on sentinel plants and after identification we found that the majority of the winged aphid colonists were either *Lipaphis pseudobrassica* (Davis) and *Pemphigus sp. (*likely *Pemphigus populi-transversus* (Riley)).

Natural Enemy Abundance

Over the field experiment, 414 predators and 4175 parasitoids were captured on our yellow sticky traps. Of the 414 predators 30% were ladybeetles and around 55% were spiders the remaining 15% were comprised of fireflies and lacewings. There was no effect of our treatments on the overall abundance of predators ($F_{6, 104} = 0.43$, p = 0.065; **Figure 3.4**), and abundance did not vary significantly by week ($F_{4, 104} = 2.08$, p = 0.089; **Figure 3.8**). There were 7 species of ladybeetles represented by the 122 total adult ladybeetles identified throughout the experiment. In particular, our treatments had no effect on the abundance of *H. axyridis*, the predator we used as our treatment source ($F_{6, 104} = 4.1801$, p = 0.943), of which only 20 were found. There was also no impact of treatment on the other 6 species of ladybeetles identified. Parasitoid abundance, while higher than predators, was also not influenced by treatment ($F_{6, 104} = 1.033$, p = 0.360; **Figure 3.4**). Abundance of parasitoids did vary significantly by week ($F_{4, 104} = 162.1$, p < 0.0001; **Figure 3.9**), with a peak in week 5.

Impact of Predators and Predator Cues on Yield

Yield was calculated from dry weights of collard plants that were directly transplanted into the ground of each plot at the beginning of the experiment. Overall, yield was not influenced by our treatments ($F_{2,218} = 2.47$, p = 0.088; **Figure 3.5**), yet dry weights were approximately 8% lower in both the chemical cue and predator treatments compared to the control where yield was the highest.

DISCUSSION

The ability to recognize the presence of predators and respond to this risk by altering traits that reduce the probability of being eaten is an important contributor to the evolutionary arms-race between predators and their prey (Lima and Dill 1990, Stankowich and Blumstein 2005). While recent advances in the field have contributed to our understanding of non-consumptive effects in terrestrial insect systems, most studies to date have been performed in the laboratory and greenhouse over short periods of time (Hermann and Landis 2017). Our major goal was to assess how non-consumptive effects might influence the community of herbivores commonly found on brassica plants and how those effects might cascade to influence other trophic levels within the food-web (namely, natural enemies and host-plants).

Overall, there was a surprisingly low population of our focal herbivore, *P. rapae*, observed throughout the field experiment which was represented by a total of 1480 eggs found during the entire experiment. Low population numbers could be a result of multiple factors including a low period in the seasonal population of this herbivore, due to the fact that our land did not previously consist of host plants for this insect or perhaps in part due to the drought that occurred in our study location in the summer of 2016 (MSU Enviroweather station, 42.6734, -84.4870).

Contrary to our predictions, we saw no effect of our treatments on the number of eggs laid by *P. rapae* and these dynamics did not change throughout the week between our two sampling times (three and five days after plant deployment). The lack of

treatment effect in egg laying might be a result of limited prey vulnerability. Since the adult stage of this herbivore is not at risk by ladybeetles itself, it is possible that it would not alter egg laying in response to risk for its progeny (as seen in Chapter 2). However, *P. rapae* adults have been shown to reduce egg laying in response to other ladybeetle predators, *Coleomegilla maculata*, that only threaten the egg and larvae stage (Layman and Lundgren 2015).

The number of eggs increased positively with increasing distance from the treatment plant, irrespective of treatment, which indicates that adult butterflies might oviposit preferentially at the field edge. Edge effects on the distribution and colonization of insects have been demonstrated in other systems (Tscharntke et al. 2002). However, such edge effects might be apparent with initial colonization and subsequent spatial distribution might be more even across the system (Boiteau 2005). Future studies might reduce the potential influence of edge effects by initiating experiments in a continuous field of host-plants where plots are not separated by a plant-free border as we had in our experiment.

In accordance with our hypothesis, aphid colonization on sentinel plants was influenced by our predator treatments, albeit only on the third day after plants and treatments were deployed. After five days exposed to our predator treatments in the field, aphid numbers were no longer affected by treatment. There are several examples of nonconsumptive effects of ladybeetles on aphids in the literature (Dixon and Agarwala 1999, Weisser et al. 1999, Mondor et al. 2005, Kaplan and Thaler 2010, Wilson and Leather

2012, Kersch-Becker and Thaler 2015a), though none of these studies were carried out in an open-field and all focused on the wingless morphotype of the aphid. In our study, we examined initial colonization in the field by winged, dispersal morphotypes. Therefore, our results uniquely add to the body of literature demonstrating that non-consumptive effects of ladybeetles on aphids are possible by adding that these dynamics can change over time. Examining the longer-term impacts of predators and predator cues on the overall success of aphid populations will be a necessary next step to examine if the combination of predator consumption and non-consumptive effects have a net negative impact on this herbivore.

Studies published to-date largely design experiments that test the interaction between a single predator and prey, and by doing so ignore the potential for interactive effects of these interspecific interactions on other important members of the food-web. In our system, we provided the possibility for interactions between all members of naturally occurring herbivores, predators and parasitoids. However, there was no effect of our treatments on the abundance of predators and parasitoids found in the field. As previously mentioned, the main herbivore in this system was exceptionally low in numbers during our experiment and this factor alone could have contributed to the abundance of natural enemies colonizing our field.

While we expected there to be a temporal effect of the predators and predator cues within the week, we did not predict the extreme variability of insect numbers we observed across weeks. This result highlights the importance to extend the length of

experiments looking at the role of predators on prey in agroecosystems because a simple snap-shot in time would likely provide a result that is not only difficult to reproduce, but also not representative of the full effect (or lack-there-of) in the system. Many experiments are limited across both space and time, perhaps over or under estimating effects between specific predator-prey pairs. In addition, those that force artificial arenas also prevent natural enemy dispersal, increasing the potential for habituation to cues associated with risk detection.

Lastly, overall plant yield was not different between treatments, suggesting that the addition of predators and predator cues, in this experiment, were not sufficient in altering prey performance in a way that affected their host plants. Since the only interaction that we found was with aphids, and aphid feeding is less likely to directly affect host plant biomass, it is not surprising that yield was unaffected.

Improving the Outcomes of Field Experiments Across Space and Time

Field studies which aim to understand the interactions between multiple trophic levels are difficult to design and perform. While the methodologies used to examine both the consumptive and non-consumptive effect of predators, and possible cascading effects, in the field. In particular, the main predators and prey within the system to be studied should be closely examined prior to field manipulation to ensure that non-consumptive effects are relevant and predicted to occur. Then including behavioral observations in the field, the use of pitfall traps and other sampling methods to better capture the resident predator community, deployment of sentinel egg cards to assess predation rate and

measuring both herbivory (leaf area removed by prey) and ultimate yield should provide more clarity in these studies.

Conclusions

In agricultural systems, the net effect of predators on prey is of particular importance since predators are often manipulated as biological control agents in an effort to reduce herbivorous pest pressure on economically important crops. We conducted an experiment in an open-field setting, that spanned the growing season for the host plant used by both P. rapae and aphid herbivores. We manipulated the presence of H. axyridis predators and predator cues to examine the possibility for NCE's to affect the colonization and subsequent damage caused by these herbivores while also examining possible impacts on the community of natural enemies in the open-field throughout the growing period. While our results predominantly show a lack of NCEs on P. rapae, we present evidence that many factors might influence the outcome of predator-prey relationships and that our current methods of examining these interactions in the laboratory and field mesocosms might not be representative of outcomes in natural settings. Therefore, there is a strong need for improved methodologies in field trials as combined with the use of theoretical modeling to better investigate the role of predator non-consumptive effects that incorporate naturally occurring insects at larger spatial and temporal scales.

FIGURES



Figure 3.1. (A.) Field configuration to examine the effect of predators and predator chemical cues on herbivore colonization and natural enemy abundance. Brown space indicates bare ground soil surrounding plots. Each square is an experimental plot. (B.) Configuration of experimental plots: central red circle depicts the sentinel plant that housed the treatment, dark green circles indicate placement of sentinel plants (at 0.5, 1, 2 and 4m radially in each direction) and light green lines indicate rows of planted collards. (C.) Diagram of sentinel plants that were placed within the planted collard field, in the ground, each week for the duration of the experiment (5 weeks).



Figure 3.2. Mean (\pm SEM) eggs laid by naturally occurring *P. rapae* butterflies on sentinel *Brassica oleracea* (Georgia collard) plants in response to a plant treated with either no predators or cues (Control), a plant with a blend *H. axyridis* chemical cues (Chemical Cue), or with five adult *H. axyridis* bagged to the plant. Eggs were counted on sentinel plants after they were placed in the field and exposed to the treatments for A) three days or B) after five days.



Figure 3.3. Mean (\pm SEM) naturally colonizing winged aphids on sentinel *Brassica* oleracea (Georgia collard) plants were counted in response to a plant treated with either no predators or cues (Control), a plant with a blend *Harmonia axyridis* chemical cues (Chemical Cue), or with five adult *Harmonia axyridis* bagged to the plant. Eggs were counted on sentinel plants after they were placed in the field and exposed to the treatments for A) three days or B) after five days. *Bars* mean (\pm SEM). (* indicates significant differences at p<0.05 following GLM).



Figure 3.4. Mean (\pm SEM) natural enemies were captured on yellow sticky traps each week for 5 consecutive weeks to assess the impact of a plant treated with either no predators or cues (Control), a plant with a blend *Harmonia axyridis* chemical cues (Chemical Cue), or with five adult *Harmonia axyridis* bagged to the plant.



Figure 3.5. Mean (\pm SEM) collard yield was measured by dry weight (g) of plants grown for five consecutive weeks in the field in response to either no added predators or cues (Control), the addition of *Harmonia axyridis* chemical cues (Chemical Cue), or with five adult *Harmonia axyridis* bagged to the central plant in each experimental plot.



Figure 3.6. Mean (\pm SEM) eggs laid by naturally occurring *Pieris rapae* butterflies on sentinel plants on sampling 1 (day 3) and sampling 2 (day 5) in the field, across five consecutive weeks.



Figure 3.7. Mean (\pm SEM) naturally occurring aphid colonists found on sentinel plants on sampling 1 (day 3) and sampling 2 (day 5) in the field, across five consecutive weeks.



Figure 3.8 Mean (\pm SEM) number of naturally occurring predators recovered from yellow sticky traps placed in the field for five-day exposures across five consecutive weeks.



Figure 3.9 Mean (\pm SEM) number of naturally occurring parasitoids recovered from yellow sticky traps placed in the field for 5 d exposures across five consecutive weeks.
CHAPTER 4: PREDATION RISK DIFFERENTIALLY AFFECTS THE BEHAVIOR AND PHYSIOLOGY OF TWO DISTINCT APHID MORPHOTYPES

INTRODUCTION

Among the most interesting interspecific interactions in insect ecology is the ongoing battle between predators and prey. In the past few decades, the complexity of these interactions has been emphasized as more research has begun to focus on the impact of predation risk on the behavior and physiology of prey (Hermann and Landis 2017). In essence, the net effect of predators includes both the density -mediated (consumptive effect) and trait -mediated (non-consumptive) effects they can have on prey and thus a greater understanding of predator-prey relationships can be gained by considering both of these important modes of interaction (Peacor and Werner 2001, Werner and Peacor 2003a, Peckarsky et al. 2008). Non-consumptive effects occur when perceived predation risk causes prey to change their phenotype (i.e. behavior, life-history, morphology) in ways that can ultimately interfere with prey performance (Lima and Dill 1990, Lima 1998b). While these effects have now been documented in several terrestrial insect systems (Hermann and Landis 2017), the bulk of the literature lies within aquatic insect and fish systems and terrestrial mammalian systems (Preisser et al. 2005b). Thus, there is still much to learn about the mechanisms that mediate predator detection, types or directionality of responses and the strength of these responses over both space and time in terrestrial insect systems. In particular, much of the current research in insect systems explores single response variables of prey which might under- or overestimate the overall impact of predation risk, especially in traits that interact across life-history.

If a single trait is measured in response to risk, we might infer a negative or positive outcome for the prey accordingly. However, other related traits that might not be measured could be equally important to measure. For example, many insects have been shown to alter feeding behavior in response to predation risk (Gonthier 2012, Thaler et al. 2012, Rypstra and Buddle 2013b, Hermann and Thaler 2014, 2018). Since this behavior comes at a potential cost for development and fitness, it would make sense for the prey to avoid colonizing areas where predation risk is high. To date, there are few studies that look at multiple relevant traits of free-living insects in a single study (but see: Hermann & Thaler, 2018). In order to measure the impact of predation risk on prey fitness, it is first necessary to understand which traits change, especially ones that might interact. For example, if predation risk influences colonization or oviposition decisions by prey, then subsequent behaviors in habitats where risk is high might not be biologically relevant to measure.

Among insects, aphids represent a unique group with a complex life-history. While many species lay eggs when needed for overwintering, the dominant form of reproduction by aphids is giving live birth to nymphs, asexually. Further, when producing nymphs, it is possible for aphid mothers to generate two distinct morphotypes – one of which is a winged morph, primarily for dispersal (alate) and the other is a more sedentary and primarily reproductive, wingless morph (aptera) (Blackman and Eastop 2000, Braendle et al. 2006). The formation of alates in aphid populations is generally considered a response to stressors (plant quality, overcrowding, pathogens and natural enemies) that allows for dispersal (Müller et al. 2001, Kunert and Weisser 2005, Hatano

et al. 2012). There are several examples of predator-induced wing formation in aphids, though most studies have focused on a single species of aphid, *Acyrthosiphum pisum* Harris (Dixon and Agarwala 1999, Weisser et al. 1999, Kunert and Weisser 2005, Mondor et al. 2005, Kaplan and Thaler 2012, Purandare et al. 2014, Kersch-Becker and Thaler 2015a). Interestingly, experiments examining the effects of predators on aphid traits have focused exclusively on the apterous morph. Aptera produce more offspring than their winged counterparts since there are significant reproductive trade-offs associated with the production of wings in alates (Johnson 1963, Groeters and Dingle 1989). Because of the physiological differences between morphotypes, we might predict that the induction of wings can lead to variation in other phenotypes as well due to energetic and immune tradeoffs. To our knowledge, there is no comparison of the impact of predators or predator cues across this polyphenism in aphids.

The objective of this study was to assess if aphid prey detect and respond to predator risk and if the responses vary between aphid morphs across several important traits. Using green peach aphids, *Myzus persicae* Sulzer, as prey and the multi-colored Asian ladybeetle, *Harmonia axyridis* Pallas as a predator, we first asked if the presence of these predators on plants would interfere with host-plant preference by adult aphids and if the responses differed between reproductive or dispersal morphs. Then, we evaluated the impact of predator cues on aphid fecundity in both morphs. Lastly, we asked if the presence of predator cues would influence aphid investment in dispersal morphs by inducing the production of alates. We predicted that both aphid morphs would indeed detect risk from predator cues and avoid these cues when choosing among host plants.

We also expected that aphid fecundity would be altered in the presence of these cues. Finally, we expected that aphids would invest more heavily in alate offspring following exposure to predator cues.

METHODS

Plants and Insects

We maintained a colony of *M. persicae* on *Brassica oleracea* (cv. Georgia collard greens) in a climate-controlled insectary (22 C; 16:8 L:D photoperiod). Collard host plants in colony cages were watered weekly and replaced periodically to avoid aphid crowding or when plant death occurred. Cages contained all ages of aphids and alate or apterous adults were collected from these cages as needed for experiments.

A colony of *H. axyridis* was established from larval and adult beetles field collected in Ingham County, Michigan. All stages of *H. axyridis* in the colony were fed a mixture of corn leaf aphids (*Rhopalosiphum maidis*) and bird cherry-oat aphids (*Rhopalosiphum padi*) which were reared on barley (*Hordeum vulgare*) plants in 10 cm round pots. Colony cages were stored in a climate-controlled growth chamber (25 C; 16:8 L:D photoperiod). Three times per week senescing barley plants that no longer harboured aphids were removed from ladybeetle cages and replaced with one to two new aphid infested plants. At this time, ladybeetle eggs were also removed and placed into a new colony cage in order to separate by life stage. Only adult male and female *H. axyridis* were used in experiments. *Brassica oleracea* plants were used in experiments as well as for *M. persicae* colonies (described above). Plants were grown from seed (Burpee, product #52159A) in Promix potting soil (Premier Horticulture Inc., Quakertown, PA, USA). Germinating seeds were placed in a climate-controlled greenhouse (25 C; 16:8 L:D) and watered daily. Once plants were established, stems were thinned to one plant per cell in a 100-cell plug tray and fertilized once weekly (20-20-20, Peters Professional Water-Soluble Fertilizer, Brantford, Ontario). Once plants were two-three weeks old and seedlings had developed true leaves, they were transferred from plug trays to 10 cm round pots where they remained until use in experiments at 4-6 weeks old.

Aphid Host Preference in the Presence of Predator Cues

Two-arm olfactometer experiments were designed to determine the effects of ladybeetle volatile odor cues on the behavior of the prey insect, *M. persicae* (for a detailed diagram, see **Figure 4.1A**). All experiments were conducted in a climate and light controlled walk-in growth chamber (25 C, 16:8 L:D photoperiod). Odor sources were placed in 35 cm tall, 615 cm wide dome-shaped glass arenas (ARS, Gainseville, Florida) set atop teflon guillotines and connected to 1.0 LPM, charcoal filtered, and humidified air flow. Guillotines were placed around the stem of the plant, sitting on the rim of the pot, allowing the foliage of the plant to enter the glass arena but excluding the pot, soil and base of the plant. Two separate odor source arenas were set-up in tandem, one for control and one for an odor treatment, 16 h prior to experimentation to allow for plant and insect acclimatization and volatile cue build-up. Control and treatment arenas were then connected via teflon tubing with each odor source supplementing airflow to an

individual arm at the end of a y-shaped olfactometer. The olfactometer consisted of an 11 cm long glass tube that branched into two 7.5 cm arms (Michigan State University, East Lansing, MI). The internal diameter of the tube and arms was 1.5 cm. In this way, each arm of the "Y" consisted of a distinct odor source that flowed down towards the base of the "Y" where insects were released and left to make a choice. For each experimental replicate, a single adult aphid was selected randomly, and placed at the far end of the olfactometer with a fine tipped paintbrush. Aphid movement towards either treatment or control arm was observed for a maximum of ten minutes. One replicate was conducted per individual aphid. Following each replicate, the y-shaped glass olfactometer was washed with both acetone and hexane and left to dry to ensure that aphids were not influenced by the movement of their conspecifics in the glassware during previous replicates. In addition, the treatment and control tubes were switched from right to left arm of olfactometer prior to each trial in order to reduce positional bias. All trials were conducted between 09:00 and 13:00 hours

Y-Tube Odor Sources

The odor sources for all y-tube assays used the same basic arena set-up which consisted of a single collard plant and a moistened cotton ball placed inside the glass chamber (described above), set up 16 h before experiments began. This basic set-up served as the control odor source. To create our predator odor treatments, we used the same basic set-up and then added five male and five female ladybeetles to create our 'predator +plant' treatment and "predator pre-treatment'. In both predator odor treatments, ladybeetles were left free-roaming in the glass arena with the collard plant for

the 16 h prior to experimentation, but for the predator pre-treatment odor source, all ladybeetles were removed just before y-tube assays began. Y-tube assays were run with a control odor source and one of the two predator treatments. All y-tube experiments were conducted with both apterous adult and alate *M. persicae*.

Aphid Performance in Response to Predator Cues in Petri Dish Arenas

We examined whether *M. persicae* would alter the number of nymphs they produce in the presence of predator cues by developing a modified petri dish arena. In this experimental arena we were able to physically separate aphid prey from ladybeetle predators while allowing volatile odors and visual cues of these predators to be experienced by the developing aphids. Petri dish arenas were made by cutting a 7 cm diameter hole in the larger half of two petri dishes. The lids were placed top to top enclosing a mesh screen and fixed together with hot melt glue (**Figure 4.1B**). A freshly excised collard leaf disc (60 mm diameter) placed directly atop moistened filter paper (Whatman 90 mm circles) cut to fit the bottom portion of the petri dish arena. Treatments were placed in the top portion of the petri dish and consisted of: 1) control (predator-free) or 2) predator (two *H. axyridis*). For each experimental replicate, a single apterous or alate aphid adult was left to reproduce over 3 d. At the end of the experiment, we counted the number of nymphs produced. For apterous aphids, n = 51 replicates were performed; for alates there were n = 59 control and n = 58 predator cue replicates.

Alate Formation in Response to Predator Cues in Petri Dish Arenas

We used the modified petri dishes (described above) to examine if predator risk affects aphid physiology. Here we exposed aphid prey to predator cues for a 3 d and monitored for induction of alate (winged) morphs. One of two treatments were placed in the top portion of the petri dish experimental arenas: 1) predator-free control treatment which contained a single moistened cotton ball or 2) predator treatment which included a two *H. axyridis* adults in the top portion along with the moistened cotton ball.

In each arena, five large aphids were randomly selected from the stock aphid colony and gently placed on the leaf disc with a fine-tipped paint brush. Aphids were then exposed to either the control or predator treatment continuously for 3 days. After 3 days, the total number of these aphids that developed wings in each treatment were counted. There were 20 replicates for each treatment.

Influence of Predator Cues on Aphid Fecundity and Alate Formation on Intact Plants

We also examined the impact of predator cues on nymph production and alate formation on intact plants, over a longer duration of time. For this experiment, we utilized 4 w old collard plants grown in smaller, 5.08 cm diameter round pots. Potted plants were placed inside 24 oz cylindrical glass ball jars (Ball®, item # 1033893) on top of one sheet of filter paper (Whatman 90mm circles). For each replicate, seven apterous adult aphids were chosen randomly from the stock colony and placed on the plants inside the jars. In each ball jar, we placed a mesh barrier between the plant and the lid of the jar,

where treatments were placed. A mesh barrier was fashioned approximately 3 cm above the top of the plant by inserting a plastic acetate ring that fit snugly in the top portion of the ball jar arena. On the top and bottom of the acetate ring, mesh was used to allow for airflow and exposure to treatments but inhibit aphid or ladybeetle movement out of the arena.

Three treatments were established: 1) a control treatment with only moistened cotton in the mesh barrier (n = 17), 2) a lethal predator treatment with one male and one female ladybeetle contained within the arena along with the aphids and the host plant (n = 16), and 3) the predator cue "risk" treatments in which one male and female ladybeetle were separated from aphids by mesh barrier (n = 18). Jars were sealed with metal ring lids that secured the mesh barrier onto the top of the jar. Jars were placed in a climate-controlled growth chamber as described above for the duration of the experiment. After the 7 d, aphids in each jar were counted and the jars were then returned to the growth chamber for an additional 7 d. After the second 7 d period jars were removed from the growth chamber and plants were removed from jars in order to obtain a total aphid count over 14 d as well as assess alate formation. Since aphids in our colonies complete a full life cycle in 7 d, this trial represents 1-2 generations of aphid production.

Statistical Analysis

All data were analyzed using JMP (JMP Pro[®], Version 12. SAS Institute Inc., Cary, NC, 1989-2007). The number of *M. persicae* entering the control versus treatment arm in the y-tube olfactometer bioassays were compared with chi-square tests. The null

hypothesis was equal entrance by aphids into both arms of the olfactometer. We used a G- test to compare the number of alates present in the predator treatment to that of the control treatment in both the short-term petri dish assay and the full-plant assay. Here, we predicted the number of alates would differ between treatments and the null hypothesis was equal numbers. For the remaining experiments, our data were not normally distributed and we were unable to normalize these data through square root or log transformation, precluding parametric tests. Therefore, we used the non-parametric Wilcoxon signed-rank test to analyze whether nymph production by both alate and apterous *M. persicae* differed from the null hypothesis of equal numbers of offspring between treatments. Finally, our longer-term nymph production and alate formation experiment data were first analyzed to compare number of aphids across our three treatments using a Kruskal-Wallis one-way analysis of variance. Then, each pair was analyzed using non-parametric Wilcoxon multiple comparisons.

RESULTS

Aphid Host Preference in the Presence of Predator Cues

When presented with a choice between a predator-free odor source or an odor source that included *H. axyridis* predators, adult apterous *M. persicae* preferred the arm with predator-free control plants ($\chi^2 = 5.12$, p = 0.024, **Figure 4.2A**). However, when the physical predators were removed from the odor source arena prior to bioassays, the adult apterous aphids no longer preferred predator-free control plants ($\chi^2 = 3$, p = 0.083,

Figure 4.2A). In contrast, alate *M. persicae* preferred to move towards plants with

predators present compared to the predator-free odor source ($\chi^2 = 7.53$, p = 0.006, Figure **4.2B**), but only when the physical predators were in the odor source arena. When predators were removed from the odor source arena prior to bioassays, we observed equal preference between the olfactometer arms was observed ($\chi^2 = 0.037$, p = 0.847, Figure **4.2B**).

Aphid Performance in Response to Predator Cues in Petri Dish Arenas

The presence of both predator cues from *H. axyridis* predators in a petri dish arena led to a 23% reduction in the overall number of nymphs produced by adult apterous *M. persicae* over 3 d compared to reproducing adult aphids in control petri dishes where predator cues were absent (Z = -4.08, p < 0.0001, **Figure 4.3A**). However, when adult alate *M. persicae* were left to reproduce in the presence of predator cues there was no discernable effect on nymph production compared to predator-free controls (Z = -0.46, p = 0.65, **Figure 4.3B**).

Alate Formation in Response to Predator Cues in Petri Dish Arenas

To investigate the potential for predation risk to induce wing formation, we exposed aphids to predator cues, by physically separating the aphids on leaf discs from ladybeetle predators in a petri dish. In this experiment, the number of individuals that produced wings after 3 d in petri dishes differed between the predator cue treatment and the predator-free control, with a five-fold increase in alate production in the predator cue treatment (G = 8.73, p = 0.003). Overall, 3% of aphids in the control treatment were

winged after 3 d whereas 15% of aphids formed wings in the treatment dishes that left aphids exposed to predator cues.

Influence of Predator Cues on Aphid Fecundity and Alate Formation on Intact Plants

Nymph production differed significantly among treatments ($\chi^2 = 32.87$, p < 0.0001, Figure 4). Pairwise comparisons of the different treatments show that the risk treatment yielded significantly more nymphs than the control and lethal treatments (Z = 3.219, p = 0.0013; Z = 4.903, p < 0.0001, respectively) while lethal treatment had the fewest aphids after 14 d. Alate formation (n = 12) was significantly increased in the risk treatment compared to both the control and lethal treatment where no alates were found during the entire experiment (G = 16.636, p < 0.0001).

DISCUSSION

This study demonstrates that *M. persicae* can detect and respond in several important ways to the cues of *H. axyridis* and that this detection has differential effects on alate and apterous morphs. Specifically, we observed behavioral changes arising from predator cues along with host-plant odors which altered the preference of aphids in a y-tube olfactometer. Interestingly, while apterous morphs avoided predators on plants by choosing to walk towards predator-free controls, alate aphids preferred plants that harboured predators. In the presence of predator cues, apterous aphid fecundity was altered by initially reducing nymph production (3 d) and subsequently increasing nymph production when in the presences of predator cues for a longer period 14 d representing

multiple generations. However, alate aphids did not alter their nymph production in the presence of predatory cues. Lastly, we found increased investment in the formation of dispersal morphs in the offspring of aphids in the presence of predator cues. Together, these results show that aphid prey are capable of using predator cues to identify risk and respond by altering behavior, fecundity and morphology.

Alate aphids were attracted to host plants with predators in our y-tube choice experiments, which was contrary to our predictions that all prey morphotypes would avoid plants with predator cues associated with them. In another systems, Colorado potato beetle, Leptinotarsa decemlineata, colonization was not affected by the presence of predators (*Podisus maculiventris*), yet subsequent behaviors such as feeding were altered once prey were established on plants (Hermann and Thaler 2018). In our study, we also measured fecundity in alate aphids in response to predator cues, but here there was no difference in nymph production compared to predator-free controls. While an attraction to host plants might not intuitively be adaptive, it is possible that alates are better equipped to avoid predators on plants due to the presence of wings. In future studies it would be necessary to observe the outcome of alate colonists on plants that contain predators to better understand this result. Further, the attraction to host plants by alates is no longer significant when predators are removed prior to experiments suggesting that physical presence of predators is necessary for the attraction to occur. Again, to gain insight on this result, work must be done to elucidate the adaptive potential of choosing a plant where predators are actively foraging.

As predicted, apterous aphid adults avoided plants that harboured predators and strongly preferred predator-free control plants. There was also a trend for these aphids to avoid plants that had previously harboured predators. Since apterous aphids lack wings and thus the ability to disperse by flight, it makes sense that they would prefer a clean, predator-free plant for reproduction over one that leaves them and their offspring at risk, as documented in other insect systems (Lee et al. 2011, Wasserberg et al. 2013, Sendova et al. 2015, Hermann and Thaler 2018). Aphid movement between plants by apterous aphids can be an important dispersal strategy in some species of aphids (Losey and Denno 1998, Kersch-Becker and Thaler 2015a). To understand if the preference we found in the y-tube olfactometer would allow for increased disperal away from predation risk, future experiments where aphids can move freely between risky and control plants would be a necessary next step. In addition, apterous adults reduced their production of nymphs in the presence of close-range predator cues over 3 days. This result followed our expectations because reducing behaviors that leave prey more apparent and thus vulnerable to predation is a common response to predation risk (Bernays 1997). While giving live-birth, aphids are likely less able to move and defend themselves and thus either avoid plants that contain predators or reducing apparency by altering behavior would be a strategy for predator evasion. However, one pitfall of this assay was that it was performed in small arenas and thus cues were very concentrated and spatially confined. Interestingly, when we scaled this experiment up to provide prey with a full plant, rather than a leaf-disc, and exposed them to the same predator cues for a longer period of time (representing 1-2 generations), apterous adults produce more nymphs in response to predator cues and significantly fewer in control treatment. Because the adult

aphids in this experiment were unable to disperse by walking to a predator-free plant, perhaps here their strategy shifts to one of bet-hedging. In this case, the more offspring produced by individual adults might allow for the population to succeed, even in the face of predation risk. Albeit uncommon, increased production of offspring under predation pressure has been found in at least one other aphid system as well (Kersch-Becker and Thaler 2015a). For example, when potato aphids (*Macrosiphum euphorbiae* Thomas) were exposed to convergent lady beetle predators (*Hippodamia convergens* Guérin-Méneville) that were rendered non-lethal through mouthpart manipulation, led to significantly higher numbers of nymphs produced by the aphids. As this field continues to expand, it will be important to better understand the factors that influence the directionality of prey trait changes in response to risk.

Investment in producing a higher proportion of dispersal morphs in response to various stressors (plant quality, crowding, alarm cues, natural enemies) has been previously demonstrated (Dixon and Agarwala 1999, Weisser et al. 1999, Kunert and Weisser 2003, Mondor et al. 2005, Kaplan and Thaler 2012, Kersch-Becker and Thaler 2015a). In our study we found that alate formation was pointedly higher in the presence of predator cues compared to controls in both our petri-dish and full plant assays. This result is highlighted in our full plant experiment where alates were only recovered in the risk predator treatment that provided only indirect predator cues. There were no alates found in control treatments or treatments with lethal predators present. In this experiment, aphid abundance was also highest in the risk treatment and since crowding can lead to increased alate formation (Purandare et al. 2014), that factor cannot be completely ruled

out. However, previous work has shown that aphids increase wing production in the presence of predators, but only when their antennae are intact (Kunert and Weisser 2005) suggesting that chemical cues are likely responsible for morph induction.

Our work provides evidence, adding to a growing body of literature, that predator cues are a factor in prey detection of predation risk and that detection can lead to varied responses in different morphotypes of the same prey. In addition, we show that several prey traits are influenced by predator cues alone, all of which are important for the success of individual aphids and could scale to interfere with the success of the population. Our study adds to a growing body of literature within insect systems that identifies the important role of predator chemical cues in predation risk related non-consumptive effects (Gonthier 2012, Hoefler et al. 2012, Ninkovic et al. 2013a, Hermann and Thaler 2014), which has direct implications for understanding fundamental insect ecology but also has practical applications in pest management and conservation efforts (Hermann and Landis 2017). Future work must look at the adaptive potential of these shifts in behavior and physiology to identify if these trait changes ultimately aid in predator avoidance and overall survival or if they are maladaptive and lead to a net negative impact on prey population growth and success.

FIGURES



Figure 4.1. (A) Schematic of the Y-tube olfactometer set-up. Air flows first through charcoal filter, is then regulated using a flow-meter, then humidified using a flask filled with distilled water and finally pumped into the glass chamber which contained odor treatments. Air is then pumped from the odor treatment chamber directly into one of the arms of the "Y". Aphids were placed individually at the base of the "Y" and monitored for their first choice into one of the arms of the olfactometer. (B) Modified petri dish used to examine aphid nymph production and alate formation in response to predator cues or predator-free controls. Left: the separated portions of the petri dish included the bottom, which contained two modified petri dish lids that held a mesh barrier between the aphid prey and the treatments; the top, this is the portion that contained the treatments which were either 1) a moistened cotton ball (control) or 2) a moistened cotton ball with two *H. axyridis* adults (predator). (The above diagrams were provided by Nick Sloff, Department of Entomology, The Pennsylvania State University)



Figure 4.2. Responses of adult apterous (top panel) and alate (bottom panel) *M. persicae* to odor sources in a two-choice y-tube olfactometer (top). Treatment plants were preexposed to 10 *H. axyridis* adult predators for 16 h and control plants were predator-free (* indicates significance at p < 0.05 following chi-square test of goodness of fit).



Figure 4.3. Nymph production by single *M. persicae* (A) apterous morphs or (B) alate morphs in a petri dish arena. Aphids were exposed to either a predator-free control or a predator treatment consisting of two *H. axyridis* ladybeetle predators for three consecutive days (* indicates significance at p < 0.05 as indicated by the non-parametric Wilcoxon signed-rank test).



Figure 4.4. Nymph production by seven *M. persicae* apterous aphids exposed to either a predator-free control, two *H. axyridis* ladybeetle predators (lethal), or two *H. axyridis* ladybeetle predators confined in a mesh barrier (risk) for 14 consecutive days (* indicates significance at p < 0.05 following a non-parametric Kruskal-Wallis, one-way analysis of variance).

CHAPTER 5: CONCLUSIONS AND CLOSING REMARKS

Interactions between predators and their prey have been key factors in driving diversification and adaptive radiations of taxa since the Cambrian Era when the diversity of metazoan fauna exploded. The evolutionary arms race between predators and prey is evident through the emergence of adaptations such as claws and sharp teeth allowing some organisms to catch and consume food whereas spines, tough shells and increased locomotion allowed others to escape with their lives. Today, humans have found several ways to harness the ecological dynamics between predators and prey. A wide variety of predators are used in personal defense, to gain or gather sustenance in hunting, to control nuisance animals and even to reduce losses of livestock and food crops. Despite predatorprey dynamics being well studied and well utilized, we typically think of predators only affecting prey through direct consumption. However, as the arms race suggests, prey are not defenseless and can initiate defensive behaviors and physiology in an effort to avoid being eaten. Yet, the initiation of anti-predator defenses come at a cost to prey and the effects of predators on prey survival and performance that are unrelated to direct consumption are called non-consumptive effects.

Non-consumptive effects are widely recognized across taxa from elephants to arthropods in both aquatic and terrestrial environments. However, there are disproportionately fewer studies in terrestrial insect systems. An understanding of NCEs is of particular importance in agroecosystems where predatory insects are manipulated to control insect pests. To date, in agricultural pest management predators have been utilized to gain suppression of herbivorous pests through direct consumption. However,

there is also a possibility to disturb or disrupt insect prey using predator cues that lead to NCEs in a way that would benefit the cropping system. The research presented in this dissertation focused on evaluating the impact of predator NCEs on important insect prey in cropping systems. However, the results herein provided evidence that the occurrence of NCEs are dependent on prey species identity and that manipulation of predator cues can influence other members of the naturally occurring community in which they are deployed. These results point to three major areas with which we should focus future research on NCEs in terrestrial insect systems.

First, we must gain a better understanding of what drives strong or weak NCEs. As demonstrated in Chapter 2, even if a predator (*Harmonia axyridis*) and its potential prey (*Pieris rapae*) have spatial overlap and an overlapping phenology there may not be any influence of the predator or their cues on prey survival and performance. The lack of NCEs in this system was unexpected and allows us to consider several factors that could influence both the strength and the occurrence of these interactions. Future work that identifies systems where NCEs do not occur will allow us to understand if factors such as coevoutionary history between predator-prey pairs, the overall strength of the consumptive effect in a given system, specific functional traits of the predator or prey and perhaps even the influence of the surrounding habitat or landscape might influence NCEs. The ability to predict the occurrence and strength of NCEs will be especially important if manipulation of predator cues is desired in open-field settings to control pests. In agricultural systems, management measures are typically applied to control one or few key insect pests, but insect communities can be highly diverse and thus, there is a

possibility for directed manipulations to also interfere with other members of the food web.

Second, it is important to understand the cascading community responses to predator manipulations if application of such cues is desired in agricultural systems. Even with the dominant practice of monoculture crop plantings, there exist complex food-webs consisting of a variety of animals, both predators and prey. When a member of the food web is disrupted or removed it is possible for trophic cascades to occur. The best-case scenario for a trophic cascade in an agroecosystem would be if following the introduction of a predator, the success of the major pest insect would be reduced, which in turn would benefit crop plant yield by relieving it of herbivory through both consumptive and nonconsumptive effects. However, food webs rarely consist of a single plant, herbivore and predator. As demonstrated in Chapter 3, the manipulative increase of predator cues in an open field setting can have non-target consequences on additional pests or predators in that system, which may or may not lead to benefits in yield. It is thus crucial for researchers to consider the community-wide influence that these manipulations might have on each level of the trophic chain.

Lastly, in order to investigate community-wide impacts and the potential for cascading effects, we must scale up experiments to more realistic spatial and temporal scales. It is clear that NCEs occur in several insect systems, yet the majority of work has been done over extremely short durations (<24 h) in laboratory or greenhouse settings. While short-term manipulations in controlled settings will allow researchers to tease apart

mechanisms for prey detection of predator risk, actual impacts on prey survival and performance must also be observed in nature to impact prey populations in a meaningful way.

Overall, evidence that predator non-consumptive effects might be useful in pest manipulation is mounting. However, there is still much to learn prior to successful application. Similar to the how controlled releases and ecological impact surveys are carried out prior to beneficial insect release, we should begin to consider the ways that NCEs could carry significant impacts through the ecosystem in both natural and argoecosystems. Importantly, this work will bring us closer to disentangling the complex interactions between predators and prey which can greatly benefit our understanding of basic and applied insect ecology. APPENDIX

APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: _____ #2019-02 _____

Author and Title of thesis: Sara L Hermann Impact of Predation Risk on the Behavior and Physiology of Insects in Agricultural Systems

Museum(s) where deposited: Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens: Family	Genus-Species	Life Stage	Quantity	Preservation
Coccinellidae	Harmonia axyridis	adult	10	pinned
Pieridae	Pieris rapae	adult Larvae	10 10	pinned alcohol
Aphidae	Myzus persicae	adult	10	alcohol

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