

HABITAT MANAGEMENT AND BIOLOGICAL CONTROL INFLUENCE *PIERIS RAPAE*
(LEPIDOPTERA: PIERIDAE) HOST PLANT CHOICE AND PERFORMANCE

By

Margaret Lund

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ABSTRACT

HABITAT MANAGEMENT AND BIOLOGICAL CONTROL INFLUENCE *PIERIS RAPAE* (LEPIDOPTERA: PIERIDAE) HOST PLANT CHOICE AND PERFORMANCE

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In recent years, our understanding of predator-prey interactions has grown in recognizing that predators can affect prey species both through consumptive and non-consumptive effects. While consumptive effects result in direct consumption of a prey species, non-consumptive effects result in a behavioral or physiological prey response to predation threat, and may have an overall larger impact on prey success than predator consumptive effects. Additionally, there is a gap in our understanding of how habitat management practices, such as cover crop mulches or nutrient management, might influence these interactions. My research aims to fill these knowledge gaps by 1) determining the impact of habitat, host plant, and predator cues, alone and in combination, on *P. rapae* oviposition in greenhouse choice tests and field observations; 2) investigate how top-down (predator) cues and bottom-up (plant) cues interact to influence *P. rapae* adult and larval behavior; 3) determine consumptive and non-consumptive effects of different predator species, alone and in combination, on *P. rapae*; 4) investigate how habitat management practices influence predator – prey interactions; 5) identify habitat domains of three common natural enemies and how their interaction might alter these domains; 6) and determine how habitat management practices influence wild natural enemy abundance.

Oviposition preference of *P. rapae* was observed in greenhouse choice tests in 2015-2017 and in field observations in 2016 to determine the impacts of plant size, habitat structure, plant nitrogen, and predator cues on host plant choice. In both greenhouse and field experiments, *P. rapae* preferred plants that were large in size compared to small plants, and plants without added

habitat structure (no plastic leaves or cover crop mulch). Plant size and nitrogen had a synergistic effect on host plant choice, with large high nitrogen plants accruing more eggs than either cue alone. Predator cues had no significant effect on oviposition.

Single predator species consumptive and non-consumptive effects were measured in environmental chamber bioassays in 2017-2018 and in field experiments in 2016-2018. Adult and larval *P. rapae* altered their preference for bottom-up factors (plant nitrogen) under predation threats from differing predator species, preferring high nitrogen plants when threatened by *Hippodamia convergens*, but not *Podisus maculiventris*. Additionally, larvae consumed more leaf tissue and grew larger when threatened by *H. convergens*, but leaf tissue consumption and larval growth did not change under threat by *P. maculiventris*, suggesting that larvae may change their behavior if they are able to quickly outgrow life stages vulnerable to predation.

Multiple species assemblage consumptive and non-consumptive effects on *P. rapae* were observed in environmental chamber bioassays in 2016 and in field experiments in 2016-2017. Predator habitat domains were measured in 2017, and a wild natural enemy analysis was performed in the field in 2017. *Hippodamia convergens* present with *P. maculiventris* had the highest level of control on *P. rapae* larvae. Lycosidae negatively impacted *P. rapae* consumption in almost all predator assemblages, and both *H. convergens* and *P. maculiventris* altered their behavior when present in cages with Lycosidae. Habitat management in field experiments did not impact predator effects; however, habitat management in field plots did influence abundance of some natural enemies. Overall, the results of this research provide a deeper understanding of the effects of habitat management and predator species identity on predator consumptive and non-consumptive effects on *P. rapae*, and have implications for cole crop growers who may want to utilize habitat management strategies to aid in *P. rapae* pest management.

This dissertation is dedicated to my parents for their constant love and support, and for teaching by example that you can achieve anything with hard work.

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CHAPTER 1: Introduction to habitat management and predator-prey interactions

Habitat management and biological control

The goal of habitat management in agroecosystems is to alter the vegetation in order to improve availability of the resources required by crops and beneficial arthropods (Landis et al. 2000). Habitat management is an important technique for conservation biological control, because many agroecosystems do not provide adequate resources for natural enemies needed to control the pest species (Landis et al. 2000). There are many different management techniques which can improve the habitat for crops and natural enemies; for example, different tillage techniques, planting of native plants, cover cropping, and intercropping. Conservation biological control efforts combined with effective habitat management techniques can lead to increased beneficial insects and decreased specialist pest species (Beirne 1975, Hall and Ehler 1979, Altieri 1999).

The *resource concentration hypothesis* states that herbivore species that are specialists on a narrow range of crops prefer and are more likely to remain in areas where hosts plants are concentrated, such as monocultures (Root 1973, Sheehan 1986, Björkman et al. 2010). Concentrated host plants provide ample oviposition sites for adults as well as an abundance of uninterrupted food source for the larvae. Cover crops, planted as intercrops between crop rows, may work to lower pest populations by either disrupting the oviposition behavior of the adults, or acting as a trap crop which draw the ovipositing adults away from the original host plant (Finch and Collier 2000, Björkman et al. 2010, Finch and Collier 2012). One specialist herbivore, *Pieris rapae* L. (Lepidoptera: Pieridae) relies on glucosinolate cues in its host plants for oviposition

(Renwick and Chew 1994), so in this case it is likely that planting cover crops within the field repels butterflies from the crop due to the disruption in chemical cues.

While the resource concentration hypothesis focuses on the pest species, the *enemies hypothesis* predicts that natural enemies will be more prevalent in habitats with increased complexity and diversification due to an increase in available prey, nectar sources, and microhabitats (Root 1973, Sheehan 1986, Björkman et al. 2010). For example, in more complex habitats *P. rapae* populations may be negatively impacted due to increased predator foraging on *P. rapae* eggs and small larvae. Although resources may be more abundant for natural enemies in a diverse habitat, an alternative hypothesis suggests that the increase in plant surface area in a diverse habitat may counteract the success of biological control (Sheehan 1986, Altieri 1999, Björkman et al. 2010).

Influence of habitat management on host plant selection

During host plant finding and oviposition, insects utilize a variety of cues in order to find high-quality habitat with low predation pressure (Rausher 1979, Feeny et al. 1989, Janz and Nylin 1997). Insects may experience cues simultaneously or sequentially, and encounter different cues at different distances (Thompson and Pellmyr 1991, Battaglia et al. 2000). Long-distance cues, such as habitat structure, plant density, or plant size (Myers 1985, Meiners and Obermaier 2004) may have a large initial impact on host plant finding, and determine what short-distance cues the insect will encounter. Short-distance cues, such as plant nutrient levels, plant texture, and plant volatiles (Tahvanainen and Root 1972, Minkenberg and Ottenheim 1990, Rojas et al. 2003), are encountered within close proximity or upon contact with a host plant, and are the final cues encountered before host plant choice.

Habitat management strategies may alter these cues by increasing habitat complexity, and changing subsequent long-distance visual cues as well as short-distance cues such as plant nutrient levels and volatile cues. The *plant vigor hypothesis* suggests that plants with vigorous growth resulting in large size should be favorable to herbivores over plants with lower growth rates (Price 1991). If habitat management increases plant nutrient levels or growth, herbivores may choose these plants over other neighboring fields. However, according to the *resource concentration hypothesis*, herbivores that are specialists will prefer to remain in habitats where host plants are concentrated (Root 1973), suggesting that increasing habitat complexity with habitat management could deter specialist herbivores. Additionally, according to the *enemies hypothesis*, increased habitat management could draw in more predator species which could produce higher amounts of short-distance cues, also resulting in deterrence of herbivores.

Impact of habitat management on predator consumptive effects

The *enemies hypothesis* focuses on the direct interaction between natural enemies and habitat management, but adding organic resources to a crop field can have an indirect impact on natural enemies by changing the plant quality and thus affecting herbivores and their interactions with natural enemies (Kaplan and Thaler 2010). There are several mechanisms to explain how changes in plant quality may impact the relationship between herbivores and natural enemies: (1) low quality plants may delay herbivore development thereby increasing exposure time to enemies (*slow-growth high mortality hypothesis*; Clancy and Price 1987, Kaplan and Thaler 2010); (2) herbivores may have the ability to sequester plant toxins for use in their own defense (Kaplan and Thaler 2010); (3) locally-induced plant resistance or drop in plant quality may cause increased movement of herbivores, which increases apparency to visually-oriented predators

(Anholt and Werner 1998, Kaplan and Thaler 2010); (4) herbivores feeding on plants may cause cues released from the damaged plants that attract natural enemies (Kaplan and Thaler 2010). Specifically looking at mechanisms 1 and 3, the quality of the crop may greatly influence consumptive behavior of predator species on prey. In low plant quality situations, larvae feeding on the plants may be forced to move and locate better sources of food, otherwise risk death either by enemies due to underdevelopment and increased exposure, or by a lack of nutrition and starvation. However, moving to find new food sources is a risk in itself due to the increased likelihood of being spotted and consumed by a foraging predator (Anholt and Werner 1998, Bernays 1998, Kaplan and Thaler 2010). In these cases, the prey is forced to make a choice on what is more important.

Impact of habitat management on predator non-consumptive effects

In addition to predators affecting prey through direct consumption, they can alter the behavior and physiological status of their prey through non-consumptive effects (NCE; or trait-mediated effects) (Beckerman et al. 1997, Werner and Peacor 2003, Preisser et al. 2005). NCEs are those resulting in a change in prey behavior, morphology, or predation response (Preisser et al. 2005, Luttbeg and Kerby 2005). NCEs may work directly, through a change in prey density caused by changes in the traits of the prey species, or indirectly, by changes in resource density of the prey resulting from changes in feeding behavior or emigration (Preisser et al. 2005, Luttbeg and Kerby 2005). When NCEs are present, prey species react to the physical or chemical presence of predators, whether it be fleeing a habitat, decreasing movement, or a change in foraging behavior (Laundre et al. 2010, Bucher et al. 2014). However, non-consumptive effects may vary depending on predator and prey characteristics (Buchanan et al. 2017, Hermann and

Landis 2017). For example, prey species may only be vulnerable to predation during specific life stages for some predators, but vulnerable during all life stages to others. If prey species are vulnerable during all life stages, these prey may decrease movement or activity in order to become more inconspicuous or reduce the release of plant volatiles (Lima and Dill 1990), but if prey are only vulnerable during specific life stages (e.g. early life stages), prey may consume more in order to grow larger and become less vulnerable to predation (Xiong et al. 2015).

While NCEs do not result in an immediate direct decrease in pests, behavioral and physiological changes comprise a large amount of the effects on pests from predators (Lee et al. 2005, Luttbeg and Kerby 2005, Priesser et al. 2005). Direct consumptive effects were found to make up 40 percent of the total effect of predators on the survival and density of prey, while indirect NCEs made up 57 percent of the total effect of predators on resource density (Preisner et al. 2005, Luttbeg and Kerby 2005). In addition, prey species may have stronger behavioral responses to predators when the prey have an unlimited food source compared to being starved (Kaplan and Thaler 2010, Anholt and Werner 1998). When food sources are plentiful and of good quality, the prey species may be more capable of postponing eating in order to avoid predation whereas prey that are on low quality food sources must make the choice of whether it is more important to eat or respond to predator presence. For example, *Manduca sexta* caterpillars spend 34% more time feeding on low quality than high quality plants due to the need to intake nutrients (Kaplan and Thaler 2010). There was also a larger difference in leaf consumption of the caterpillars in the presence of a non-lethal predator on high quality plants than on low quality plants (Kaplan and Thaler 2010).

When taking other habitat management factors into account, such as cover crops, more inferences can be made. Assuming that cover crops draw in beneficial enemies, this could lead to

greater NCEs on the prey species. If the cover crops increase the amount of time the predators are spending in or around the crop, then this increases the exposure of the prey species to the predator and may increase the behavioral changes that take place. Conversely, cover crops and complex habitats may lead to higher protection of the prey from the predators, reducing behavioral changes (Warfe and Barmuta 2004). However, cover crops may also work indirectly by adding nitrogen and other nutrients to the soil, thus altering the physiological status of the main crop, producing a stronger, higher quality plant.

In the presence of fertilized crops, herbivores may have an increased growth rate (Hsu et al. 2009). Larvae are subjected to a less significant threat of starvation when on high quality crops compared to lower quality crops. If larvae are feeding on a low quality crop and in the presence of a predator, they have to take into account both the threat of consumption and the threat of starvation if they choose to change feeding behavior to avoid predation. This is explained by the *slow growth/high mortality hypothesis*, which suggests slow growth and prolonged duration on a crop increases the likelihood of mortality, either through direct consumption or through other manners such as starvation (Clancy and Price 1987). With a higher threat of starvation, it is likely that larvae will continue feeding in the presence of predators, increasing their chances of predation. In this case, the indirect effects of cover crops may help protect the prey by providing better habitat. With increased plant quality, the larvae have a lower chance of starvation and may be more willing to change their behavior. In this case, the indirect effects of the cover crop may increase behavioral changes and NCEs of predators on prey species.

Habitat management and multiple predator effects

In field settings it is uncommon to find prey with only one predator. A general prediction is that there is a median of 2-3 predator species per prey species (Schoener 1989, Sih et al. 1998).

Therefore, it is not only important to understand single predator-prey interactions, but to understand interactions between prey and multiple predators. Reactions of prey to multiple predators can be summarized by three multiple predator effects (MPEs): substitutable, risk-reducing, and risk-enhancing (Sih et al. 1998, Schmitz 2007, Grabowski et al. 2008, McCoy et al. 2012). Substitutive effects are observed when the risk of mortality to prey in the presence of multiple predators is equal to that of the average risk imposed by each predator alone (Sih et al. 1998, Schmitz 2007). Risk-reducing MPEs, or antagonistic effects, result in lower prey mortality than seen in the presence of a single predator, while risk-enhancing MPEs, or synergistic effects, result in higher prey mortality than occurs with a single predator (Sih et al. 1998, Schmitz 2007, Grabowski et al. 2008).

The effects of MPEs depend upon predator hunting strategies, and predator and prey habitat domain (Schmitz 2007). Habitat domains include both the microhabitat choice of the animal and their extent of spatial movement within that microhabitat (Schmitz 2007, Miller et al. 2014). The habitat domain of prey may shift in response to a predator, and a predator's habitat domain is dependent upon its hunting strategy. For example, sit-and-wait predators remain in one location and wait for prey to approach them, sit-and-pursue predators remain in one location until prey are in the vicinity at which time they will pounce, and active hunters continuously move throughout their environment (Schmitz 2007, Miller et al. 2014). Active hunters will hold a larger habitat domain than sit-and-wait or sit-and-pursue predators, as they are continuously moving throughout the environment. When multiple predators are present, these hunting

strategies can either work with or against each other in decreasing prey populations, and along with prey habitat domain, determine whether multiple predators will result in higher or lower numbers of prey compared to the presence of a single predator.

Substitutable MPEs are common when multiple predators inhabit different spatial locations (Schmitz 2007). When the habitat domains of multiple predators don't overlap, it is difficult for prey to avoid predators, and predators do not have to compete directly for food. Thus, predation pressure in any one location would be equal to the average risk of each predator individually (Schmitz 2007). Risk-reducing MPEs occur most often when predator habitat domains are completely overlapping, resulting in intraguild predation and interference competition among the predators, making it easier for prey to avoid predation (Schmitz 2007; Grabowski et al. 2008). Predators that occupy the same habitat have to choose between hunting or protecting their hunting grounds from other predators. This takes away from time spent actively hunting, and may result in reduced prey mortality compared to when only a single predator is present. Risk-enhancing MPEs again occur with overlapping predator domains, but with prey also exhibiting a narrow domain, and generally in high numbers (Schmitz 2007). This restricts escape of prey from predators. Often in risk-enhancing MPEs, behavioral changes of prey due to NCEs of one predator can lead to a greater risk of predation by a second predator because of their low mobility (Sih et al. 1998). However, research suggests that high levels of pest suppression are not as reliant on the species richness of predators, and instead more focus should be put on the identity of the enemies present (Straub and Snyder 2006). High species richness may lead to increased competition among predators, but with low species diversity and the presence of a few key predators, interference may be low leading to increased pest suppression (Straub and Snyder 2006). However, risk-enhancing MPEs are less common than

risk-reducing MPEs due to interference among predators (Sih et al. 1998). Under risk-reducing MPEs, prey often benefit from behavioral changes in the presence of predators. They have a greater ability to compensate and adjust their habitat to avoid predation (Sih et al. 1998).

Habitat management adds a level of complexity to the interactions of multiple predators and prey. Multiple predators often result in decreased mortality of prey due to interference among predators. However, encounter rates with predators are decreased when in more complex habitats (Finke and Denno 2002, Langellotto and Denno 2004, Grabowski et al. 2008). This suggests that with increased habitat complexity predator effects will be stronger and risk-reducing MPEs are less likely (Grabowski et al. 2008). However, whether or not habitat complexity will strengthen or weaken trophic cascades is dependent on predator identity within the ecosystem (Finke and Denno 2002). In some cases, increased complexity may lead to risk-reduced MPEs due to difficulty in locating prey or maneuvering the ecosystem (Warfe and Barmuta 2004). In invertebrate systems, intraguild predation among predators is common, resulting in decreased suppression of the herbivore (Schmitz 2007, Grabowski et al. 2008). However, research suggests that in more complex systems, intraguild predation may decrease, while predator interactions with the herbivores increases (Finke and Denno 2002, Langellotto and Denno 2004). This suggests that habitat complexity within an agroecosystem may not only be beneficial for drawing in natural enemies, but that it may also enhance biological control within the ecosystem (Langellotto and Denno 2004).

Model system studied

This research tests the effects of increased habitat structure on predator – prey interactions on *P. rapae* in Brassicaceae. In 2015, cabbage production in Michigan was valued at

over \$16 million (USDA NASS 2015). Pests of cabbage and other brassicas, such as *P. rapae*, have the potential to be highly detrimental to the crop. Many predators feed upon *P. rapae*, but this research primarily focuses on *Hippodamia convergens* Guérin-Ménéville (Coleoptera: Coccinellidae), *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), and Lycosidae (Araneae: Lycosidae), as each has been proven to feed upon *P. rapae* under natural settings in Michigan (Szendrei et al. 2014).

Research Objectives

This research investigated biological control strategies on *P. rapae* in Brassicaceae, determine the role of plant quality and habitat management on herbivore pest behavior, as well its role on consumptive and non-consumptive effects of predators. It also aimed to determine the role of habitat management techniques on multiple predator effects on herbivore pest behavior. This was accomplished through the following objectives:

Objective 1: Determine the effects of predators, plant quality, and cover crop mulch on *P. rapae* oviposition.

- A. Measure effects of plant size, habitat complexity, and plant nitrogen on oviposition in two – choice tests in the greenhouse.
- B. Measure effects of plant size, habitat complexity, and predator presence on oviposition in four – choice tests in the greenhouse.
- C. Survey the presence and preference of *P. rapae* in different cover crop treatments in the field under natural settings through visual observation of field plot visits by adults, as well as egg and larval counts.

Objective 2: Determine the effects of predator identity and plant nitrogen on consumptive and non-consumptive predator effects on *P. rapae*.

- A. Measure the impacts of plant nitrogen and predator species on adult *P. rapae* oviposition in greenhouse choice tests.
- B. Measure the impacts of plant nitrogen and predator chemical threats from two predator species on *P. rapae* larvae in environmental chamber choice tests.
- C. Measure the impact of predator species on *P. rapae* larval survival, leaf consumption, and weight in no-choice environmental chamber bioassays.
- D. Measure the impact of predator species and plant nitrogen on *P. rapae* larval survival and weight in no-choice field experiments.

Objective 3: Determine the impacts of multiple predator communities and habitat management strategies on consumptive and non-consumptive predator effects on *P. rapae*.

- A. Measure the impact of multiple predator communities on *P. rapae* survival and weight in environmental chamber bioassays.
- B. Measure the impact of multiple predator communities, cover crop mulch, and plant nitrogen on *P. rapae* survival and weight in field experiments.
- C. Measure the habitat domains of different predators of *P. rapae*.
- D. Survey wild natural enemy communities to determine impact of cover crop mulch and plant nitrogen on natural enemy abundance.

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CHAPTER 2: Cue hierarchy for host plant selection in *Pieris rapae*

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Introduction

Female insects utilize a variety of cues in locating host plants during oviposition, seeking high-quality habitat with low predation pressure (Rausher 1979, Feeny et al. 1989, Janz and Nylin 1997). Cues may derive from habitat structure (Meiners and Obermaier 2004), plant size (Myers 1985), plant texture (Rojas et al. 2003), plant volatiles (Tahvanainen and Root 1972), plant nutrient levels (Minkenberg and Ottenheim 1990), or natural enemies (Silberbush and Blaustein 2011). Females may experience multiple cues simultaneously or sequentially, and rely on hierarchical categorization to rank different cues for oviposition choice. The majority of the current literature on insect oviposition focuses on a single cue or on host species identity (Courtney et al. 1989), instead of reflecting natural circumstances where insects encounter and evaluate multiple cues presented by a host species and the surrounding environment before selecting a host.

Cue hierarchies in insect oviposition are often driven by the physical distance between the insect and the plant (Thompson and Pellmyr 1991, Battaglia et al. 2000). During host finding, insects first select a habitat or host patch based on cues perceived at long distances. Therefore, cues such as habitat structure, plant density, and plant size may have a large initial impact on

female choice. However, these cues may have varying effects depending on herbivore biology; with generalist herbivores often preferring complex habitats due to the potential for refuge from predation (Tschanz et al. 2005), and specialists frequently deterred by the presence of non-host plants (Root, 1973, Björkman et al. 2010). Volatile odor cues may also be perceived at longer distances if emitted at high concentrations, or if the insect's sensory modalities are sensitive to particular molecules; however, volatile cues released at low quantities may only be perceived as the insect moves closer to the host plant (Feeny et al. 1989). When long-distance cues elicit a positive response, females alight on the plant and further explore short-distance cues, which require close proximity or contact (Rausher 1979, Singer 1986).

In some insect species contact cues are important for oviposition decisions. For example, when buckeye butterflies, *Juonia coenia* Hübner, were simultaneously presented high and low nitrogen plants, they preferred high nitrogen plants (Prudic et al. 2005). *Pieris* spp. butterflies (Lepidoptera: Pieridae) also utilize contact cues by using their tarsi to sense levels of plant compounds which help stimulate oviposition (Du et al. 1995, Städler et al. 1995). In other species, long-distance cues drive oviposition decisions; in the case of Glanville fritillary butterflies, *Melitaea cinxia* (L.), plant size was a more important stimulus than chemical qualities, therefore larger plants received more eggs than small plants regardless of plant secondary metabolites (Reudler Talsma et al. 2008).

The plant vigor hypothesis suggests that plants with vigorous growth resulting in a larger size compared to the average growth rate should be favorable to herbivores (Price 1991). This proposes that plant cues which indicate more vigorous growth should elicit a higher herbivore preference than cues that indicate a lower or average growth rate. For example, according to the plant vigor hypothesis, we would expect long-distance cues such as large plant size (Reuler

Talsma et al. 2008) and short-distance contact cues associated with high nitrogen content (Prudic et al. 2005) to be preferred over smaller plant size or low nitrogen plants, as large size and high nitrogen content suggest higher plant vigor.

In addition to plant cues, risk posed by natural enemies may also play an important role in female oviposition decisions. The presence of predators may cause a female to avoid a host plant or fail to oviposit, as prey species should prefer enemy-free space with reduced vulnerability to predation (Jeffries and Lawton 1984, Nomikou et al. 2003). For example, *Eunica bechina* (Hewitson) butterflies, after inspecting a host, laid fewer eggs on plants with predatory ants compared to those with no predators or with non-predatory ants (Sendoya et al. 2009). Although the range of predator cues is less understood, their importance on herbivore oviposition is gaining recognition in the literature.

Here, we examined the effect of habitat structure (presence or absence of plastic leaves or cover crop mulch), plant size, plant nitrogen level, and predators, alone and in combination, on *Pieris rapae* L. (Lepidoptera: Pieridae) oviposition in greenhouse and field experiments, to gain a better understanding of the relative importance of these cues for host choice, and how multiple cues may change *P. rapae* behavior compared to a single cue. We used *P. rapae* due to its importance as an agricultural pest as well as its use as a model organism (Renwick and Radke 1988, Städler et al. 1995; Layman and Lundgren, 2015). We hypothesized that long-distance visual cues, such as habitat structure and plant size, may be assessed first and therefore have a stronger influence on *P. rapae* oviposition than short-distance cues, such as plant nitrogen levels and predator presence, and that a combination of multiple cues would have a different impact on oviposition than single cues in isolation.

Material and methods

Herbivore colony

Pieris rapae were obtained from a greenhouse colony kept at Michigan State University, East Lansing, MI, USA. Insects originated from Michigan State University's Farms and were in continuous culture since 2015. Field-caught *P. rapae* adults were added to the colony in 2016 to maintain genetic diversity. Larvae were reared on collard greens (*Brassica oleracea* L. cv. Georgia, Brassicaceae; W. Atlee Burpee & Co., Warminster, PA, USA) ad libitum, and adults were fed a honey or sugar water solution. Greenhouse temperatures were kept between 22 and 30°C.

Oviposition choice tests in greenhouse

A series of choice tests was conducted between December 2015 and January 2017, in mesh cages (122 × 70 × 70 cm; Nasco, Fort Atkinson, WI, USA) in a greenhouse (25-30 °C, L16:D8 photoperiod). Georgia collards were grown individually from seed in perlite soil mixture (Suremix Perlite; Michigan Grower Products, Galesburg, MI, USA) in 7-l plastic pots (Elite Nursery Containers; International Greenhouse Company, Danville, IL, USA). Four- to 6-week-old collards were used for all choice tests.

To determine effects of plant size on oviposition, choice tests included 'large' collards (5- to 6-week-old plants with 8-10 fully extended true leaves) and 'small' collards (4-week-old plants with 4-6 fully extended true leaves). We used only small plants for tests that did not concern effects of plant size.

To evaluate the role of plant nitrogen status on oviposition choice, plants received either no nitrogen ('low nitrogen') or organic blood meal ('high nitrogen'; 15 g 12N:0P:0K per pot;

The Espoma Company, Millville, NJ, USA), added at 2-2.5 and 3-3.5 weeks after planting. Blood meal was applied to the surface of the soil around the plants and worked into the top 2-3 cm of the soil with a fork. Soil around low nitrogen plants was also disturbed with the fork to ensure equal aeration between treatments. Low-nitrogen plants were used in choice tests not evaluating nitrogen effects. Unless plant size was tested in combination with nitrogen effects, choice tests were conducted when plants were 4-weeks-old. When testing the effects of nitrogen without the effects of plant size, plants were selected to be similar in size. Prior to experiments, low- and high-nitrogen collards were tested for overall plant nitrogen content (A&L Great Lakes Laboratories, Fort Wayne, IN, USA) to establish differences in nitrogen content.

To assess how oviposition is affected by habitat structure, female butterflies were offered collards with or without plastic leaves surrounding them to simulate the presence of mulch (20 green plastic leaves, 1.5 × 8.5 cm; article number 708925, Hobby Lobby, Oklahoma City, OK, USA). Plastic leaves were pushed ca. 4 cm deep into the soil to stand vertically, and placed randomly in the pot around the collard. Before each use, plastic leaves were washed in 95% hexane to remove chemical cues from the surface.

In choice tests evaluating predator effects, each collard had a white mesh bag (3.79 l; Master Craft Manufacturing, South El Monte, CA, USA) covering one fully expanded leaf at the top of the plant, and plants with predators had five convergent lady beetles, *Hippodamia convergens* Guérin-Ménéville (Coleoptera: Coccinellidae) (Rincon-Vitova Insectaries, Ventura, CA, USA) added to the bag. Due to the white color of the mesh bags, visibility of lady beetles by herbivores was likely low, but the open mesh allowed herbivores to detect chemical cues of the predator. Lady beetles were maintained according to the protocol outlined by Bryant et al. (2014). In all choice tests, one 3- to 6-day-old mated female *P. rapae* was released in the center

of each cage and left to oviposit for 24 h. Afterwards, the eggs on each plant were counted.

Two-choice greenhouse tests

Two-choice tests were run with two collards of different treatments placed 60 cm apart in mesh cages as described above. Fourteen types of plant treatment were offered (Table 1), consisting of combinations of cue type and cue number. Cue types were plant size, plant nitrogen, and habitat structure, and either one cue, or a combination of two, or all three cue types were offered to butterflies simultaneously.

Four-choice greenhouse tests

The first set of four-choice tests had in each cage a collard alone, a collard with predators, a collard with 20 plastic leaves, and a collard with predators and 20 plastic leaves. All treatments contained collards of the same size grown without added nitrogen. The four collard treatments were placed randomly in the same cage in a staggered, zig-zag formation, so that each collard was 30cm away from the next. Cages used in four-choice tests were the same as those used in two-choice tests.

A second set of four-choice tests was run with differently sized plants and predators. Butterflies were given the choice between a large collard, a large collard with predators, a small collard, and a small collard with predators. No plastic leaves were present in this test.

Field experiment

In order to evaluate the effects of plant nitrogen and habitat structure on *P. rapae*, an experimental cabbage (*B. oleracea* var. Farao; Bejo Seeds, Oceana, CA, USA) field was

established at Michigan State University's Horticulture Teaching and Research Center (Holt, MI, USA). Seeds were grown in the greenhouse and 4-week-old transplants were planted in the field on 6 July 2016, in a randomized complete block design with four blocks and five treatments (Table 2). Treatment plots in each block measured 3×6 m, and blocks were spaced 4.5 m apart. Treatments consisted of a factorial combination of two levels of cover crop [cereal rye, *Secale cereale* L. (Poaceae), or none] and two levels of nitrogen (no nitrogen or pelleted chicken manure). A treatment with a mixture of rye and hairy vetch (*Vicia villosa* Roth, Fabaceae) without any additional nitrogen fertilization was also included. Cover crops were seeded in fall 2015, with rye sown only between – and vetch only within – future cabbage rows. In spring 2016, all cover crops were flail mowed 2 weeks prior to cabbage planting. Cover crop treatments were tilled in-rows with a 25-cm-wide strip-tiller (Table S2.1), resulting in rye residue remaining on the soil surface as a mulch between rows, and vetch incorporated into the soil as a nitrogen source. Treatments without cover crops were rototilled. Additional details of field and crop management activities are provided in Table S2.1.

Observations of feral adult *P. rapae* landing behavior were conducted for eight consecutive weeks in July-September, 2016. All observations were done under sunny or partly cloudy weather conditions between 12:00 and 14:00 h. The plants in each plot visited by butterflies were counted by recording each time an individual butterfly landed on a plant within each plot. Each block was observed for 30 min. Multiple landings on an individual cabbage were recorded as only one landing per butterfly, as females often fly around and land on cabbages multiple times prior to acceptance and oviposition in order to evaluate plant and environmental cues (Renwick & Chew 1994). It is possible for butterflies to choose to oviposit multiple times in a row on the same plant, but this is difficult to determine without checking for eggs after each

landing; we only counted an individual plant once per butterfly. However, multiple landings were recorded for each butterfly if they visited multiple cabbages in each treatment. We counted the *P. rapae* eggs and larvae on five random plants in each plot weekly for seven consecutive weeks in July-August, 2016. Four cabbages from each treatment were collected and tested for overall plant nitrogen content (A&L Great Lakes Laboratories) to establish differences in nitrogen content among treatments.

Statistical analysis

Two-choice ovipositional preferences were analyzed with a χ^2 test to determine differences in number of eggs laid between choices due to high variation in eggs laid across replications (Renwick and Radke, 1988; Sadek et al., 2010). Observed values were the sum of all eggs across all replications of a given choice, and the expected values were the sum of all eggs of both choices divided by two. Additionally, effect size of plant size (large vs. small), nitrogen (high vs. low), and plastic leaves (present vs. absent) on oviposition was determined with an independent t-test. Effects of adding cues to a focal cue (for example, plastic leaves and size together compared to size alone) were also tested for each cue type (size, nitrogen, and plastic leaves) with a linear mixed effects model, where the focal and additional cues were fixed factors, and date was a random factor. Differences among means of tested factors were determined with post-hoc pairwise comparisons (Tukey's HSD; $\alpha = 0.05$; package 'multcomp', Hothorn et al. 2008; 'lsmeans', Lenth 2016).

Four-choice ovipositional preferences were analyzed with a Kruskal-Wallis test to observe overall differences among the four choices. A Dunn's test (package 'dunn.test', Dinno 2016) was run to determine differences among the four choices. Additionally, overall effect size

of plant size, predator presence, and plastic leaves on oviposition was determined with an independent t-test.

Pieris rapae landings in the field were analyzed with a generalized linear mixed effects model (package ‘lme4’, Bates et al. 2013) with a Poisson distribution, with treatment (cover crop mulch and nitrogen) as a fixed factor, and week, block, and plot as random factors. *Pieris rapae* egg and larval numbers in the field were analyzed with a generalized linear mixed effects model with a Poisson distribution, with mulch and nitrogen as fixed factors, and week, block, and plot number as random factors. A post-hoc means comparison was used to determine significant differences among treatment means ($\alpha = 0.05$). The overall difference in plant nitrogen content of cabbages was determined with a linear mixed effects model with treatment as a fixed factor, and block as a random factor. A post-hoc pairwise comparison was used to determine differences in plant nitrogen among treatments ($\alpha = 0.05$). All statistical analyses were conducted in R v.3.3.2 (R Core Team, 2017).

Results

Two-choice tests

When *P. rapae* were presented with a single cue in two-choice tests, they preferred to oviposit on large compared to small plants (Table 2.1), on plants without compared to with plastic leaves (Table 2.1), and low- compared to high-nitrogen plants (Table 2.1). However, having multiple cues presented together influenced preference; for example, when nitrogen and size cues were presented together, *P. rapae* laid twice as many eggs on large high-nitrogen plants compared to small low-nitrogen plants, even though they preferred low-nitrogen plants when nitrogen was presented alone (Table 2.1). Also, females laid 36% more eggs on large high-

nitrogen plants with plastic leaves compared to small low-nitrogen plants with no plastic leaves (Table 2.1), again negating their preference for low-nitrogen plants and no plastic leaves when only one cue was present.

Table 2.1. *Pieris rapae* two-choice oviposition tests observing differences in collard plant size (large vs. small), plant nitrogen (high vs. low), and plastic leaves (presence vs. absence). Choices with no plant size listed were both small plants, choices with no nitrogen level listed were both low nitrogen. The effect of plastic leaves was tested with both large and small plants. The difference in eggs laid indicates the difference in the total number of eggs per choice (in parentheses the proportion of eggs per choice) on the preferred vs. the non-preferred plants. Differences in total number of eggs laid were analyzed using χ^2 tests ($\alpha = 0.05$).

	Preferred			Not preferred			Difference in eggs laid	n	χ^2	d.f.	P
	Plant size	Plastic leaves	Nitrogen	Plant size	Plastic leaves	Nitrogen					
One cue	Large	-	-	Small	-	-	101 (0.09)	30	9.13	1	<0.01
	Large	No	-	Large	Yes	-	192 (0.13)	30	25.82	1	<0.01
	Small	No	-	Small	Yes	-	203 (0.18)	28	35.80	1	<0.01
	-	-	Low	-	-	High	162 (0.15)	33	23.90	1	<0.01
Two cues	Large	No	-	Small	Yes	-	924 (0.72)	24	669.10	1	<0.01
	Large	Yes	-	Small	No	-	120 (0.06)	36	7.35	1	0.01
	Large	-	Low	Small	-	High	157 (0.09)	30	14.37	1	<0.01
	Large	-	High	Small	-	Low	449 (0.34)	27	153.08	1	<0.01
	-	No	Low	-	Yes	High	258 (0.28)	24	71.73	1	<0.01
	-	No	High	-	Yes	Low	33 (0.05)	32	1.49	1	0.22
Three cues	Large	No	Low	Small	Yes	High	403 (0.44)	28	175.58	1	<0.01
	Large	No	High	Small	Yes	Low	1216 (0.70)	23	852.74	1	<0.01
	Large	Yes	Low	Small	No	High	158 (0.19)	32	29.93	1	<0.01
	Large	Yes	High	Small	No	Low	283 (0.15)	29	42.76	1	<0.01

When analyzing the effect of plant size across all cue combinations from all two-choice tests, we found that females laid on average about 15 more eggs on large plants than on small plants ($t = 6.91$, d.f. = 442, $P < 0.01$; Figure 2.1A), resulting in about double the number of eggs on large plants. When parsing out the effect of the additional cues' (plastic leaves and nitrogen) interactions with size on oviposition, we found that when plants were small, females laid about half as many eggs on plants with plastic leaves compared to plants without them ($t = 4.82$, d.f. = 476, $P < 0.01$; Figure 2.2A). However, when both plastic leaf and nitrogen cues were present with small plants, there was no impact on the number of eggs laid compared to when small plants were present with no other factors ($t < 1.67$, d.f. = 476, $P > 0.69$; Figure 2.2A). When large plants were offered, the presence of plastic leaves did not change the number of eggs laid ($t = 1.53$, d.f. = 476, $P = 0.77$; Figure 2.2A), but females laid about 40% more eggs on high- compared to low-nitrogen plants ($t = 3.83$, d.f. = 476, $P < 0.01$; Figure 2.2A).

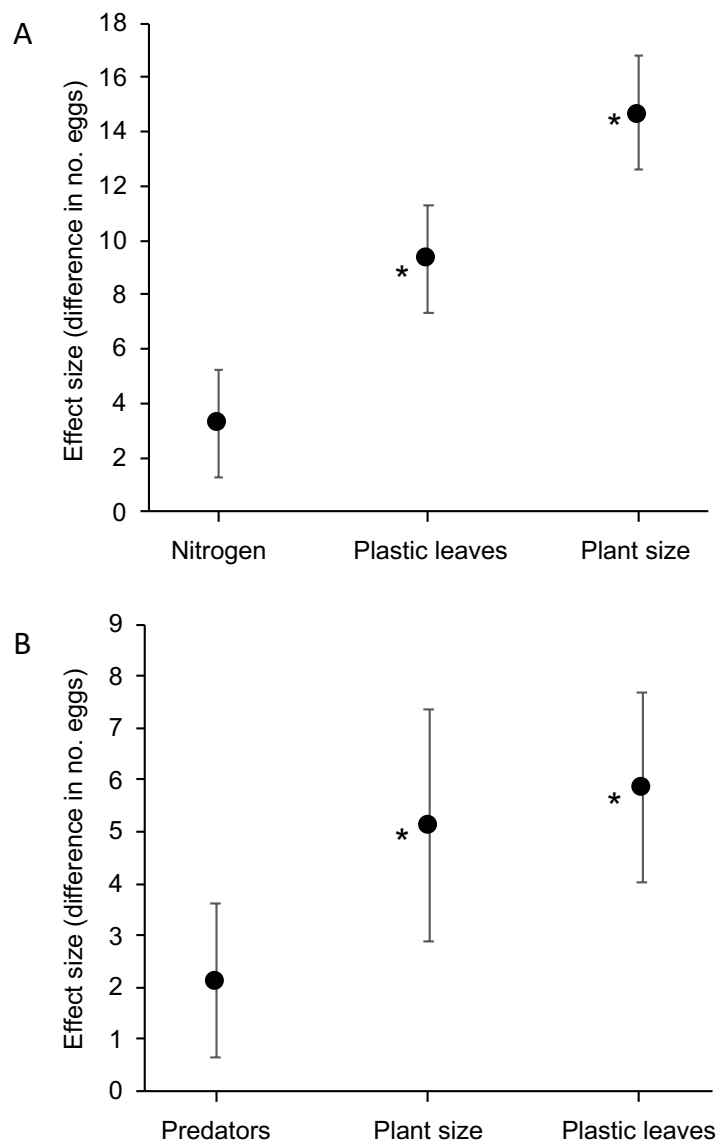


Figure 2.1. The effect sizes of various cues on *Pieris rapae* oviposition in (A) dual choice and (B) four-choice tests, indicated by the mean ($\pm$ SEM) difference in the numbers of eggs laid when a cue was present compared to when it was absent (plant nitrogen: high vs. low; plant size: large vs. small), or when a cue was absent compared to when it was present (plastic leaves: absent compared to present; predators: lady beetles absent compared to present). Asterisks represent differences that are significantly different from zero (t-test: $P < 0.05$).

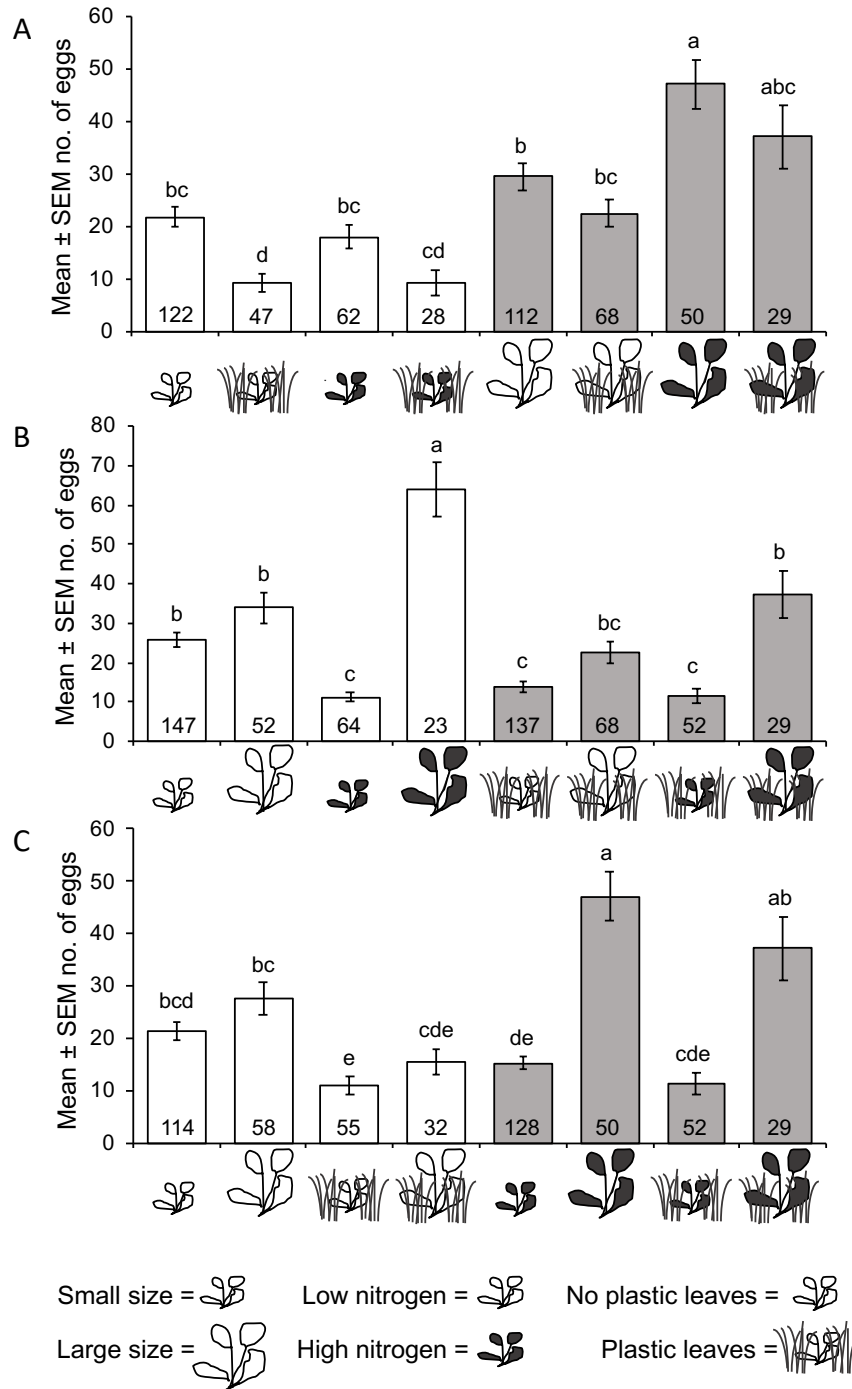


Figure 2.2. Mean ($\pm$ SEM) number of eggs laid by *Pieris rapae* on collards across all greenhouse two-choice tests measuring the effects of plant size, plastic leaves, and plant nitrogen, and their interactions. The panels are based on all dual choice tests where (A) plant size, (B) plastic leaves, and (C) nitrogen was the focal factor, and observed effects of the two other cues on this focal factor. Grey bars indicate the presence of a cue (i.e., large size in panel A, plastic leaves present in panel B, high nitrogen in panel C). The numbers inside each bar indicate the numbers of test replications. Different letters above bars within a panel indicate significant differences among treatments (Tukey's HSD: $P < 0.05$).

Across all cue combinations in all two-choice tests, females on average laid about nine more eggs on plants where plastic leaves were absent instead of present ($t = 4.71$, d.f. = 545, $P < 0.01$; Figure 2.1A), close to doubling the number of eggs present. When parsing out effects of additional factors (plant size and nitrogen), in the absence of plastic leaves we found that 40% fewer eggs were laid on small high-nitrogen plants ($t > 3.66$, d.f. = 527, $P < 0.01$; Figure 2.2B), and almost twice as many eggs were laid on large high-nitrogen plants compared to low-nitrogen plants ($t > 5.22$, d.f. = 527, $P < 0.01$; Figure 2.2B). However, when plastic leaves were present, females preferred large high-nitrogen plants over small plants ($t > 4.05$, d.f. = 527, $P < 0.01$; Figure 2.2B), although large low-nitrogen collards did not differ from any other treatment with plastic leaves ($t < 2.87$, d.f. = 527, $P > 0.07$; Figure 2.2B).

In choice tests examining nitrogen effect alone (single cue), females preferred low- over high-nitrogen plants (Table 1), but with data combined from all two-choice tests that included nitrogen differences as a factor in addition to other cues, there was a slight preference, though not significant, for high- over low-nitrogen plants ($t = 1.62$, d.f. = 476, $P = 0.11$; Figure 2.1A), with about three more eggs (16% increase) laid on nitrogen treated plants. When analyzing how the presence of other factors (plant size and plastic leaves) influenced nitrogen effects on oviposition, we found that small low-nitrogen plants with plastic leaves received about half as many eggs on average compared to treatments without plastic leaves ($t > 3.72$, d.f. = 474, $P < 0.01$; Figure 2.2C). However, when low-nitrogen plants were large and had plastic leaves, the number of eggs laid was not different compared to other low-nitrogen plants regardless of other factors ($t < 1.55$, d.f. = 474, $P > 0.77$; Figure 2.2C). In the case of high-nitrogen plants, females laid about 3× as many eggs on average on large plants compared to small ones, regardless of the presence of plastic leaves ($t > 3.90$, d.f. = 474, $P < 0.01$; Figure 2.2C). The addition of blood meal to collards

resulted in the percent nitrogen in the high-nitrogen plants (8.42%), almost twice as high as the low-nitrogen treatment (4.51%).

Four-choice tests

In the first oviposition four-choice test, *P. rapae* laid over twice as many eggs on plants without than on plants with plastic leaves ($\chi^2 > 1.84$, d.f. = 1, $P < 0.03$; Table 2.2). Regardless of whether plastic leaves were present or absent, predators had no effect on the number of eggs laid ($\chi^2 < 0.73$, d.f. = 1, $P > 0.23$; Table 2.2). In the second four-choice test, females laid about twice as many eggs on large plants without predators than on small plants with or without predators ($\chi^2 > 2.35$, d.f. = 1, $P < 0.01$; Table 2.2). However, the number of eggs was not different between large plants with predators vs. small plants with or without predators ($\chi^2 < 1.14$, d.f. = 1, $P > 0.12$; Table 2.2). Regardless of plant size, there was no effect of predators on the number of eggs ($\chi^2 < 1.30$, d.f. = 1, $P > 0.10$; Table 2.2). Across all cue combinations in both four-choice tests combined, females preferred plants without over plants with plastic leaves ($t = 3.17$, d.f. = 75, $P < 0.01$), and large over small plants ($t = 2.29$, d.f. = 69, $P = 0.03$), though only slightly preferred plants without over plants with predators present ($t = 1.42$, d.f. = 155, $P = 0.16$; Figure 2.1B).

Table 2.2. *Pieris rapae* ovipositional four-choice tests observing the effects of collard plant size (large vs. small), plastic leaves (presence vs. absence), and predators (lady beetles present vs. absent) on the no. eggs laid. The first test had all large plants, the second test had no plastic leaves. The total number of eggs laid is indicated for all replications for each test. A Kruskal-Wallis test was performed for each four-choice test to determine significant differences among treatments. Subsequently a Dunn's test was used to determine significant differences between individual treatments within a test, indicated by different letters following the number of eggs within a test ($\alpha = 0.05$).

Test	Size	Plastic leaves	Predator	Total no. eggs laid	n	χ^2	P
1	-	No	No	237a	21	12.30	0.01
	-	No	Yes	169a			
	-	Yes	No	105b			
	-	Yes	Yes	55b			
2	Large	-	No	263a	19	7.82	0.05
	Large	-	Yes	194ab			
	Small	-	No	123b			
	Small	-	Yes	139b			

Field experiment

Plant nitrogen testing revealed differences in nitrogen content among cabbages from different field treatments ($F_{4,12} = 20.88$, $P < 0.01$). When cover crop mulch was absent, the addition of nitrogen fertilizer resulted in about 1.5× higher nitrogen content in cabbages compared to when no nitrogen was added ($t = 4.27$, d.f. = 12, $P < 0.01$; Table 2.3), and when cover crop mulch was present, the addition of nitrogen resulted in almost 1.75× higher plant nitrogen content compared to cabbages with mulch but no nitrogen added ($t = 3.65$, d.f. = 12, $P = 0.02$; Table 2.3). However, plant nitrogen content in cabbages where vetch was incorporated did not differ from either plants with or without nitrogen added when mulch was present ($t < 2.45$, d.f. = 12, $P > 0.17$; Table 2.3), and across all field treatments, cabbages contained the highest nitrogen content in plots without mulch and with nitrogen addition ($t > 4.27$, d.f. = 12, $P < 0.01$; Table 2.3).

Pieris rapae preferred to land in plots without mulch ($\chi^2 = 4.89$, d.f. = 1, $P = 0.03$; Table 2.3) and in plots with nitrogen added ($\chi^2 = 5.61$, d.f. = 1, $P = 0.02$; Table 2.3). The interaction between mulch and nitrogen was significant ($\chi^2 = 6.14$, d.f. = 1, $P = 0.01$; Table 2.3), with more landings in plots without mulch and with nitrogen compared to all other plots except plots containing rye mulch with nitrogen ($z > 2.85$, $P < 0.04$). *Pieris rapae* laid about 30% more eggs in plots without mulch compared to plots containing mulch ($\chi^2 = 4.34$, d.f. = 1, $P = 0.04$; Table 2.3), whereas nitrogen level alone had no effect on oviposition ($\chi^2 = 1.56$, d.f. = 1, $P = 0.21$; Table 2.3). Across all treatments, females laid about twice as many eggs on average in plots without mulch and nitrogen added compared to plots containing rye mulch between-rows and vetch in-rows ($\chi^2 = 10.78$, d.f. = 4, $P = 0.03$; Table 2.3). Additionally, 25% more *P. rapae* larvae were observed in treatments without mulch compared to treatments with mulch ($\chi^2 = 6.60$, d.f. = 1, $P = 0.01$; Table 2.3), and about 40% more in treatments with compared to without nitrogen ($\chi^2 = 5.16$, d.f. = 1, $P = 0.02$; Table 2.3). Around 85% more larvae were found in plots without mulch and with nitrogen added compared to all plots except plots without nitrogen, regardless of the presence of mulch ($\chi^2 = 19.11$, d.f. = 4, $P < 0.01$; Table 2.3).

Table 2.3. Field observation of *Pieris rapae* mean ($\pm$ SEM) number of adult landings (per 30 min), eggs laid and larvae assessed (both per five plants) in five treatments comprised of various combinations of nitrogen (added or not; content measured for each treatment), and cover crop mulch factors. Cover crops were planted in or between crop rows. Means within a column followed by different letters are significantly different between treatments (Tukey's HSD: $P < 0.05$). Significant effects of mulch, nitrogen, and mulch*nitrogen interaction on the numbers of landings, eggs, and larvae were determined using a generalized linear mixed effects model (GLME), and are indicated by P-values. ns, $P > 0.05$

Cover crop mulch				Mean $\pm$ SEM		
Between-row	In-row	Nitrogen addition	% nitrogen	Adult landings	Eggs	Larvae
None	None	-	3.53b	2.56 $\pm$ 0.55b	4.43 $\pm$ 1.09ab	1.32 $\pm$ 0.32b
None	None	Yes	5.18a	4.75 $\pm$ 0.77a	6.29 $\pm$ 1.53a	2.46 $\pm$ 0.57a
Rye	None	-	1.93c	2.90 $\pm$ 0.57b	3.39 $\pm$ 0.76ab	1.36 $\pm$ 0.32b
Rye	None	Yes	3.35b	3.03 $\pm$ 0.63ab	4.64 $\pm$ 1.15ab	1.71 $\pm$ 0.29ab
Rye	Vetch	-	2.40bc	2.88 $\pm$ 0.60b	2.93 $\pm$ 0.65b	1.14 $\pm$ 0.30b
GLME						
Cover crop mulch				0.03	0.04	0.01
Nitrogen				0.02	ns	0.02
Mulch*nitrogen				0.01	ns	0.05

Discussion

Our results demonstrated that *P. rapae* oviposition is a complex process that involves decision making and hierarchical categorization of cues to select a host plant (Wicklund 1981, Singer 1986, Hoffmann and Resh 2003, Janz 2003, Thöming et al. 2013). We also determined that their response to a single cue is different than to multiple cues, when making oviposition decisions. Cues perceived at longer distances, such as habitat structure and plant size, were assessed before contact cues, such as nitrogen content. Plant size had the largest effect on the number of eggs laid in two-choice tests, and had a similar effect to plastic leaves in our four-choice tests. Whereas butterflies generally preferred large compared to smaller plants, the presence of plastic leaves deterred them from laying eggs on collards of any size in both two-

and four-choice tests. Long-distance cues play an important role in host selection in other insects as well, such as wood-boring beetles, *Brachys tessellatus* (Fabricius), whose host preference hierarchy changes when presented with plants at longer distances (Waddell and Mousseau 1996). The results of two- and four-choice tests were consistent with our field results, where, overall, *P. rapae* landed more often and laid more eggs in plots without cover crop mulches than in plots where mulches were present. These results support our hypothesis that cues that are assessed first at longer distances have a strong impact on oviposition, and the presence of non-hosts disrupts the host selection process even before landing and contacting surfaces (Finch and Collier 2000).

Long-distance visual cues may initially attract insects to plants, but contact chemical cues might modify their host choice (Janz 2003). In our studies, cues secondarily assessed at shorter distances and on contact had inconsistent effects on *P. rapae* oviposition. In two-choice tests, when only presented with differing nitrogen cues, females laid more eggs on low- compared to high-nitrogen plants. However, when observing the effects of nitrogen across all two-choice tests where additional cues were also present, females laid more eggs on high- compared low-nitrogen plants. This was also observed in the field, with more *P. rapae* adults landing in plots with added nitrogen. This contradiction suggests that in some cases long-distance cues (e.g., plant size and plastic leaves) may be altering the effect of nitrogen. Research shows that increased nitrogen content may lead to lower levels of glucosinolates, a secondary plant compound assessed by *P. rapae* during oviposition (Du et al. 1995, Rosen et al. 2005). This could explain why in the presence of only nitrogen cues lower nitrogen plants were preferred, but when other preferable long-distance cues were also present, their preference changed.

In our studies, when long-distance cues were not preferred (e.g., small plants), the addition of nitrogen did not increase plant attractiveness, but when plants were large, the addition

of nitrogen increased the average number of eggs laid by about two-fold compared to both small and large plants without nitrogen. This suggests a context-dependent synergy between nitrogen and plant size as oviposition cues and that plant size may be indicative of the nitrogen content only when certain cues are present. Visual and chemical cue synergy has a behavior-modifying effect in many insects (Blackmer and Cañas 2005, Raguso and Willis 2002, Fukaya et al. 2004). For example, oviposition by the tomato fruit borer, *Neoleucinodes elegantalis* (Guenée), varies with the availability of visual (visibility of host) and chemical cues (hexane extract of tomato fruit) and the two cues have a synergistic effect on oviposition rates: tomato fruit borer laid more eggs when both visual and chemical cues were present together, compared to when either was presented separately or both were absent (Teles Pontes et al. 2010).

It is possible that some of our results could be a consequence of random insect movements leading to more frequent encounters with larger than with smaller plants (Hern et al. 1996). Additionally, we recognize that size and nitrogen content are often positively related in plants, as seen in our field study, where plants treated with nitrogen were larger than those that were left untreated (Figure S2.1). As nitrogen and size were controlled independently in our greenhouse two-choice tests, and females preferred low nitrogen when plants were similar in size, but high nitrogen when plants were large, we conclude that both cues can independently influence oviposition. Furthermore, it is possible that females with large egg loads in their abdomens at the time of release in caged experiments may have a higher acceptance for lower-quality hosts, and dump eggs on plants they otherwise would not prefer (Courtney et al. 1989). In order to minimize this effect, we standardized the age of the butterflies used in experiments. Data collected from our choice tests suggests that this standardization did reduce potential egg load effects (data not shown).

Plant cues appeared to have a larger impact on *P. rapae* oviposition than predator cues. Although there was a slight numerical reduction in average eggs laid by *P. rapae* when predators were present compared to when they were absent, this effect was not significant. This may be because predator cues presented were not strong enough to be recognized as a threat, predators were not visible enough for a visual predator cue to register, or that *P. rapae* may need both visual and chemical predator cues to recognize a threat. A study observing Colorado potato beetle, *Leptinotarsa decemlineata* (Say), found that these insects consumed significantly less leaf tissue when both visual and chemical predator cues were present compared to a no-predator control, but not in the presence of visual predator cues alone, suggesting that both visual and chemical predator cues may be important in affecting prey behavior (Hermann and Thaler 2014). Conversely, a study investigating oviposition rates of *P. rapae* in the presence of pink spotted lady beetle, *Coleomegilla maculata* De Geer, and green peach aphids, *Myzus persicae* (Sulzer), found that lady beetle presence alone did not reduce egg numbers, but when both lady beetles and aphids were present, *P. rapae* laid fewer eggs than on controls (Layman and Lundgren 2015). This suggests that predator cues alone may not be strong enough to deter butterflies, but other alarm pheromones, emitted by plants or other herbivores, may be important cues. If so, future studies may need to focus on effects of predator-prey interactions and predation, instead of just predator presence, on oviposition choice.

Conclusions

Female arthropods use a wide variety of cues during oviposition ranging from long-distance (e.g., visual) cues (Myers 1985, Meiners and Obermaier 2004) to short-distance (e.g., chemical or tactile) cues (Minkenberg and Ottenheim 1990, Silberbush and Blaustein 2011).

During host finding, females experience cues in a sequence during alighting, which creates a hierarchy for finding an optimal host plant (Singer 1986, Battaglia et al. 2000, Janz 2003). Our results indicated that long-distance cues may have had a larger impact on *P. rapae* oviposition than cues assessed at shorter distances, that cues are context dependent, and synergy between preferred long- and short-distance cues may influence final oviposition rates. As most literature on insect oviposition focuses on a single environmental cue (Minkenberg and Ottenheim 1990, Layman and Lundgren 2015) or on insect species identity (Courtney et al. 1989), these novel results implicate the importance of studying the effects of multiple cues in studies on host preference instead of single isolated ones, as insects encounter multiple cues under natural circumstances, and synergy between different cues may influence preference over single cues. Additionally, these results may have implications for agricultural pest management strategies, for example, utilizing agricultural techniques such as cover crops and mulches to help deter these pests in field settings.

APPENDIX

Supplementary Tables for Chapter 2

Table S2.1. Timeline of operations performed to prepare and manage cabbage field plots used in this study at Michigan State University's Horticulture Teaching and Research Center in Holt, MI, USA during the 2016 growing season.

Field operation	Additional information	Source(s)	Date
Pelleted chicken manure (4 3 2: N P K) applied	Applied by hand at rate of 33.6 kg N ha ⁻¹	Herbruck's Poultry Ranch, Saranac, MI, USA	10 Sept 2015
Rye (variety not stated) cover crop planted	Drilled between rows with a 3 m drill (John Deere, Moline, IL, USA)		10 Sept 2015
Hairy Vetch (VNS) cover crop planted	Sowed with a Jang seeder in the in row zone	Jang Automation Company, Cheongju, Sth Korea	10 Sept 2015
Cabbage ('Farao') transplants started	Sown in 128-cell flats with Morgan's potting mix	Morgan's Composting, Sears, MI, USA	7 June 2016
Cover crops flail mowed			14 June 2016
Fertilized	45 kgs k20/ha as K-Mag (all); 67 kgs of N/ha as 13-0-0 (treatments: rye cover crop + nitrogen, no cover crop + nitrogen)		29 June 2016
Tillage	Strip tillage with Hiniker 6000; conventional tillage with rototiller	Hiniker Agricultural Equipment, Mankato, MN, USA	29 June 2016
Cabbage transplanted in field	Transplanted by hand		6 July 2016
Between-row cultivation	Hillside rolling cultivator	Hillside Cultivator Company, Lititz, PA, USA	13 July 2016
Between-row cultivation (2)	Hillside rolling cultivator		19 July 2016
Hand weeded in-row zones			25 July 2016
BT sprayed			29 July 2016
Sidedress N application	67 lbs/ha as 13-0-0 (all treatments except rye cover crop with no nitrogen added)		4 Aug 2016
BT sprayed (2)			8 Aug 2016
Handweeded in-row zones			10 Aug 2016
Harvest			22 Sept 2016

Supplementary Figures for Chapter 2

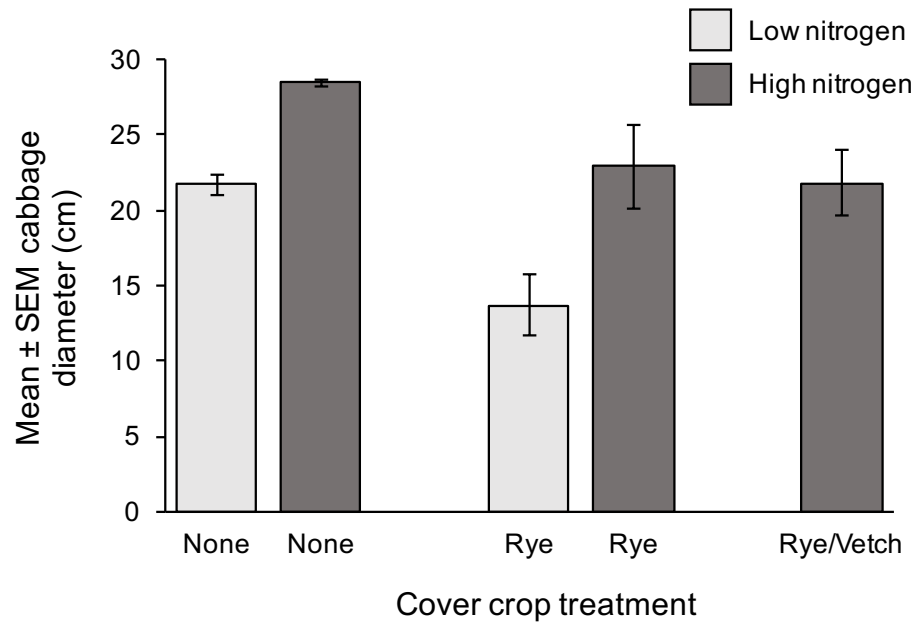


Figure S2.1. Mean ($\pm$ SEM) cabbage diameter for three cover crop treatments, no cover crops, rye cover crops, and a rye/vetch cover crop mix, under low and high nitrogen treatments. There was no low nitrogen treatment for the rye/vetch cover crop mix.



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CHAPTER 3: Predation threat and predator identity modify bottom-up effects on a specialist herbivore

Introduction

Predators can affect their prey through direct consumption (consumptive effects) as well as through inducing a fear response in prey, which present as behavioral or physiological changes (non-consumptive effects) (Werner and Peacor 2003, Priesser et al. 2005). Consumptive effects result in a direct reduction in the size of the prey population, and are often studied when observing predator-prey interactions, though, in recent years, there has been growing consensus in the literature that non-consumptive effects may have similar or larger impacts on overall success of prey species (Werner and Peacor 2003, Priesser et al. 2005, Thaler and Griffen 2008). A recent meta-analysis found that non-consumptive effects in arthropods were impactful across a range of predator and prey characteristics, with behavioral non-consumptive effects stronger than physiological ones, and effects on prey activity stronger when the predator and prey have a shared evolutionary history (Buchanan et al. 2017). Even though prey are not being directly consumed, non-consumptive effects may still lead to a decrease in prey survival (Siepielski et al. 2014) or fecundity (Peckarsky et al. 1993), as well as a change in growth or development time (Xiong et al. 2015), or change in activity (Kaplan et al. 2014). However, the type and magnitude of these responses are likely to vary depending on environmental factors.

Non-consumptive effects of predators may differ depending on top-down factors such as predator species characteristics (Hermann and Landis 2017). For example, predator hunting mode (Preisser et al. 2007, Miller et al. 2014) or feeding strategy (McClure and Despland 2011) may alter perceived predation threat levels for an herbivore species, leading to a change in prey

fear response to different predators. In a study observing prey (*Malacosoma disstria* caterpillars) behavioral response to three different predators, spiders (*Thanatus vulgaris*), spined soldier bugs (*Podisus maculiventris*) and parasitoid wasps (*Hyposoter fugitivus*), caterpillars held onto their silk to evade predation when threatened by spiders, but not the other two predators, and showed overall more efficient evasive behaviors when threatened by spined soldier bugs compared to the other predators (McClure and Despland 2011). This study demonstrates that non-consumptive effects of predators on prey differ depending on predator species. Additionally, prey species may only be vulnerable to predation during specific life stages for some predators but vulnerable during all life stages to another. If a prey species is vulnerable to predation throughout all of its herbivorous life stages, we may expect prey to reduce feeding in order to be more inconspicuous due to decreasing movement or activity and potentially reducing the release of plant volatiles (Lima and Dill 1990). However, if a prey species is only susceptible to predation in its early life stages, larvae may consume more in order to outgrow the vulnerable stage (Xiong et al. 2015). While research with individual predator-prey pairs has gained more attention in the literature in recent years, there is relatively little focus on if and how prey species alter their behavior towards predator species representing varying levels of predation threat.

Prey response to predators may also be altered depending on bottom-up factors, for example, if plant nutritional quality is poor, larval growth rate may decrease, subsequently leaving them vulnerable to predation for a longer period of time (slow growth high mortality hypothesis; Clancy and Price 1987). Prey may also be detected by predators through plant volatiles released during feeding. For example, herbivores can attract natural enemies to their host plant through plant volatiles released in response to feeding damage (Vet and Dicke 1992). In the case of poor plant quality, larvae spend more time feeding to obtain nutrients, which may

lead to increased predator consumptive effects due to an increase in plant volatiles. However, with low plant quality we may see a decrease in larval behavioral response to predation threat as prey energetically prioritize feeding over evading predators (Anholt and Werner 1995, Kaplan and Thaler 2010, Hawlena and Schmitz 2010). For example, when presented with low nutrients, tadpoles (*Rana catesbeiana*) showed higher foraging activity and subsequent mortality to predators compared to tadpoles presented with high quality food which could afford to alter their behavior and decrease their activity levels under a predation threat (Anholt and Werner 1998). Thus, bottom-up factors affect prey response to predators, but it is currently unclear if these responses are consistent across different predator threats.

In many insect species, larval stages are less mobile compared to the adult life stage, and are usually confined to a host plant chosen by the female during oviposition. Therefore, we may expect to see females choosing host plants that provide the highest quality food source and enemy-free space for their offspring. The ‘mother knows best’ hypothesis predicts that adult oviposition choice will match larval host preference (Valladares and Lawton 1991) and that females are able to evaluate predation threat to their offspring, even in cases when they are not vulnerable to the predator. For example, gravid Colorado potato beetles (*Leptinotarsa decemlineata*) adjusted their behavior by consuming less leaf tissue and ovipositing fewer egg clutches on host plants where a predation threat was present, suggesting that adults may change their behavior to benefit their offspring (Hermann and Thaler 2018). However, mothers may also choose a different strategy by spreading their offspring among plants of different quality and predation threat levels (risk-spreading hypothesis; Levins 1962). In this case we may expect to see no clear host preference by females, and larvae will adjust their behavior later in response to different top-down and bottom-up factors. For example, in the absence of predators or predator

threat, Colorado potato beetles spread their egg clutches out among different levels of plant quality (Hufnagel et al. 2017). There are relatively few studies observing simultaneously how the combination of top-down and bottom-up factors may influence non-consumptive effects on adult choices and the success of larval life stages.

In this study we aim to determine how plant nitrogen (N) levels and predation threat (via chemical cues or predator presence) by two arthropod predators representing different feeding modes and levels of threat, the convergent lady beetle (chewing, *Hippodamia convergens* Guérin-Ménéville, Coleoptera: Coccinellidae) and the spined soldier bug (piercing-sucking, *Podisus maculiventris* Say, Hemiptera: Pentatomidae) impact adult and larval host plant choice and subsequent behavior of early life stages of the imported cabbageworm (*Pieris rapae* L., Lepidoptera: Pieridae). Adult *P. rapae* lay single eggs on leaves, and recent research suggested that plant cues (bottom-up factors) have a larger impact on oviposition than predator cues (top-down factors), but when presented with high quality plants, plants without predators may be slightly preferred (Lund et al. 2019). Therefore, we hypothesized that female choice would be driven by plant N more than predation threat. On the other hand, *P. rapae* larvae require high quality plants to grow, and are relatively immobile; therefore they are more vulnerable to predation than adults. This herbivore is only vulnerable to predation by *H. convergens* during early instar stages (Evans 2009), but vulnerable to predation by *P. maculiventris* during all larval stages (Mukerji and LeRoux 1969). Due to the increased exposure and susceptibility to predation, we hypothesized that *P. rapae* larvae would prefer host plants that are high in N and without predation threat. We also expected that they would show different behavioral responses to predation threats by *H. convergens* and *P. maculiventris* where plant quality could be an important factor when threatened by *H. convergens* but less so for *P. maculiventris*. We expected

that larvae may consume more leaf tissue in order to grow larger to evade predation to *H. convergens*, but consume less leaf tissue to remain more inconspicuous to *P. maculiventris*. We also studied how these behaviors were altered to changes in plant N. Under low N conditions we expected larvae to respond less to predators compared to high N conditions due to the stress of feeding on a low quality food source. To test these hypotheses we observed *P. rapae* host choice and behavior in greenhouse and environmental chamber bioassays, and mirrored bioassays in a field setting to observe the impacts of different field management strategies.

Methods

Insects and collard maintenance

Pieris rapae were reared in a greenhouse colony at Michigan State University as outlined in Lund et al. (2019). In short, insects originated from Michigan State University's Farms and were continuously kept in colony since 2015, with field caught insects added yearly to maintain genetic diversity. Larvae were reared on collard greens (*Brassica oleracea* cv. "Georgia"; W. Atlee Burpee & Co., Warminster, PA) *ad libitum*, and adults were fed a honey or sugar water solution.

Two different predators were used in experiments, the convergent lady beetle, *H. convergens*, and the spined soldier bug, *P. maculiventris*. *Hippodamia convergens* adults were sourced from a biological agent supplier (Rincon-Vitova Insectaries, Ventura, CA), and 3rd instar *P. maculiventris* were obtained from a colony and reared according to a standard protocol (Coudron et al. 2002).

Potted Georgia collards for colony rearing and experiments were raised according to Lund et al. (2019). In summary, plants were grown in a greenhouse (25-30 °C, 16:8 light/dark

photoperiod) received either no supplemental N (“low N”) or organic blood meal (“high N” ; 15 g/pot, 12:0:0 N:P:K , The Espoma Company, Millville, NJ) applied when collards were 3-3.5 weeks old. Blood meal was gently worked in to the top 2-3 cm of the soil using a fork, and plants with no N also had the top 2-3 cm of soil fluffed at this time to provide equivalent soil aeration. This was done to ensure blood meal was well incorporated into the soil, and to help reduce odors let off by the blood meal and maintain similar soil appearances in both high and low N treatments, both of which could influence *P. rapae* behavior. All collards used in greenhouse and environmental chamber experiments were 4-5 weeks old.

Adult choice greenhouse bioassays (Figure 3.1a)

Two different four-choice trials were conducted in a greenhouse (25-30°C, L:D 16:8) at Michigan State University from 22 May 2017 – 31 March 2018 to determine adult female *P. rapae* oviposition preference among high and low N plants both in the presence or absence of the two predator species.

The first trial consisted of four treatments: low N collard, high N collard, low N collard with *H. convergens*, and high N collard with *H. convergens*. Each of these four treatments was replicated twice within one mesh cage for a total of eight potted collard plants per cage (122 x 70 x 70 cm, Nasco, Fort Atkinson, WI), and 22 cages were set up total. Plants with predators had five *H. convergens* confined to one new fully emerged collard leaf with a white mesh paint strainer bag (3.79 l; Master Craft Mfg. Co., South El Monte, CA) just before adult *P. rapae* were added. The bag was tied closed to ensure predators remained on the leaf for the full 24 hours the cages were set up, and collards without predators had an empty mesh bag covering one leaf. One adult 3-6 day-old, naïve, mated female *P. rapae* was added per cage and left for 24 hours to

oviposit on the collards. After 24 hours, *P. rapae* were removed, and the number of eggs on each plant in the cage was counted and recorded.

The second trial tested the effects of *P. maculiventris* and plant N on *P. rapae* oviposition choice with the following treatments: low N collard, high N collard, low N collard with *P. maculiventris*, high N collard with *P. maculiventris*. Cages were set up as in the previous trial, except three third instar *P. maculiventris* were added to the mesh bags. Three *P. maculiventris* were used due to availability of predators, and because they tend to be cannibalistic in higher abundance. After 24 hours, *P. rapae* eggs were counted and recorded for each plant. Choice tests using *P. maculiventris* were replicated in the same way as *H. convergens* choice-tests, with a total of 22 cages.

Four-choice ovipositional preferences were analyzed using a Kruskal-Wallis test to observe overall differences in total egg numbers among the four treatments. A Dunn's test was run using the 'dunn.test' package (Dinno 2016) to determine differences among the four choices ($\alpha = 0.05$). Separate analyses were done for data with *H. convergens* and *P. maculiventris*.

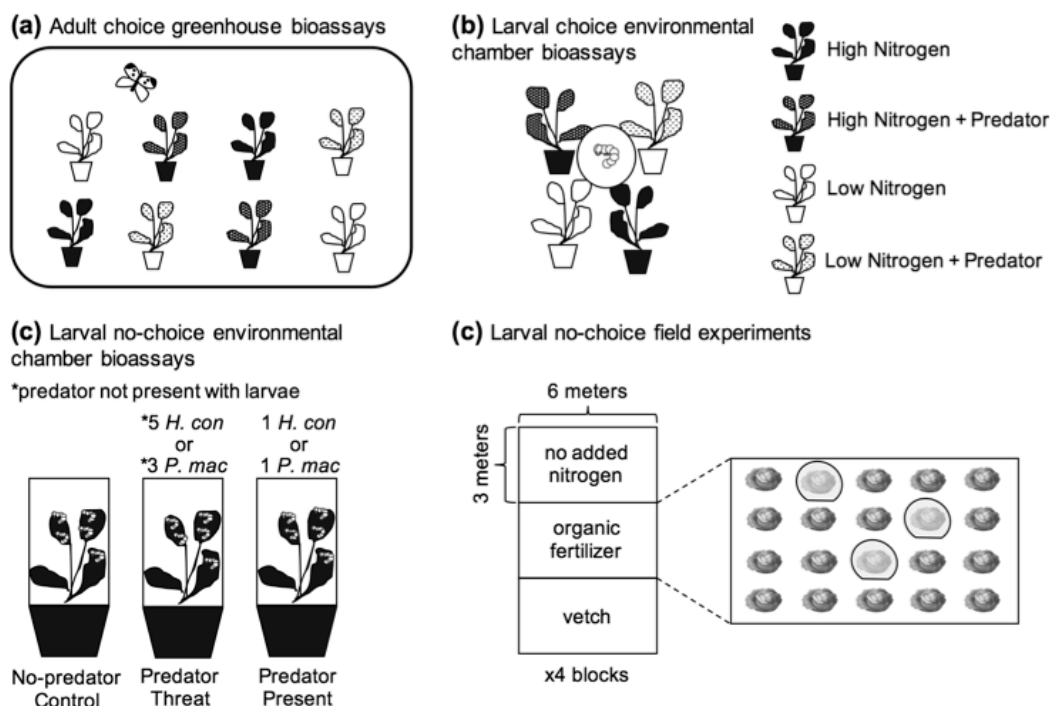


Figure 3.1. Overview of experimental set up used to study the effects of plant nitrogen and predator identity on *P. rapae* host plant choice, growth, and behavior. (a) Adult *P. rapae* choice tests in the greenhouse to evaluate effect of plant N and predator threat on oviposition. (b) Larval *P. rapae* choice in an environmental chamber to evaluate effects of plant N and predator threat on larval host plant choice. (c) Larval *P. rapae* no-choice bioassays to evaluate the effect of predator threat and presence on larval survival, growth, and leaf consumption. (d) Larval no-choice field experiments to evaluate the effect of plant N source and levels, and predator threat and presence on larval survival and growth in a field setting.

Larval choice environmental chamber bioassays (Fig. 3.1b)

Pieris rapae neonate larval choice tests were conducted in an environmental chamber at 25°C, 16:8 L:D. The first trial was set up between 20 May 2017 – 1 June 2017 to determine plant N and *H. convergens* predator effects on larval *P. rapae* choice. Four treatments were used: low N collard, high N collard, low N collard with *H. convergens*, and high N collard with *H. convergens*. Potted plants for larval choice tests were not placed in cages; treatments with predators had five *H. convergens* bagged to one collard leaf with a mesh bag for 24 hours prior

to the start of the experiment. After 24 hours, *H. convergens* were removed and choice tests were set up so that predator chemical cues were present on the leaf as a potential predator threat for the larvae. A piece of tape was added to the underside of the top 1.5 cm of one leaf per treatment (in predator treatments this was the leaf that *H. convergens* had been allowed to walk on for 24 hours), and each leaf was taped to a piece of Whatman filter paper (70cm), so that all four treatments were attached to one filter paper, creating a bridge among the four leaves. One neonate *P. rapae* larva was placed in the center of the filter paper bridge with a paintbrush, and left for 24 hours (Fig. S3.1). After 24 hours, each plant was checked to find the larva, and the larval choice was recorded. Larval choice tests with *H. convergens* were replicated 28 times.

A second trial was run from 14 February 2018 – 2 March 2018 to test N and *P. maculiventris* predator effects on *P. rapae* larval choice. Plant treatments with *P. maculiventris* were the same as with *H. convergens*, except three third instar *P. maculiventris* were bagged to each plant for 24 hours. Larval choice tests with *P. maculiventris* were replicated 28 times.

Four-choice larval preferences were analyzed using a chi-square test to determine overall differences in total larval choices. Separate analyses were performed for *H. convergens* and *P. maculiventris* choice tests.

Larval no-choice environmental chamber bioassays (Fig. 3.1c)

Bioassays were conducted in an environmental chamber (25°C, 16:8 L:D) to determine *H. convergens* and *P. maculiventris* consumptive and non-consumptive effects on *P. rapae* larval survival, growth, and leaf consumption. Bioassays were run from 28 August 2017– 1 September 2017 (*H. convergens*) and 20 April 2018 – 1 May 2018 (*P. maculiventris*). The three predation treatments were ‘no-predator control’, ‘predator threat’ (predator removed), and ‘predator

present', which were replicated 20 times, all replications set up at the same time, for *H. convergens*, and 25 times for *P. maculiventris*, split between two groups set up 7 days apart due to predator availability. All experiments were set up in a randomized complete block design. For each treatment, an acetate tube (11 cm diam., 21 cm tall; ACCO Brands, Inc., Apollo, Lincolnshire, IL) was placed around each collard and pushed 1-2 cm into the soil and covered with a lid to ensure insects could not escape. In predator threat treatments, predators were added to cages (five adult *H. convergens* or three third instar *P. maculiventris*) for 24 hours prior to experimental set up, and removed just before larvae were added so that no predators were in the cages with larvae. In predator present treatments one predator, either an adult *H. convergens* or third instar *P. maculiventris*, was added to the cage with larvae. No predators were added to control cages. Five *P. rapae* neonate larvae were added to each plant with a paintbrush and were left for 4 days, after which all predators were removed and larvae were counted, collected, and weighed. Photos were taken of each leaf on collards in each treatment, and Image J (Version 1.50i; National Institutes of Health, USA) was used to calculate the leaf area consumed.

Larval survival was analyzed with a linear mixed-effects model, using the 'lmer' function in the 'lme4' package (Bates et al. 2013), with predator treatment as a fixed factor and block as a random factor. Treatment means were compared using 'lsmeans' with the false discover rate adjustment method.

Larval weight was analyzed with a linear mixed-effects model with predator treatment as a fixed factor and block as a random factor, using the 'lmer' function in the 'lme4' package. Weight data for *H. convergens* and *P. maculiventris* predator treatments was ln transformed to achieve assumptions of normality. Treatment means were compared using 'lsmeans' with the false discovery rate adjustment method.

Collard leaf consumption was analyzed using a linear mixed-effects model with predator treatment as a fixed factor and block as a random factor. Leaf consumption data was square root transformed for *H. convergens*, and ln transformed for *P. maculiventris* to achieve assumptions of normality. Treatment means were compared using ‘lsmeans’ with the false discovery rate adjustment method.

Larval no-choice field experiments (Fig. 3.1d)

No-choice experiments were conducted during the 2016, 2017, and 2018 growing seasons in an organic cabbage (*Brassica oleracea* var. “Farao”; Bejo Seeds, Inc., Oceana, CA) field located at the Michigan State University Horticulture Teaching and Research Center in Holt, MI, to test N management practices and *H. convergens* and *P. maculiventris* consumptive and non-consumptive effects on *P. rapae* survival and growth.

Cabbage seedlings were grown in a greenhouse (25/20 °C; 16/8 daylength) in 98 cell plug trays for 4-weeks and transplanted to the field on 6 July 2016, 29 June 2017, and 5 July 2018 in a randomized complete block design with four blocks and three treatments. Treatment plots in each block measured 3 x 6 m, and blocks were spaced 4.5 m apart. Each plot consisted of 4 rows of cabbage with a between row spacing of 76 cm, and an in-row spacing of 36 cm. Nutrient treatments consisted of three levels of N: 1) no N applied during cabbage production; 2) an organic fertilizer consisting primarily of hydrolyzed feather and blood meal (NatureSafe 10-2-8 and 13-0-0, Darling Ingredients, TX) applied at 134 kg N/ha in two split applications; and 3) hairy vetch (*Vicia villosa*, VNS, Albert Lea) drilled during the previous year’s fall, mowed in the spring, and incorporated with strip tillage approximately 2 weeks prior to transplanting. The organic fertilizer treatment received 67 kg N/ha just before transplanting, and an additional 67 kg

N/ha at 29, 26 or 36 days after transplanting, in 2016, 2017 and 2018, respectively. In the fall prior to cabbage production in each year, the entire experimental area was fertilized with 840 kg/ha of pelleted 4-3-2 chicken manure (Herbruck's Poultry Ranch, Saranac, MI) and drill-planted with a winter rye (*Secale cereale*) cover crop at 67 kg/ha. Rye was planted only in the zone between future cabbage rows by blocking drop tubes on the grain drill. In hairy vetch treatments, vetch was sown at 23 kg/ha at the same time as rye, but only in the in-row zone. Although not widely adopted, this practice of zonal cover crop planting is useful for avoiding interference of rye with strip tillage and transplanting operations, while maintaining the benefits of rye mulch between crop rows (Lowry and Brainard, 2017). In the following spring yearly, all cover crops were flail mowed two weeks prior to cabbage planting, and strip-tilled (Hiniker 6000 strip tiller equipped with a shank, offset disks, and a rolling basket) to create a tilled zone approximately 25 cm-wide and 25 cm deep for subsequent cabbage transplants. Tilled strips were centered at 76 cm, resulting in rye residue remaining on the soil surface as a mulch between rows, and vetch incorporated into the soil as a N source. Additional details of yearly field and crop management activities are provided in Appendix II, Table S3.1.

Experiments were set up in each plot between 1 – 20 August 2016 (11 replications), 12 – 29 August 2017 (12 replications), and 5 – 26 August 2018 (10 replications). Predation treatments consisted of a 'no-predator control', 'predator threat', and 'predator present'. Predators were adult *H. convergens* in 2016 and 2017, and third instar *P. maculiventris* in 2018. Before the start of the experiment, each cabbage was inspected for arthropods, and any found were removed. In predator threat cages, cabbages were covered with 50 x 55 cm white mesh bags (Hummert International, Earth City, MO), and either five (*H. convergens*) or three (*P. maculiventris*) predators were added. Cages were tied closed around the base of the cabbage so the entire

cabbage head was enclosed, and predators were allowed to walk around on cabbages for 48 hours. After 48 hours, all predators were removed, and larvae were added. Five *P. rapae* first instars were placed onto each cabbage with a paintbrush, after which cabbages were covered with the bags. ‘Predator present’ cages received one predator at this time. Experiments were run for 3 days, after which the bags were removed, and all remaining larvae were counted, collected, and weighed.

A linear mixed effects model was used to determine if year had a significant effect on *P. rapae* larval survival and weight. Year was determined to have a significant impact, so data was analyzed independently for each field season. Larval survival and weight data for each year was analyzed with a linear mixed effects model, using the ‘lmer’ function in the ‘lme4’ package, with predator and nutrient treatment as fixed factors, and block as a random factor. Survival data was not transformed for any year. Weight data was ln transformed in 2016, 2017 and 2018 to improve the assumption of normality. Treatment means were compared using ‘lsmeans’ with the false discovery rate adjustment method.

Plant nitrogen content

Both low and high N collards from the greenhouse were submitted for N analysis to measure N content (A&L Great Lakes Laboratories, Fort Wayne, IN). The overall difference in plant N content of collards was analyzed using a t-test.

One cabbage per nutrient treatment per block was collected for N analysis (A&L Great Lakes Laboratories, Fort Wayne, IN) from the field experiment during each growing season. The overall difference in plant N content of cabbages was determined using a linear mixed effects model with nutrient treatment as a fixed factor, and block as a random factor. A post-hoc

pairwise comparison was used to determine differences in plant N among treatments. All statistical analyses were conducted using R version 3.3.2 (R Core Team 2017; $\alpha = 0.05$).

Results

Adult choice greenhouse bioassays

Adult female *P. rapae* oviposition choice in tests with *H. convergens* was significantly different among collard treatments ($\chi^2 = 19.84$, $df = 3$, $P < 0.01$; Figure 3.2a), with females laying about 2.5 times more eggs on high N plants ($P = 0.01$) and high N plants with *H. convergens* ($P < 0.01$) than low N plants with predators, and almost 3.5 times more eggs on high N plants ($P < 0.01$) and high N plants with *H. convergens* ($P < 0.01$) than low N plants without predators. Overall, females laid more eggs on high N plants than low N plants ($\chi^2 = 229.51$, $df = 1$, $P < 0.01$), but the presence of *H. convergens* had no overall effect on oviposition ($\chi^2 = 0.28$, $df = 1$, $P = 0.60$).

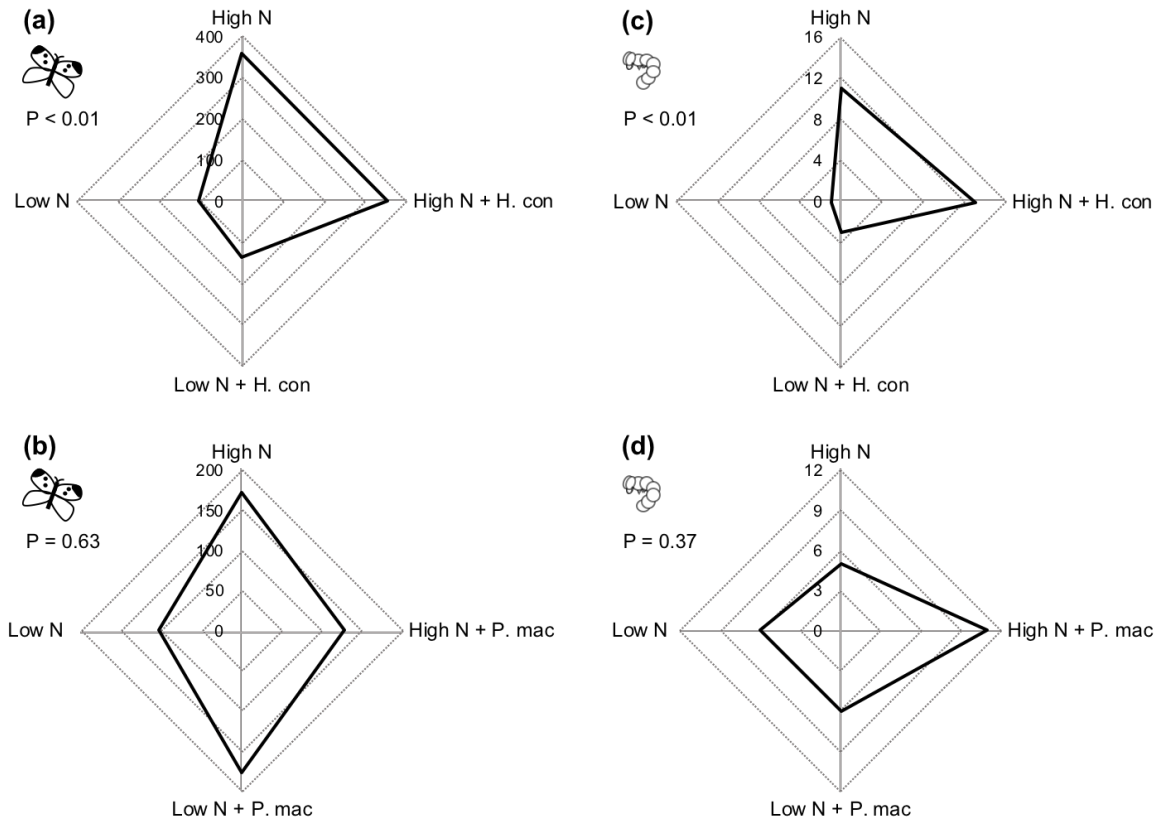


Figure 3.2. Results of four-way *P. rapae* choice tests with potted plants in the greenhouse using high N collard, low N collard, high N collard with predator threat (either *H. convergens* or *P. maculiventris*) and low N collard with predator threat. (A) Adult *P. rapae* choice tests with *H. convergens*, (B) adult *P. rapae* choice test with *P. maculiventris*, (C) *P. rapae* larval choice tests with *H. convergens*, and (D) *P. rapae* larval choice test with *P. maculiventris*. In predator threat treatments, predators were bagged on one collard leaf immediately before butterflies were added in choice tests, and 24 hours before in larval choice tests; predators were removed after this time in larval choice tests, but in adult choice tests predators were left in the bags on the plants. Each graph represents either the total number of eggs laid (A and B) or number of larval choices (C and D) along each axis for the four choices presented ($\alpha = 0.05$). H. con. – *Hippodamia convergens*, P. mac – *Podisus maculiventris*, N – nitrogen.

In adult choice tests using *P. maculiventris*, *P. rapae* females did not lay significantly more eggs on any of the treatments ($\chi^2 = 1.75$, $df = 3$, $P = 0.63$; Figure 3.2b). Neither plant N

($\chi^2 = 0.83$, $df = 1$, $P = 0.45$) or *P. maculiventris* presence ($\chi^2 = 1.35$, $df = 1$, $P = 0.25$) had any effect on *P. rapae* oviposition choice.

Larval choice environmental chamber bioassays

In *P. rapae* larval bioassays with *H. convergens*, treatments had a significant effect ($\chi^2 = 14.86$, $df = 3$, $P < 0.01$; Figure 3.2c), with 46% of larvae choosing high N plants with predators, 39% high N plants, 11% low N plants with predators, and 4% low N plants. Overall, N had a significant impact on larval choice ($\chi^2 = 229.51$, $df = 1$, $P < 0.01$), while *H. convergens* presence had no influence on larval choice ($\chi^2 = 0.57$, $df = 1$, $P = 0.45$).

Larvae did not have a preference for any treatment in the bioassays with *P. maculiventris* ($\chi^2 = 3.14$, $df = 3$, $P = 0.37$; Figure 3.2d). Neither N ($\chi^2 = 0.57$, $df = 1$, $P = 0.45$) or *P. maculiventris* threat ($\chi^2 = 1.29$, $df = 1$, $P = 0.26$) influenced larval choice.

Larval no-choice environmental chamber bioassays

The presence and threat of *H. convergens* did not impact survival of *P. rapae* larvae ($F = 1.47$, $df = 2$, 38, $P = 0.24$; Figure 3.3a). Larval weight was significantly affected by *H. convergens* presence ($F = 3.66$, $df = 2$, 217, $P = 0.03$; Figure 3.3b), with weight increasing by 42% in cages where *H. convergens* were present ($t = 2.4$, $df = 220$, $P = 0.03$), and 30% in predator threat cages ($t = 2.29$, $df = 215$, $P = 0.03$) compared to no-predator controls. The mean leaf tissue consumed by larvae was impacted by treatment ($F = 3.67$, $df = 2$, 38 $P = 0.03$; Figure 3.3c), with 58% more tissue consumed by larvae in *H. convergens* threat treatments compared to no-predator control ($t = 2.69$, $df = 38$, $P = 0.03$), and only 40% higher in predator present treatments compared to controls ($t = 1.61$, $df = 38$, $P = 0.17$).

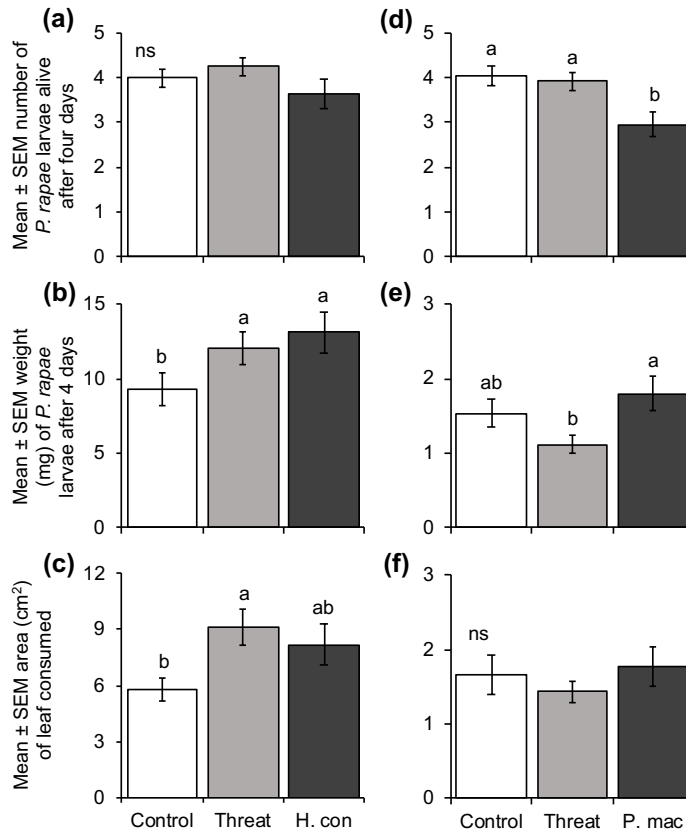


Figure 3.3. Results of environmental chamber bioassays observing the effects of *H. convergens* and *P. maculiventris* on *P. rapae* larval survival (A, D), weight (B, E), and collard leaf consumption (C, F) after 4 days. Effects were observed across three predation treatments: no-predator control, predator threat, and predator present (either *H. con* - *H. convergens* or *P. mac.* - *P. maculiventris*). Bars with different letters are significantly different from each other, 'ns' indicates that treatments were not significantly different ($\alpha = 0.05$).

In bioassays using *P. maculiventris*, predator presence significantly affected larval survival ($F = 6.59$, $df = 2, 48$, $P < 0.01$; Figure 3.3d), with an average of about three out of five larvae surviving after 4 days where *P. maculiventris* were present compared to control ($t = 3.31$, $df = 48$, $P = 0.01$) and threat ($t = 2.94$, $df = 48$, $P = 0.01$) treatments, (four larvae survived on average). Larval weight also varied across *P. maculiventris* predator treatments ($F = 3.80$, $df = 2$,

254, $P = 0.02$; Figure 3.3e). Mean larval weight increased by 60% in predator present compared to threat treatments ($t = 2.63$, $df = 254$, $P = 0.03$), although larval weight was not significantly different in control compared to either threat ($t = 1.97$, $df = 252$, $P = 0.08$) or predator present ($t = 0.83$, $df = 255$, $P = 0.41$) treatments. Collard leaf consumption by larvae was not different across *P. maculiventris* treatments ($F = 0.19$, $df = 2, 40$, $P = 0.83$; Figure 3.3f).

Larval no-choice field experiments

There were significant differences in larval survival ($t = 4.49$, $df = 197$, $P < 0.01$) and weight ($t = 19.22$, $df = 315.05$, $P < 0.01$) between 2016 and 2017, so data from the two years were analyzed separately. In 2016, *H. convergens* predator treatment did not affect larval survival or weight, and nutrient treatment did not affect larval survival (Table 3.1). Additionally, when observing the impact of both predator and nutrient treatment together on larval survival, we saw no significant effects (Table 3.1; $F_{4,80} = 0.59$, $P = 0.67$; Figure 3.4a). However, nutrient treatment significantly impacted larval weight with about a 30% overall increase in weight in plots with a vetch cover crop, and a 24% increase in plots with organic fertilizer compared to plots with no N (Table 3.1). When observing the effects of both predator and nutrient treatment on larval weight, in no-predator control plots we saw a similar trend, with a significant increase in larval weight in vetch treatments compared to both no N (60% increase; Fig. 3.4b; $t = 3.53$, $df = 286$, $P < 0.01$) and organic fertilizer (30% increase; $t = 2.22$, $df = 285$, $P = 0.04$; Figure 3.4b) treatments. However, there were no differences in larval weight among predator threat or predator present treatments across the different nutrient treatments.

Table 3.1. Main effects and means comparisons for predation treatment (no-predator control, predator threat, and predator present) and nutrient treatment (no N added, organic fertilizer, and hairy vetch) on *P. rapae* larval survival and weight in no-choice field experiments using bagged cabbage plants in 2016, 2017, and 2018. In 2016 and 2017 *H. convergens* were used as predators, and *P. maculiventris* was used in 2018. Numbers in a row followed by different letters represent significant differences within main effects ($\alpha = 0.05$); ns – not significant. P – predator main effect; N- nutrient main effect.

		Main effects			Treatment means comparison					
		F	df	P-value	P: N:	No Predator No N	Threat Fertilizer	Present Vetch	t	P-value
2016										
Survival	Predator	0.97	2, 80	0.38		3.36 ± 0.20 ns	3.00 ± 0.23 ns	2.94 ± 0.26 ns	< 1.29	> 0.41
	Nutrient	1.23	2, 80	0.30		3.36 ± 0.23 ns	3.09 ± 0.22 ns	2.85 ± 0.26 ns	< 1.57	> 0.36
	P x N	0.59	4, 80	0.67						
Weight	Predator	0.40	2, 285	0.30		3.72 ± 0.28 ns	3.79 ± 0.36 ns	4.32 ± 0.36 ns	< 0.88	> 0.74
	Nutrient	5.63	2, 286	< 0.01		3.37 ± 0.27 b	4.16 ± 0.34 ab	4.36 ± 0.31 a	> 0.93	< 0.05
	P x N	1.64	4, 285	0.16						
2017										
Survival	Predator	0.33	2, 88	0.72		4.00 ± 0.24 ns	3.94 ± 0.19 ns	3.78 ± 0.18 ns	< 0.78	> 0.84
	Nutrient	0.33	2, 88	0.72		4.00 ± 0.20 ns	3.78 ± 0.19 ns	3.94 ± 0.23 ns	< 0.78	> 0.84
	P x N	1.73	4, 88	0.15						
Weight	Predator	1.01	2, 392	0.36		0.39 ± 0.03 ns	0.52 ± 0.06 ns	0.43 ± 0.04 ns	< 1.34	> 0.42
	Nutrient	0.06	2, 392	0.94		0.45 ± 0.05 ns	0.49 ± 0.05 ns	0.40 ± 0.04 ns	< 0.34	> 0.89
	P x N	2.86	4, 392	0.02						
2018										
Survival	Predator	3.91	2, 78	0.02		4.47 ± 0.14 a	3.90 ± 0.24 ab	3.60 ± 0.27 b	> 0.95	< 0.05
	Nutrient	0.95	2, 78	0.39		4.20 ± 0.22 ns	3.77 ± 0.23 ns	4.00 ± 0.23 ns	< 1.38	> 0.52
	P x N	0.85	4, 78	0.50						
Weight	Predator	0.82	2, 339	0.44		2.53 ± 0.25 ns	2.40 ± 0.24 ns	2.29 ± 0.24 ns	< 1.13	> 0.39
	Nutrient	5.20	2, 340	< 0.01		2.73 ± 0.25 a	1.85 ± 0.22 b	2.62 ± 0.25 a	> 0.29	< 0.05
	P x N	0.76	4, 339	0.55						

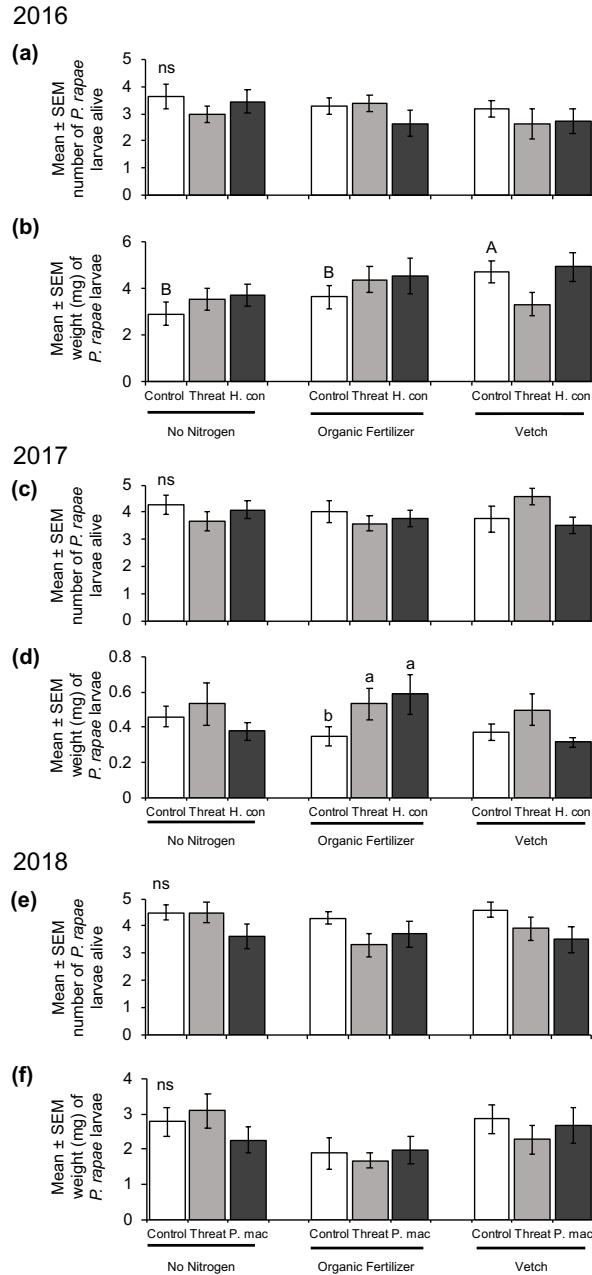


Figure 3.4. *Pieris rapae* larval survival (A, C, E) and weight (B, D, F) in an experimental cabbage field in 2016 (A, B), 2017 (C, D), and 2018 (E, F) after 4 days. Larval survival and weight were observed across three predation treatments: no-predator control, predator threat, and predator present (either *H. con* - *H. convergens* or *P. mac* - *P. maculiventris*), each replicated within three nutrient treatments: no N added, organic fertilizer, and hairy vetch. In 2016 and 2017 *H. convergens* were used and in 2018 *P. maculiventris* were used in predator threat and predator present treatments. Uppercase letters represent significant differences among nutrient treatment within a predation treatment, and lowercase letters represent significant differences among predation treatment within a nutrient treatment, while 'ns' indicates that treatments were not significantly different ($\alpha = 0.05$).

In 2017, larval survival and larval weight were unaffected by predator treatment (Table 3.1; survival: $F_{2,88} = 0.33$, $P = 0.72$, weight: $F_{2,391} = 0.95$, $P = 0.39$) and nutrient treatment (Table 3.1; survival: $F_{2,88} = 0.33$, $P = 0.72$, weight: $F_{2,391} = 0.07$, $P = 0.93$). Additionally, when looking at the overall impact of predator treatment with nutrient treatment on larval survival, we found no significant effects (Table 3.1; $F_{4,88} = 1.73$, $P = 0.15$; Figure 3.4c). However, larval weight was influenced by the interactive effects of predator and nutrient factors. In particular, we found that larval weight increased by 70% in predator present treatments but only in plots treated with organic fertilizer ($t = 2.74$, $df = 392$, $P = 0.01$; Figure 3.4d), and by 50% in predator threat treatments ($t = 2.62$, $df = 392$, $P = 0.01$; Figure 3.4d) compared to no-predator controls.

During the 2018 field season, *P. maculiventris* predator treatment had a significant impact on larval survival (Table 3.1; $F_{2,75} = 4.36$, $P = 0.02$), with a 20% decrease in treatments where *P. maculiventris* were present compared to no-predator controls. However, there was no difference in larval weights among predator treatments (Table 3.1; $F_{2,75} = 1.06$, $P = 0.35$). While nutrient treatment did not significantly affect larval survival (Table 3.1; $F_{2,333} = 0.68$, $P = 0.51$), there were differences in larval weight among nutrient treatments (Table 3.1; $F_{2,333} = 5.36$, $P = 0.01$) with larvae weighing about 30% less in organic fertilizer plots compared to no added nutrients ($z = 2.58$, $P = 0.03$) or vetch plots ($z = 2.48$, $P = 0.04$). When looking at interactive effect of both predator and nutrient treatment we found no significant effect on either larval survival (Table 1; $F_{4,78} = 0.85$, $P = 0.50$; Fig. 4e) or larval weight (Table 3.1; $F_{4,339} = 0.76$, $P = 0.55$; Figure 3.4f).

Plant nitrogen content

The N content of collards used in all of our bioassays are reported in Chapter 2. Cabbage N content in field trials was affected by N treatment in 2016 ($F_{2,9} = 4.98$, $P = 0.03$) and 2018 ($F_{2,9} = 6.52$, $P = 0.02$), but no effects of N treatment on cabbage N were detected in 2017 ($F_{2,9} = 1.19$, $P = 0.35$). In 2016, cabbages treated with organic fertilizer had 1.7 times higher N ($3.35\% \pm 0.96$) than cabbages without added N ($1.93\% \pm 0.34$; $P = 0.3$), but cabbages grown in the vetch treatment did not contain significantly more N than cabbages without added N ($2.40\% \pm 0.48$; $P = 0.58$). In 2018, cabbages grown in vetch treatments had twice as high N content ($3.10\% \pm 0.75$) compared to cabbages grown without N addition ($1.61\% \pm 0.26$; $P = 0.01$), while organic fertilizer treated cabbages were similar in N content to both cabbages with no N added and cabbages grown in the vetch treatment ($2.44\% \pm 0.62$; $P = 0.17$).

Discussion

In this study we found that bottom-up and top-down factors have synergistic effects when determining the outcomes of predator-prey interactions, and that predator identity is key in regulating the way prey respond to these environmental factors. We found that the effect of host plant quality on herbivore choice did not differ in the presence or absence of predation threat, but was modified by predator identity, with low quality food sources being more acceptable in the presence of *P. maculiventris* than *H. convergens*. Additionally, larval response to predators differed depending on a combination of plant quality and predator identity, with larvae showing altered behavior to predation threat under different plant N sources in field trials, dependent on predator identity. Much of our current understanding of predator non-consumptive effects and prey response to environmental factors stems from studies analyzing the impacts of a single

predator species (Trussell et al. 2006, Thaler et al. 2012, Kaplan et al. 2014, Xiong et al. 2015). The results found in this study suggest that predator identity, not just predator presence, is a key factor driving how prey species respond to top-down and bottom-up factors. Therefore, it may be hard to draw general conclusions on the outcome of non-consumptive effects in studies using only one predator species.

In choice tests with *P. rapae* adults and first instars, *H. convergens* threat itself did not affect plant choice, but when *H. convergens* cues were present both life stages preferred high over low N plants. This finding partially supports the ‘mother knows best’ hypothesis as it applies to a focus on plant quality (Thompson 1988, Jaenike 1990, Renwick and Chew 1994), and suggests that *P. rapae* are primarily influenced by bottom-up factors such as plant N compared to top-down effects by *H. convergens*. However, a *P. maculiventris* predation threat caused females to change their oviposition strategy to risk-spreading (Levins 1962), distributing eggs evenly among the four choices. Interestingly, *P. maculiventris* threat also caused *P. rapae* larvae to choose evenly among the four choices, suggesting that top-down effects by *P. maculiventris* have stronger effects than bottom-up factors such as plant N, causing *P. rapae* to modify their host plant choice when threatened by this predator. It is noteworthy that the threat of predation by *H. convergens* did not appear to impact host plant choice by adults and larvae, as we expected both to prefer enemy-free space (Jeffries and Lawton 1984, Thompson 1988, Denno et al. 1990), but there are a few reasons this might be the case. First, it is possible that chemical cues emitted by *H. convergens* (or visual cues for adult choice tests) were not strong enough for the adults and larvae to perceive when making a choice, and therefore predation threat did not impact herbivore choices. On the other hand, it is possible that the threat of predation by this predator did not have as large of an impact on herbivore choices as bottom-up factors, since

choosing a high quality host plant might be initially more important especially when the predator eats the early life stages, thus it is important for the herbivore to grow fast and evade the threat of predation. We consider the larval behavioral response as seen in their feeding activity and growth lends support for this second hypothesis.

Hippodamia convergens is capable of consuming only small *P. rapae* (1st or 2nd instar) (Evans 2009), while *P. maculiventris* is capable of consuming all larval stages (Mukerji and LeRoux 1969). Based on our results, we found that *P. rapae* may respond differently to these two different threats. *Hippodamia convergens* predation threat (chemical cues only) caused larvae to consume more leaf tissue and grow larger than larvae that were not exposed to a predation threat. When *H. convergens* were present with larvae in bioassays, larvae again weighed more than larvae without a predation threat, and were consuming numerically (though not significantly) more leaf tissue. These results support our hypothesis that under the threat of a predator of early instars, it might be more beneficial for *P. rapae* to consume more leaf tissue in order to outgrow vulnerable life stages. The fact that larvae were consuming the most leaf tissue in predation threat cages, but weighed the most in predator present cages suggests that there may be physiological changes occurring in the larvae when *H. convergens* are present in the environment (Hawlena and Schmitz 2010). However, *P. rapae* responses changed when threatened by *P. maculiventris* predation; compared to control cages without predator cues, larvae did not consume or weigh more when a *P. maculiventris* threat or predator was present. When looking at the numerical trends in leaf consumption and weight gain across the predator treatments, the change in weight mirrored leaf consumption (Fig. 3e and f), suggesting that weight changes are due to leaf consumption and not physiological changes. Based on these results, *P. rapae* larvae may not benefit from changing their behavior to consume more leaf

tissue to evade predation when threatened by a species such as *P. maculiventris*, which can consume all life stages.

Some of the results from our environmental chamber studies were mirrored in our field trials with similar patterns in herbivore response to predation threat across our N treatments. In 2016 and 2017, there was a similar trend for larval weight in the presence of *H. convergens* in the organic fertilizer treatments compared to our no-choice environmental chamber bioassays (larvae increased in size in predator threat and predator present cages compared to controls; compare Fig. 3.3b to 3.4d). We also observed similar results to our no-choice environmental chamber bioassays in 2018 in the organic fertilizer and vetch treatments for *P. maculiventris* (*P. maculiventris*: compare Fig. 3.3e to 3.4f). The cabbage plants in the organic fertilizer field treatments were most similar in N content to the blood meal treated plants we used in no-choice environmental chamber bioassays, so *P. rapae* larval leaf consumption (in environmental chamber bioassays) and growth in these treatments are likely indicative of how these larvae are able to respond to these two predators when a higher quality diet is available. These results suggest that the behavior observed in adult and larval choice tests for both predators may be due to larval performance in the presence of the two predator species. When a *H. convergens* threat is present, it may be most beneficial to lay more eggs on or move to higher nitrogen plants, since larvae are able to consume more and grow larger on plants with added nitrogen as seen in our environmental chamber and field experiments. However, when threatened by *P. maculiventris*, a risk-spreading behavior may be a better option for survival, as larvae do not appear to alter their consumption or growth in response to this predator when on high or low nitrogen plants. Therefore, depositing eggs on one plant type likely will not increase larval chances of survival when *P. maculiventris* are present.

Several studies observing effects of a predation threat on feeding and growth rate in terrestrial arthropod systems have used *P. maculiventris* predation cues, and have found a decrease in both feeding and growth rate of prey species (Thaler and Griffen 2008, Thaler et al. 2012, Kaplan et al. 2014). This study fills a gap in our understanding of how prey respond to predator threats by demonstrating that these responses may change depending on predator identity. In a study observing non-consumptive effects of *Harmonia axyridis* (Coleoptera: Coccinellidae) on *Helicoverpa armigera* (Lepidoptera: Noctuidae), the caterpillars also increased development when under the threat of predation (Xiong et al. 2015), similar to our findings of increased consumption and weight gain when threatened by *H. convergens*. Additionally, while the literature suggests that *P. maculiventris* decreases prey feeding and growth, we did not find this in our results. However, past studies have focused on alternative prey species (Thaler et al. 2012, Hermann and Thaler 2014, Kaplan et al. 2014), so it is possible that *P. rapae* may not respond to *P. maculiventris* in the same way as other prey species. It is important to note that larvae in our study consumed less leaf tissue and weighed less in bioassays using *P. maculiventris* compared to *H. convergens*. We suspect this is due to these trials being run at different times of year, resulting in different quality leaf tissue of our collards which were grown in summer for *H. convergens* trials, and early spring for *P. maculiventris*. However, it is unlikely that leaf consumption and weight gain trends were affected since we found similar trends in our field trials.

The results from our field experiments were variable, and measured responses were generally weaker than in our no-choice environmental chamber bioassays. Due to cooler temperatures and consistent rain in 2017 during our experiments, larval development was slow over the course of the experiment, but trends in their development on organic fertilizer treated

plants were similar to the results from the no-choice environmental chamber bioassay, suggesting that larvae were responding to predation threat by *H. convergens* even under field conditions. In general, field experiments in ecology often show weaker responses than laboratory experiments because they are influenced by greater variation in biotic and abiotic factors (Calisi and Bentley 2009).

In conclusion, the herbivore we studied not only responded differently to the two predator species representing varying levels of threat, but predator identity also influenced prey response to environmental factors such as plant quality. A recent meta-analysis on top-down and bottom-up effects on terrestrial herbivores found that bottom-up forces were stronger than top-down forces for specialist chewing herbivores (Vidal and Murphy 2018). We found some support for this with *H. convergens* threat, as *P. rapae* showed a stronger response towards plant N than predation threat. However, we found that with a different predator species, *P. maculiventris*, top-down factors were stronger and thus modified bottom-up effects. This is significant, because as we start considering applying our understanding of predator non-consumptive effects in pest management strategies, we have to account for the fact that a typical herbivore is attacked by a variety of predator species that represent different threats and alter the way herbivores perceive host plant suitability (Schoener 1989, Sih et al. 1998). Therefore, understanding how different predators impact prey responses to top-down and bottom-up factors allows us to get a better idea of the broader ecological impact of predator-prey interactions. We suggest future studies take predator identity into consideration in the context of bottom-up and top-down effects on prey species in order to obtain a clearer understanding of the interaction between predator non-consumptive effects and other environmental factors.

APPENDIX

Supplementary Tables for Chapter 3

Table S3.1. Schedule of major field operations, 2016-2018.

Event	2015-16		2016-17		2017-18	
	Date	DAT	Date	DAT	Date	DAT
Cover crops planted	09/10/15	-300	09/01/16	-301	09/01/17	-307
Cover crop biomass sampled	06/07/16	-29	05/26/17	-34	06/07/18	-28
Cover crops mowed	06/14/16	-22	05/30/17	-30	06/08/18	-27
Cabbage transplants started in greenhouse	06/08/16	-28	05/31/17	-29	06/08/18	-27
Strip tillage	06/29/16	-7	06/13/17	-16	06/30/18	-5
Organic fertilizer application (trt 2)	06/30/16	-6	06/28/17	-1	07/02/18	-3
Cabbage transplanted	07/06/16	0	06/29/17	0	07/05/18	0
First in-row mechanical cultivation (Finger)	NA	NA	07/09/17	10	07/13/18	8
Second in-row mechanical cultivation (Finger)	NA	NA	07/18/17	19	07/24/18	19
First between row cultivation (Lilliston)	07/19/16	13	07/24/17	25	07/25/18	20
Second between row cultivation (Lilliston)	08/05/16	30	NA	NA	NA	NA
First hand weeding	07/25/16	19	07/25/17	26	08/03/18	29
Cabbage fertilizer side-dress application (trt 2)	08/04/16	29	07/25/17	26	08/10/18	36
Second hand weeding	08/05/16	30	08/03/17	35	NA	NA
BT application	07/29/16	23	08/11/17	43	08/01/18	27
Yield and quality assessment	09/22/16	78	09/20/17	83	09/14/18	71

Supplementary Figures for Chapter 3



Figure S3.1. Experimental design for our larval choice environmental chamber bioassays as also represented in Fig. 1b. Four plant treatments (high nitrogen (N) + predator, high N no predator, low N + predator, low N no predator) were attached to a piece of filter paper with a small piece of tape. One neonate *P. rapae* larva was placed in the center of the filter paper (circled in red), and left to make a choice. After 24 hours plants were checked for the larva and a choice was recorded.

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LITERATURE CITED

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CHAPTER 4: Predator species identity impacts multiple predator effects on *Pieris rapae*

Introduction

Biological control of pests has been established as an important tool in insect pest management (Gurr and Wratten 1999, Landis et al. 2000, Gurr et al. 2017), and in recent years our understanding of predator-prey interactions, and associated predator consumptive and non-consumptive effects has deepened (Priesser et al. 2007, Buchanan et al. 2017, Ingerslew and Finke 2018). However, much of the current literature on predator-prey relationships focuses on single predator – single prey interactions instead of the impact of multiple predator species. It is estimated that for every prey species, there are around 2-3 predator species (Schoener 1989, Sih et al. 1998), therefore understanding how multiple predator species, or predator communities, can work to alter prey behavior and decrease prey survival is critical to increase the efficiency of biological control tactics.

While single predator species may be effective at controlling pest populations, these outcomes may differ when present in predator communities. Multiple predator effects (MPEs) may be substitutive, risk-reducing, or risk-enhancing (Sih et al. 1998, Schmitz 2007, Grabowski et al. 2008, McCoy et al. 2012). Substitutive MPEs are observed when the risk to prey in the presence of multiple predators is similar to that of the average risk to prey by each predator alone (Sih et al. 1998, Schmitz 2007), while risk-reducing MPEs result in lowered prey mortality in the presence of multiple predators than a single predator, and risk-enhancing MPEs (synergistic effects) result in higher prey mortality in the presence of multiple predators (Sih et al. 1998, Schmitz 2007, Grabowski et al. 2008). These MPEs depend upon a variety of factors including predator and prey habitat domain, predator hunting mode, and intraguild predation. Habitat

domains include the microhabitat choice of a species as well as their movement within these microhabitats (Schmitz 2007, Miller et al. 2014). If a predator and prey species occupy the same habitat domain, predators can consume those prey species. If habitat domains of multiple predators do not overlap with each other, but do overlap with prey, we may see substitutive MPEs since predation in any one location is equal to that of a single predator (Schmitz 2007). However, when predator habitat domains overlap, risk-reducing or risk-enhancing MPEs may occur. Risk-reducing MPEs are most common when predator habitat domains overlap completely, resulting in interference among predator species and intraguild predation, making it easier for prey to avoid predation (Schmitz 2007, Grabowski et al. 2008). However, if predator habitat domains overlap, but prey are available in high numbers and in a narrow range or have limited mobility, multiple predators may have risk-enhancing effects, and any behavioral changes in prey in response to one predator may increase risk of predation by a second predator (Sih et al. 1998, Schmitz 2007).

While these predator effects on prey can reduce prey populations through direct consumption (consumptive effects), predators can also cause behavioral or physiological changes in prey when threatened by predation, known as predator non-consumptive effects (Werner and Peacor 2003, Priesser et al. 2005). While predator consumptive effects are often used as a measure of success in biological control, recent literature suggests that non-consumptive effects may have just as large or larger of an impact on prey species survival (Werner and Peacor 2003, Priesser et al. 2005, Siepielski et al. 2014), growth (Xiong et al. 2015), and fecundity (Peckarsky et al. 1993). Predator non-consumptive effects have been linked to a variety of predator and prey characteristics (Buchanan et al. 2017), that can be altered by both predator hunting mode and habitat domain (Preisser et al. 2007). However, most of our current understanding on non-

consumptive effects revolves around single prey – single predator pairs (Thaler et al. 2012, Hermann and Thaler 2014, Kaplan et al. 2014), not multiple predator communities.

While insect predators have been found to be effective at controlling pest species through consumptive and non-consumptive effects, habitat simplification has threatened the effectiveness of biological control overall by limiting natural enemy habitat in and around agricultural fields (Landis et al. 2000, Gurr et al. 2017). The ‘enemies hypothesis’ predicts that natural enemies will be more prevalent in habitats containing increased complexity due to higher prey availability, nectar sources, and microhabitats (Root 1973, Sheehan 1986, Björkman et al. 2010). Therefore, there has been a push for increased habitat management (altered vegetation) in and around agricultural fields in order to improve the availability of resources required by these insect predators (Landis et al. 2000). There has been consensus in the literature that increasing habitat complexity can increase predator abundance (Rypstra et al. 1999, Langelloto and Denno 2004, Bryant et al. 2014), as well as reduce intraguild predation (Finke and Denno 2002), and increase prey suppression (Finke and Denno 2002, Lundgren and Fergen 2010) by reducing predator interference. However, the impact of habitat management on predator non-consumptive effects is relatively unknown. Additionally, research on MPEs suggests that predator species identity within a predator community may play a more important role in prey suppression than overall predator diversity within that community (Straub and Snyder 2006), suggesting that enhancing landscapes to increase overall predator populations could negatively affect biological control if the predator assemblages do not include the ideal species identities for pest suppression. Therefore, more research is needed to better understand the relationship between habitat management practices and the non-consumptive effects of multiple predators.

In this study we aimed to determine the consumptive and non-consumptive effects of predator communities composed of known predators of the cabbage white, *Pieris rapae* L. (Lepidoptera: Pieridae), convergent ladybeetle, *Hippodamia convergens* Guérin-Ménéville (Coleoptera: Coccinellidae), spined soldier bug, *Podisus maculiventris* Say (Hemiptera: Pentatomidae), and wolf spiders, Lycosidae (Araneae) (Szendrei et al. 2014). We investigated how the consumptive and non-consumptive effects of these predators are altered by predator species richness or identity, and how habitat management practices such as cover crop mulches and nitrogen application may impact consumptive and non-consumptive effects. Through a series of laboratory studies we measured the impact of single and multiple predator species on *P. rapae* larvae, and determined how predator habitat domains change based on the composition of their communities. We also measured the impacts of two habitat management strategies, cover crop mulch and fertilization, on wild natural enemy populations, as well as single and multiple predator consumptive and non-consumptive effects to determine how these habitat management strategies influence predator-prey interactions.

Materials and Methods

Arthropod rearing and collection

Pieris rapae were reared in a greenhouse colony in continuous culture from 2015-2017 at Michigan State University, in East Lansing, Michigan. Insects originated from Michigan State University's Student Organic Farm (East Lansing, MI), and field caught adults were added to the colony in 2016 and 2017 to maintain genetic diversity. Larvae were fed Georgia collards (W. Atlee Burpee & Co., Warminster, PA) *ad libitum* and adults were fed a 20% sugar water solution. In this study, all *H. convergens* were acquired from Rincon-Vitova Insectaries (CA),

third instar *P. maculiventris* (Hemiptera: Pentatomidae) were obtained from a colony, and reared according to a standard protocol (Coudron et al. 2002), and female Lycosidae (Araneae) were collected from the Michigan State University's Arboretum. All Lycosidae were verified to family before use in experiments; multiple species were used due to low availability of a single species from collected individuals.

All *H. convergens* were stored in a refrigerator for up to two weeks before being used in experiments, and were fed a 20% honey water solution. Any *H. convergens* used in experiments were removed from the fridge 24-48 hrs prior, where they were kept in a room with 16:8 L:D and starved up until the experiment. All *P. maculiventris* nymphs were stored in an environmental chamber (16:8 L:D, 25 °C) in cups containing a wet cotton ball for water and a mealworm, *Tenebrio molitor*, for food until use in experiments. Lycosids were captured 24-48 hours before use, and were stored in cups with a wet cotton ball for water in a room with 16:8 L:D, and were starved until use in experiments.

Habitat domain bioassays

Predator habitat domains were determined using environmental chamber bioassays. Plastic tubes, as described in the environmental chamber bioassay experiment above (Figure 4.1), were placed around single 4-week-old collards, and either a single predator (one *H. convergens*, *P. maculiventris*, or lycosid), or a combination of two predators (one *H. convergens* and one *P. maculiventris*; one *H. convergens* and one lycosid; one *P. maculiventris* and one lycosid) were placed in the tube, for a total of 6 predator cages (3 single predator and 3 multiple predator). In this experiment we wanted to observe interactions between two predator species in order to better understand how species responded to one another. Due to this, as well as ease of

collecting observations, we did not include a cage with all three predator species present. Cages were replicated a total of 17 times over two separate dates (29 June, and 26 July 2017). Cages were observed every 6 hours, starting immediately after set-up, for 24 hours (1pm, 7pm, 1am, 7am, 1pm), and predator location in each cage was recorded. Observations made at 1am were made under red light, while all others were made under normal light conditions. Predator location was indicated by ‘zone’ (Figure 4.1): zone 1 – bottom 6 cm of tube including the soil surface and collard stem, zone 2 – middle 6 cm of cage including collard foliage, zone 3 – top 6 cm of cage including top of the collard foliage and top of the cage. If a predator died, we stopped recording their location for all subsequent observations. In habitat domain bioassays, an additive design was used (only one insect present in single-predator cages, and two insects present in two-predator cages) in order to better understand habitat domains of predators when alone, and how they may be altered in the presence of a different predator; if two predators were present in single predator cages, their behavior may have been modified.

To determine the effect of time (6-hour intervals) and treatment on predator location (habitat domain), we used a linear mixed effect model, using the ‘lmer’ function of the ‘lme4’ package, with time and treatment as fixed factors and block as a random factor. There was no significant effect of time on observed predator location, so we further analyzed data pooled across all time intervals. Differences in distribution of each predator across different habitat zones when a second species was present together in cages compared to when alone were calculated using a chi-square test. We summed the number of times a predator (*H. convergens*, *P. maculiventris*, or lycosids) was present in each zone over all time intervals when in cages alone, and used these counts as expected values. We then did the same for each predator when they were present in a cage with a different species (*H. convergens* with *P. maculiventris* or

lycosid; *P. maculiventris* with *H. convergens* or lycosid; lycosid with *H. convergens* or *P. maculiventris*), and compared these observed values to our expected values with a chi-square test in order to determine if predator habitat domains changed when in the presence of other predator species.

Environmental chamber bioassays

In order to observe effects of predator species combinations on *P. rapae* larvae survival and growth, an experiment was run in an environmental chamber (16:8 L:D, 27 °C). All bioassays used four-week old Georgia collards, which were grown individually from seed in perlite soil mixture (Suremix Perlite, Michigan Grower Products, Inc., Galesburg, MI) in 12 x 12 cm plastic pots. Tubes made from polyester plastic (11 cm diam., 21 cm tall; ACCO Brands, Inc., Apollo, Lincolnshire, IL) were placed around the collards and pushed 2-3 cm into the soil and covered with a lid to ensure insects did not escape (Figure 4.1).

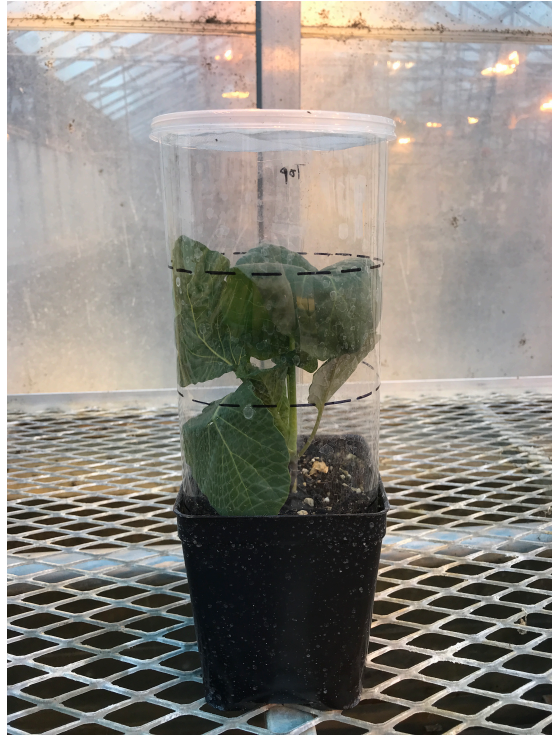


Figure 4.1. Environmental chamber bioassay and habitat domain bioassay cage. Polyester tubes with lids were placed around individual collards to contain insects. Zones were indicated by dashed lines on the tube, with zone 1 on the bottom, zone 2 in the middle, and zone 3 at the top of the cage.

Predators used in this experiment were *H. convergens*, *P. maculiventris*, and Lycosidae. The experiment consisted of 15 treatments – a predator-free control, a series of predator threats, and a series of predator present cages (Table 4.1). Threat and predator present treatments consisted of single predator species cages (each predator species alone), and multiple predator species cages (each combination of two predator species and all three species together).

Table 4.1. Environmental chamber bioassay treatments consisting of a predator-free control, a series of threat cages where predators were added 24 hours in advance and removed just before *P. rapae* larvae were added, and predator present cages where predators were present in cages with larvae. Numbers in parentheses represent the number of that predator species added to each cage.

Treatment	Presence	Predator
1	None	None
2	Threat	<i>H. convergens</i> (3)
3	Threat	<i>P. maculiventris</i> (3)
4	Threat	Lycosidae (3)
5	Threat	<i>H. convergens</i> (2) + <i>P. maculiventris</i> (1)
6	Threat	<i>H. convergens</i> (2) + Lycosidae (1)
7	Threat	<i>P. maculiventris</i> (2) + Lycosidae (1)
8	Threat	<i>H. convergens</i> (1) + <i>P. maculiventris</i> (1) + Lycosidae (1)
9	Present	<i>H. convergens</i> (3)
10	Present	<i>P. maculiventris</i> (3)
11	Present	Lycosidae (3)
12	Present	<i>H. convergens</i> (2) + <i>P. maculiventris</i> (1)
13	Present	<i>H. convergens</i> (2) + Lycosidae (1)
14	Present	<i>P. maculiventris</i> (2) + Lycosidae (1)
15	Present	<i>H. convergens</i> (1) + <i>P. maculiventris</i> (1) + Lycosidae (1)

In predator threat cages, predators were added to the cage 24 hours before larvae, and were removed right before larvae were placed in the cage so that only predator chemical cues were available to caterpillars. In predator present cages, all predators were present with larvae for the entire duration of the experiment. In this study we were more interested in observing predator species effects than predator density effects, so all single predator species and multiple predator species cages had three total predators present to create a substitutive design, and thus ensure that any effects on larval behavior were due to predator species identity instead of density (Sih et al. 1998, Griffen 2006). Numbers of each species in a cage (Table 4.1) were assigned based on predator availability as well as field predator abundance. Five *P. rapae* neonate larvae were added to each collard with a paintbrush, and left to feed for 48 hours, after which larvae remaining were counted, collected, and weighed. Remaining predators were also collected in

order to determine their survival. Treatments were arranged in a randomized complete block design, and each treatment was replicated 6 times.

Both *P. rapae* larval survival and weight in environmental chamber bioassays were analyzed using linear mixed effects models, using the ‘lmer’ function in the ‘lme4’ package (Bates et al. 2016), with predator treatment as a fixed factor, and block as a random factor. Treatment means were compared using ‘lsmeans’ with the false discover rate adjustment method. Predator community impact was analyzed using a model developed by Wootton (1997) to determine expected values of larval suppression by different communities of predators based on values observed when a single predator species was present, and compare this expected value to observed value of suppression by different multiple predator communities. Using the equation, $\ln[(N_{\text{control}} + 1) / (N_{\text{treatment}} + 1)]$, we determined the observed impact of each predator community, where N_{control} was the number of larvae surviving in predator-free control cages, and $N_{\text{treatment}}$ was the number of larvae surviving in predator present treatments. This value was calculated separately for each predator present cage for each replication. We then calculated expected value for each of the multiple predator species cages. To obtain expected values, we took the observed impact of single predator species cages divided by 3 (3 predators in each cage) to determine the impact of a single predator, and added these values together to find what impact is expected if individual predator impacts were additive. For example, to find the expected impact of our *H. convergens* + *P. maculiventris* cages, for each replication we divided the observed impact in *H. convergens* cages by 3, and divided the observed impact in *P. maculiventris* cages by 3, and added these new values together as such: 1 *H. convergens* + 1 *H. convergens* + 1 *P. maculiventris*. If observed values were higher than expected values, those predator combinations had a synergistic effect on predation, and observed values lower than expected indicate a

decreased level of control. We then performed a Mann-Whitney test for each multiple predator community to analyze differences in expected and observed predator community impacts on larval survival.

Field experiment

A field experiment was conducted in an experimental cabbage (*Brassica oleracea* var. “Farao”; Bejo Seeds, Inc., Oceana, CA) field at Michigan State University’s Horticulture Teaching and Research Center (Holt, MI) over two field seasons (2016 and 2017), to determine effects of cover crop mulch and plant nitrogen on predator community interactions with *P. rapae* larvae. Cabbages were grown from seed in a greenhouse (25/20 °C, 16/8 daylength) in 98 cell plug trays, and transplanted into the field when they were 4 weeks old (6 July 2016, 29 June 2017). Transplants were planted into a low nitrogen (no nitrogen added) and high nitrogen (organic fertilizer applied at 134 kg N/ha in two split applications; NatureSafe 10-2-8 and 13-0-0, Darling Ingredients, TX) plot, both replicated across four blocks. High nitrogen treatments received 67 kg N/ha just prior to transplanting, and an additional 67 kg N/ha at 29, or 26 days after transplanting in 2016 and 2017, respectively. Treatment plots measured 3 x 6 m, and contained 4 rows of cabbage with 76 cm between row spacing and 36 cm in-row spacing; each block was spaced 4.5 m apart.

Cages were set up in a randomized complete block design, and were replicated a total of 7 times over the 2016 season across 2 dates (set up 14 (3 reps) and 21 July (4 reps)), and replicated a total of 7 times over the 2017 season across 3 dates (set up 7 (2 reps), 14 (1 rep), and 21 July (4 reps)). Each cage was constructed to cover one cabbage plant (Figure 4.2); first, a black landscape fabric (Greenscapes Home & Garden Products, Inc., Calhoun, GA) was cut into

1 x 1 m pieces, a small hole was cut in the center to allow the cabbage to fit through, and fabric was placed on the soil around cabbages. Next, for structural support, two rebar (0.6 m length) were hammered into the soil at two corners of the landscape cloth, and PVC pipe (1 m length) was hammered into the soil over the rebar, and in the remaining two corners to stand roughly 0.6 m above the ground. Cage exteriors were constructed from white insect netting fabric (Hummert International, Earth City, MO), which were placed around the PVC pipes. The bottom of the fabric exterior was buried into the soil with the outer edges of the landscape fabric to seal the bottom of the cage, and the top of the exterior was tied closed to keep insects inside.



Figure 4.2. Field experiment cages with (a) 16 treatments set up in each block. Landscape fabric was placed on the soil around each cabbage, rebar was hammered into two corners, and PVC pipes were added over the rebar and in the remaining two corners to provide the cage structure. White mesh cages were placed over the PVC pipes, and the edges of the cages along with the landscape fabric was buried into the soil. Cages were tied shut on the top. (b) Each cage was constructed around a single field-grown cabbage plant.

There were 16 treatments in field trials across a combination of three factors: nitrogen application, cover crop mulch, and predator species (Table 4.2). Eight cages were placed in the

low nitrogen field treatment and eight in the high nitrogen treatment. Four of each low nitrogen and high nitrogen cage had mulch (wheat straw, ~ 30 g; Frosty Acres, Williamston, MI) added to the cage, which was placed on top of the landscape fabric surrounding the caged cabbage, and four of each were left with no mulch. Four predator combinations were replicated each in the no mulch and mulch cages in both low and high nitrogen treatments: no-predator control, *P.*

maculiventris predator present, Lycosidae predator present, and *P. maculiventris* with Lycosidae predator present.

Table 4.2. Treatment combinations used in field trials in an experimental cabbage field in East Lansing, MI. High nitrogen treatments used cabbages treated with blood meal while low nitrogen treatments had no fertilization. Treatments with mulch had straw added to the ground surrounding the base of the cabbage. Predator treatments either contained no predators, a single predator species, or a combination of two predator species. Numbers in parentheses represent how many of that predator were added to the cage; these numbers were consistent across all treatments.

Treatment	Nitrogen	Mulch	Predator
1	Low	None	None
2	Low	None	<i>P. maculiventris</i> (2)
3	Low	None	Lycosidae (2)
4	Low	None	<i>P. maculiventris</i> (1) + Lycosidae (1)
5	Low	Mulch	None
6	Low	Mulch	<i>P. maculiventris</i>
7	Low	Mulch	Lycosidae
8	Low	Mulch	<i>P. maculiventris</i> + Lycosidae
9	High	None	None
10	High	None	<i>P. maculiventris</i>
11	High	None	Lycosidae
12	High	None	<i>P. maculiventris</i> + Lycosidae
13	High	Mulch	None
14	High	Mulch	<i>P. maculiventris</i>
15	High	Mulch	Lycosidae
16	High	Mulch	<i>P. maculiventris</i> + Lycosidae

Hippodamia convergens were not used in the field experiment due to a restricted field size. Five neonate *P. rapae* were placed on each cabbage with a paint brush, and left for 72

hours, after which time the remaining larvae were counted, collected, and weighed. Cabbages were removed from the field when the experiment was completed for a nitrogen analysis.

A linear mixed effects model was used to determine if year had a significant effect on larval survival and weight in field experiments. Year was determined not to have a significant impact, so data was combined from both years for analysis. Both *P. rapae* larval survival and weight were analyzed using a linear mixed effects model, using the ‘lmer’ function in the ‘lme4’ package, with predator treatment x mulch x nitrogen as fixed factors, and block and year as random factors.

Wild natural enemy surveys

Natural enemies were observed in a field setting in five treatments: no mulch, no mulch + nitrogen, rye cover crop, rye cover crop + nitrogen, and rye cover crop + vetch. Natural enemies were observed within a 1 m² area in each plot once a week for 8 weeks in 2017. In each plot a 1 x 1 meter quadrat constructed from PVC pipe was placed over three cabbages, so that both the in-row and between-row areas were included, and all plant material and soil surfaces within the quadrat were searched for natural enemies. Each natural enemy observed was recorded to order or family.

Counts of natural enemy abundance were summed across weeks, and communities were analyzed in response to field treatment using non-metric multidimensional scaling (NMDS, function = ‘adonis’, package = ‘vegan’). Overall difference in the total numbers of an individual natural enemy across each field treatment were analyzed using a chi-square test, and individual differences between field treatments were determined using a Dunn’s test (package = “dunn.test”). All statistics were performed using R version 3.3.2 (R Core Team 2017; $\alpha = 0.05$).

Results

Habitat domain bioassays

There were no significant differences across the 6 hour time intervals for the location of predators in habitat domains ($F_{4,650} = 1.17$, $P = 0.32$), but predator treatment had a significant effect on their distribution ($F_{8,650} = 50.85$, $P < 0.01$), so impact of predator treatment on predator location was analyzed with data pooled across all time intervals. Across all time intervals in cages only containing *H. convergens*, 36% of observed *H. convergens* were in zone 1, 12% in zone 2, and 52% in zone 3 (Figure 4.3a), and *H. convergens* were observed walking 22% of the time and not moving the other 78% of the time. Zone distribution was not affected by the addition of *P. maculiventris* in cages with *H. convergens* ($X^2 = 1.17$, d.f. = 2, $P = 0.56$; Figure 4.3a), with 38% observed in zone 1, 8% in zone 2, and 54% in zone 3. When *P. maculiventris* were present, *H. convergens* was observed walking in 18% and not moving in 82% of observations. However, we observed a significant change in distribution of *H. convergens* with the addition of lycosids ($X^2 = 12.53$, d.f. = 2, $P < 0.01$; Figure 4.3a): *H. convergens* shifted higher in the cage with 30% of *H. convergens* observed in zone 1, 1% in zone 2, and 69% in zone 3. With lycosids present, *H. convergens* also reduced movement, and were found walking in only 9% of observations, and not moving in the other 91%.

When in cages alone, *P. maculiventris* were observed in the lower zone 1 during 57% of the time, 15% of the time in zone 2, and 28% of the time in zone 3 across all time intervals (Figure 4.3b), and were observed walking 27% of the time and not moving 73% of the time. When *H. convergens* were also present in the cage, this pattern was very similar ($X^2 = 0.19$, d.f. = 2, $P = 0.90$; Figure 4.3b), with *P. maculiventris* observed in zone 1 55% of the time, 16% of times in zone 2, and 28% of times in zone 3. When *H. convergens* were present, *P. maculiventris*

were observed walking in 16% and not moving in 84% of observations. However, when lycosids were present in cages with *P. maculiventris*, we observed a habitat domain shift towards lower zones ($X^2 = 20.43$, d.f. = 2, $P < 0.01$; Figure 4.3b), with *P. maculiventris* observed in zone 1 56% of the time, in zone 2 32% of the time, and in zone 3 only 12% of the time, and observed *P. maculiventris* walking in only 9% of observations, and not moving in 91%.

In Lycosidae cages, when alone all lycosids were observed in zone 1 (Figure 4.3c), and were observed walking 6% of the time and not moving 94% of the time. When present with *H. convergens*, 1 lycosid was observed in zone 2 and all others in zone 1, and when present with *P. maculiventris*, 1 lycosid was also observed in zone 2, and all the other in zone 1 (Figure 4.3c). When present with *H. convergens*, lycosids were observed walking in 13% and not moving in 87% of observations, and when with *P. maculiventris* were observed walking in 10% and not moving in the other 90% of observations. Due to lack of variation in the data, we were not able to statistically analyze zone differences in Lycosidae cages.

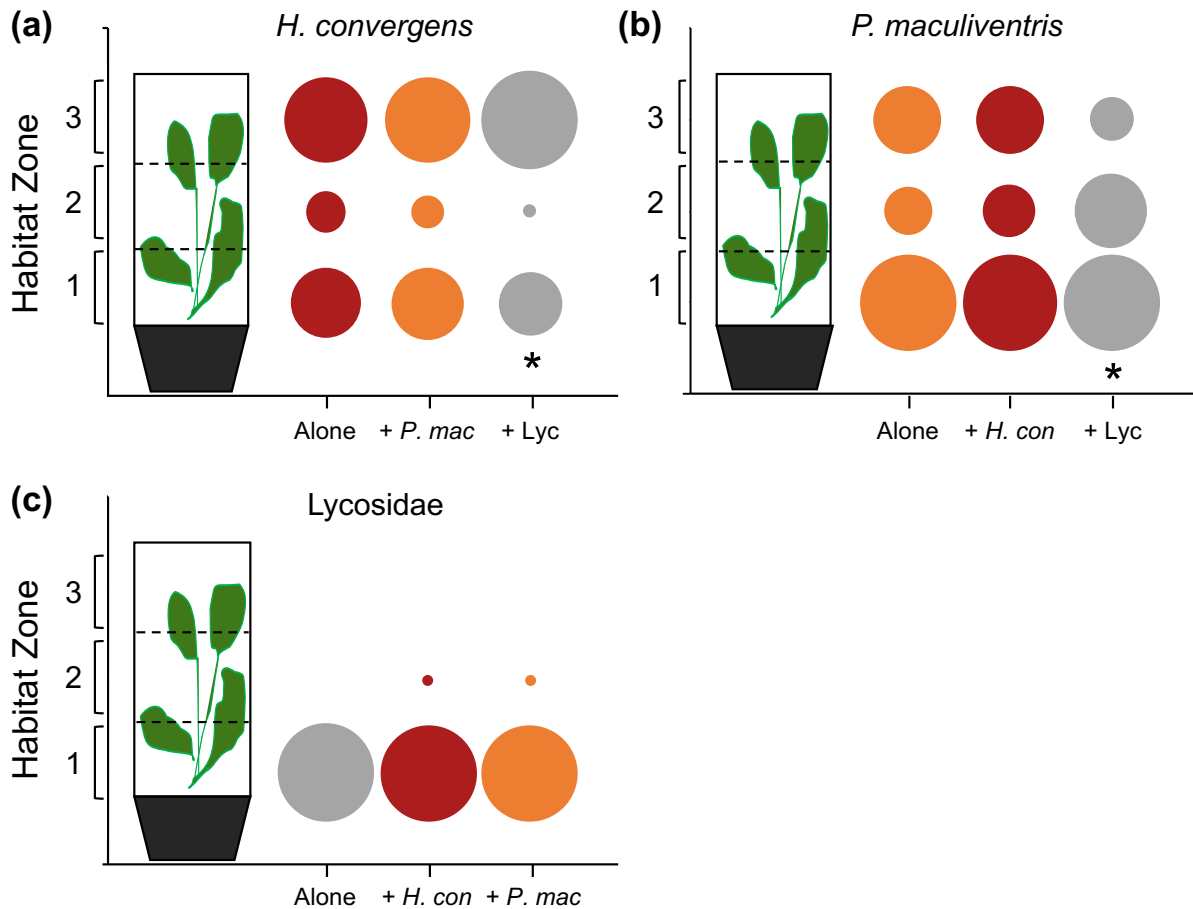


Figure 4.3. Proportions of observations of each predator [(a) *H. convergens*, (b) *P. maculiventris*, (c) Lycosidae] in different habitat zones across all time intervals. Predators were either alone in cages ('Alone'), or present with another predator ('+ *H. con*': with *H. convergens*; '+ *P. mac*': with *P. maculiventris*; '+ Lyc': with Lycosidae). Large circles represent larger proportions of observations and small circles represent smaller proportions. Significant differences in distribution when a second predator was present in the cage compared to when insects were alone in cages is indicated with an asterisk (Chi-square test; $\alpha = 0.05$).

Environmental chamber bioassays

In bioassays, *H. convergens* mortality was 11% in *H. convergens* only cages, 25% in *H. convergens* + *P. maculiventris* cages, and 33% in both *H. convergens* + Lycosidae and three predator species cages. *Podisus maculiventris* experienced 5% mortality in *P. maculiventris* only cages, 16% in *P. maculiventris* + *H. convergens* cages, 67% in *P. maculiventris* + Lycosidae

cages, and 100% mortality in three predator species cages. Lycosids experienced 45% mortality in Lycosidae only cages, and no mortality in any other predator combinations.

Predator treatment significantly affected *P. rapae* larval survival ($F_{14,70} = 6.94$, $P < 0.01$; Figure 4.4). Compared to controls, predator threat cages did not impact larval survival ($t \leq 1.84$, d.f. = 70, $P \geq 0.13$); however, many predator present cages decreased larval survival, with roughly a 70% decrease in *H. convergens* ($t = 3.46$, d.f. = 70, $P < 0.01$), *P. maculiventris* ($t = 3.23$, d.f. = 70), and *H. convergens* + Lycosidae cages ($t = 3.46$, d.f. = 70, $P < 0.01$), and an 80% decrease in *H. convergens* + *P. maculiventris* cages ($t = 3.91$, d.f. = 70, $P < 0.01$) compared to control cages without predators. While other predator present cages had no significant impact on larval survival, we observed a 14 % decrease in Lycosidae cages ($t = 0.69$, d.f. = 70, $P = 0.59$), 28 % decrease in *P. maculiventris* + Lycosidae cages ($t = 1.38$, d.f. = 70, $P = 0.25$), and a 38 % decrease in larval survival in *H. convergens* + *P. maculiventris* + Lycosidae cages ($t = 1.84$, d.f. = 70, $P = 0.13$) compared to predator-free controls. While there was an effect of predator treatment on larval survival, there was no impact on larval weight ($F_{14,234} = 1.38$, $P = 0.17$; Figure 4.5).

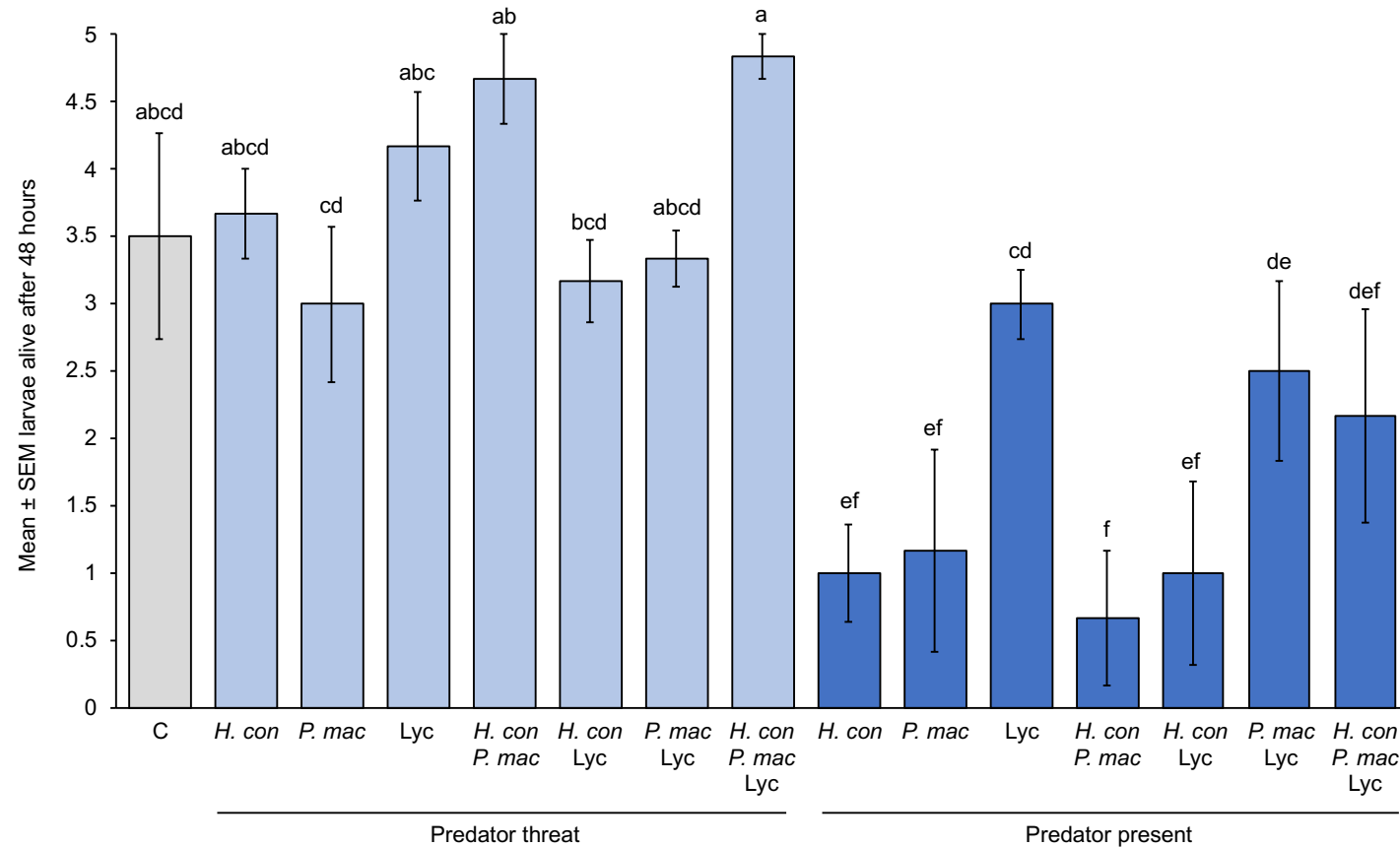


Figure 4.4. Mean $\pm$ SEM number of *P. rapae* larvae alive after 48 hours for each predator treatment. Predator cages are indicated as ‘C’: no-predator control, ‘H. con’: *H. convergens*, ‘P. mac’: *P. maculiventris*, and ‘Lyc’: Lycosidae, and marked as ‘predator threat’ or ‘predator present’ cages. Predator threat cages had predators added 24 hours before adding 5 *P. rapae* first instars and removed at time of adding the caterpillar larvae, while predator present cages had predators added when caterpillars larvae were added and were present with larvae during the entire 48 hour experimental period. Each cage contained 3 total predators: in single species cages there were 3 of one species, in cages with two species there were 2 of the first species listed and 1 of the second, and in cages with all three species there was 1 of each predator in each cage. Analysis was run across all treatments, and differences between treatments are indicated by different letters (Tukey’s HSD; $\alpha = 0.05$).

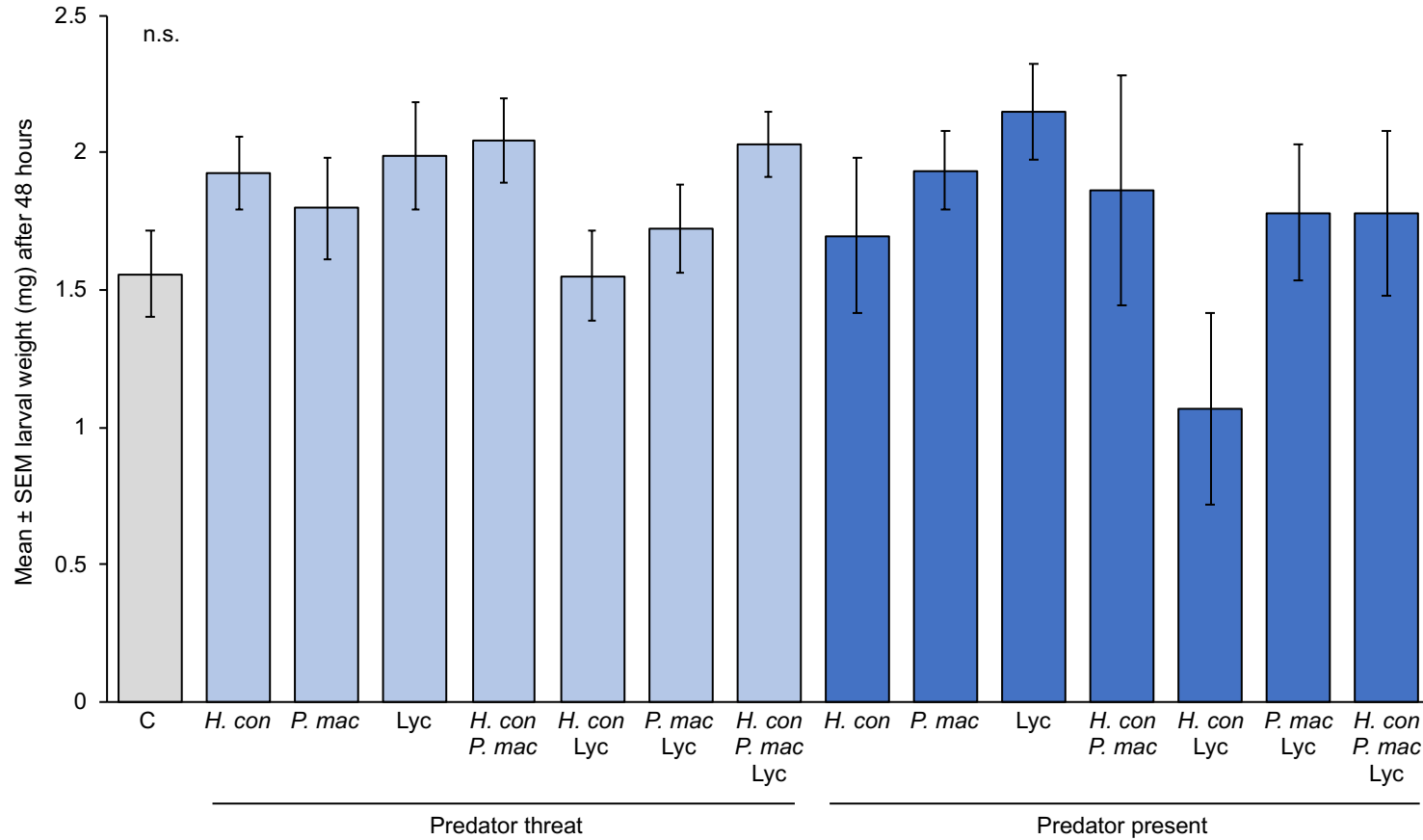


Figure 4.5. Mean $\pm$ SEM weight (mg) of *P. rapae* larvae after 48 hours for each predator treatment. Predator cages are indicated as ‘C’: no-predator control, ‘H. con’: *H. convergens*, ‘P. mac’: *P. maculiventris*, and ‘Lyc’: Lycosidae, and marked as ‘predator threat’ or ‘predator present’ cages. Predator threat cages had predators added 24 hours before adding 5 *P. rapae* first instars and removed at time of adding the caterpillar larvae, while predator present cages had predators added when caterpillars larvae were added and were present with larvae during the entire 48 hour experimental period. Each cage contained 3 total predators: in single species cages there were 3 of one species, in cages with two species there were 2 of the first species listed and 1 of the second, and in cages with all three species there was 1 of each predator in each cage. Analysis was run across all treatments (lmer; $\alpha = 0.05$).

Observed predator community impact was not significantly different than our calculated community impact for *H. convergens* + *P. maculiventris* ($W = 21.5$, $P = 0.63$; Figure 4.6), *H. convergens* + Lycosidae ($W = 24$, $P = 0.38$; Figure 4.6), *P. maculiventris* + Lycosidae ($W = 15$, $P = 0.69$; Figure 4.6), or *H. convergens* + *P. maculiventris* + Lycosidae ($W = 15$, $P = 0.70$; Figure 4.6).

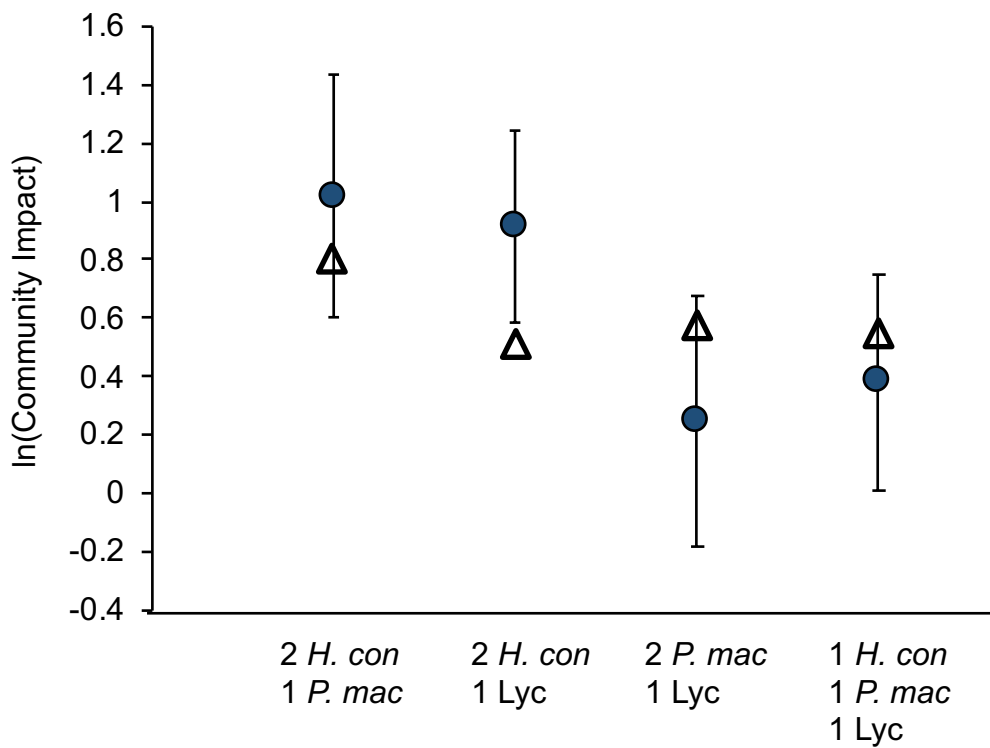


Figure 4.6. Per capita impact of predator communities on *P. rapae* larval survival. Observed values (circles) for each predator combination were determined using the equation $\ln[(N_{\text{control}} + 1) / (N_{\text{treatment}} + 1)]$ (Wootton 1997), where N_{control} was *P. rapae* survival in control cages, and $N_{\text{treatment}}$ was *P. rapae* survival in each predator cage. Expected values (triangles) were calculated using observed values from the equation for individual predator species cages, and determining expected values for an additive design. If observed values are higher than expected values, those predator combinations had a synergistic effect on predation, and observed values lower than expected indicate a decreased level of control.

Field experiment

Year did not have a significant impact on larval survival ($F_{1,12} = 0.55$, $P = 0.47$) or larval weight ($F_{1,12} = 1.69$, $P = 0.13$), so data from both years was combined for analysis. In our field trials we found no effect of plant nitrogen, mulch, or predator treatment on larval survival (nitrogen: $F_{1,195} = 3.28$, $P = 0.07$; mulch: $F_{1,195} = 0.58$, $P = 0.45$; predator treatment: $F_{3,195} = 0.82$, $P = 0.49$) or larval weight (nitrogen: $F_{1,614} = 0.19$, $P = 0.66$; mulch: $F_{1,614} = 0.41$, $P = 0.52$; predator treatment: $F_{3,614} = 0.37$, $P = 0.77$).

Wild natural enemy analysis

In all field treatments across all weeks, we observed a total of 94 ladybeetles (Coleoptera: Coccinellidae), 190 damsel bugs (Hemiptera: Nabidae), 20 parasitoid wasps (Hymenoptera), 411 ground beetles (Coleoptera: Carabidae), and 218 spiders (Araneae).

Field treatment did not significantly impact overall natural enemy community composition ($F_{4,35} = 1.05$, $R^2 = 0.11$, $P = 0.42$, Stress = 0.2). However, we observed differences in total numbers of natural enemy groups across the different field treatments. Field treatment significantly impacted ladybeetles ($X^2 = 51.64$, d.f. = 4, $P < 0.01$; Figure 4.7a), with 48% of observed ladybeetles in plots without mulch + N, compared to 19% in plots without mulch and no nitrogen ($z = 1.30$, $P = 0.10$), 18% in plots with rye + N ($z = 1.3$, $P = 0.10$), 9% in plots with rye + vetch ($z = 2.11$, $P = 0.02$), and 6% in plots with rye only ($z = 2.54$, $P = 0.01$). Field treatment did not significantly impact number of damsel bugs ($X^2 = 8.05$, d.f. = 4, $P = 0.09$; Figure 4.7b), or parasitoid wasps ($X^2 = 6.00$, d.f. = 4, $P = 0.20$; Figure 4.7c), but had a significant impact on the number of ground beetles ($X^2 = 41.03$, d.f. = 4, $P < 0.01$; Figure 4.7d), with 26% of beetles found in plots with rye mulch without N and also in rye mulch + N, compared to 24%

in plots with mulch + vetch ($z = 0.18$, $P = 0.43$), 14% in plots with no mulch + N ($z = 1.82$, $P = 0.03$), and 11% in plots without mulch or N ($z = 2.45$, $P = 0.01$). We also found a significant impact of field treatment on spiders ($X^2 = 24.52$, d.f. = 4, $P < 0.01$; Figure 4.7e), with 27% of observed spiders found in plots with rye mulch + vetch compared to 26% in plots with mulch + N ($z = 0.18$, $P = 0.43$), 22% in plots with rye mulch only ($z = 0.15$, $P = 0.44$), 17% in plots with no mulch + N ($z = 1.64$, $P = 0.05$), and 9% in plots with no mulch or N ($z = 2.27$, $P = 0.01$).

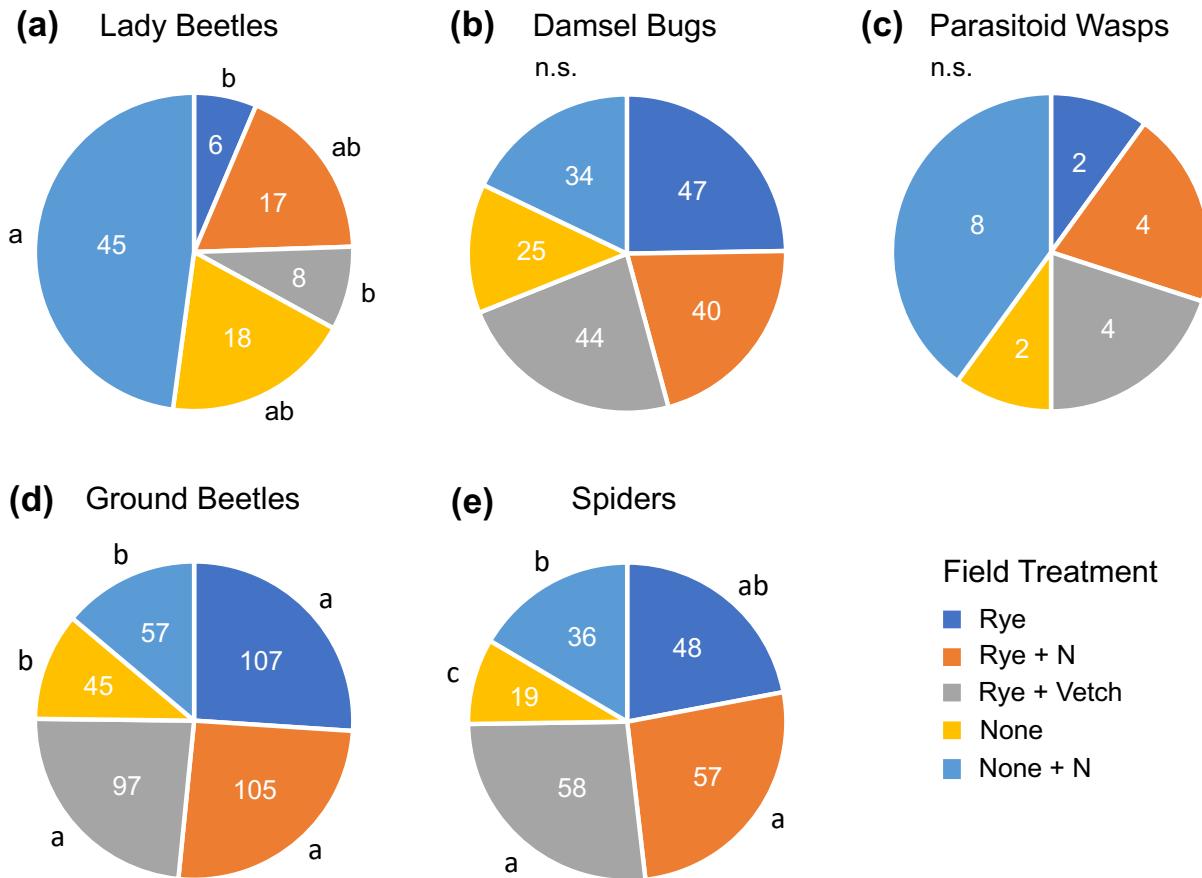


Figure 4.7. Total number of natural enemies [(a) lady beetles, (b) damsel bugs, (c) parasitoid wasps, (d) ground beetles, (e) spiders] found in five different treatments in an experimental cabbage field. Rye mulch (dark blue), rye mulch + nitrogen (N) (orange), rye mulch + vetch (grey), no mulch (yellow), and no mulch + N (light blue). Numbers in pie-charts represent the total number of the respective natural enemy found in each treatment. Letters represent differences among treatments, and “n.s.” represents no significant differences among treatments (Dunn’s Test; $\alpha = 0.05$)

Discussion

In this study, we found that predator species identity within communities has a stronger effect on MPEs than overall predator species richness. In our environmental chamber bioassays, cages with three predator species did not consume as many larvae as some cages with only two or one predator species. Both *H. convergens* and *P. maculiventris* alone, as well as *H. convergens* + *P. maculiventris* cages had increased consumption (though not significant) compared to cages with all three predators, and a significant increase in consumption compared to control cages. However, when lycosids were added in single predator Lycosidae cages, Lycosidae + *P. maculiventris* cages, and cages with all three predator species present, consumption of *P. rapae* decreased and was no longer significantly different than control cages. This suggests that wolf spiders negatively impact biological control of *P. rapae*. However, in cages containing both Lycosidae + *H. convergens* there was a significant increase in *P. rapae* consumption compared to control cages. *Hippodamia convergens* shifted their habitat domain towards higher locations in the cage away from wolf spiders that occupied the lower part of the cages. It is possible that since *H. convergens* in these cages were spending an increased proportion of their time in the canopy away from lycosids, they were able to avoid intraguild predation and have a greater effect on *P. rapae* consumption. In cages with *P. maculiventris* + Lycosidae, *P. maculiventris* shifted habitat domains and spent a larger proportion of their time in lower areas of the cage, increasing their risk of being consumed by lycosids. This was observed with 66% *P. maculiventris* mortality in *P. maculiventris* + Lycosidae cages compared to only 5% mortality in *P. maculiventris* only cages. In habitat domain bioassays, *P. maculiventris* were observed walking only 9% of the time (not moving in the other 91% of observations) when present with lycosids compared to walking in 16% and 27% of observations when with *H.*

convergens or alone, respectively. While *P. maculiventris* behavior was only recorded in habitat domain bioassays, it is possible that this reduction in activity was also occurring in environmental chamber bioassays, resulting in reduced hunting. *Podisus maculiventris* were experiencing higher mortality and reduced mobility in cages with wolf spiders, which led to a decrease in *P. rapae* consumption compared to when alone or with *H. convergens*. When all three predators were present in a cage, there was a decrease in *P. rapae* larval survival compared to controls, but this was not significant. In three predator species cages, 100% of *P. maculiventris* and 33% of *H. convergens* experienced mortality, in part due to consumption by lycosids, suggesting that intraguild predation by wolf spiders decreased overall predator numbers and diminished *P. rapae* biocontrol.

Although none of the per capita impacts of these multiple predator assemblages were significantly different than the expected values, the observed value of both *H. convergens* + *P. maculiventris* and *H. convergens* + Lycosidae fell above the expected values for these assemblages, while the observed value for both *P. maculiventris* + Lycosidae and the three predator species assemblage fell below their expected values. Since both *H. convergens* and *P. maculiventris* had significant control on *P. rapae* when alone, and even higher control when together, this suggests that these two species have a minor risk-enhancing effect on *P. rapae* survival when present together. In *H. convergens* + Lycosidae cages, *H. convergens* altered their behavior to remain in the plant canopy a larger proportion of the time, and therefore likely came into contact with *P. rapae* more frequently. It is unlikely that lycosids were frequently feeding on *P. rapae*, since when alone they did not significantly impact *P. rapae* survival compared to control cages, but changed *H. convergens* behavior which subsequently led to higher *P. rapae* consumption than expected. Intraguild predation likely contributed to reduced observed values in

P. maculiventris + Lycosidae and three predator species cages compared to their expected values. In both of these predator assemblages, there was high mortality of *P. maculiventris* due to intraguild predation by lycosids. Since *P. maculiventris* were consumed by the spiders and not actively feeding on *P. rapae*, this likely resulted in risk-reducing MPEs on *P. rapae*.

Although there were significant consumptive effects of our predator communities on *P. rapae*, we did not observe any significant non-consumptive effects. Nevertheless, in most predator threat and predator present cages we observed an increase (9 - 40%) in *P. rapae* weight compared to control cages where no predator cues were present. This suggests that the presence of these predator cues (chemical cues only in threat cages, visual and chemical in predator present cages) has a slight, though not significant, influence on *P. rapae* weight (Chapter 2). *Pieris rapae* larvae weighed the most overall in cages with wolf spiders (40% increase compared to control cages). While consumption of *P. rapae* larvae in Lycosidae cages was not different than no-predator controls, we observed 44% lycosid mortality in these cages. The lycosids were voracious, especially when in cages with other lycosids, and likely emitted some chemical cues when consuming one another (Persons et al. 2001). It is possible that these cues resulted in non-consumptive effects on *P. rapae*, causing them to grow larger either through physiological changes or behavioral changes such as increased leaf consumption. The exception to increased weight in our threat and predator present treatments was in the *H. convergens* + Lycosidae threat and predator present cages. *Pieris rapae* in the *H. convergens* + Lycosidae threat cages weighed the same as no-predator control cages, and larvae in *H. convergens* + Lycosidae cages weighed 31% less compared to no-predator controls. In habitat domain bioassays, *H. convergens* altered their distribution in cages by moving towards the top of the cage away from lycosids. In addition, *H. convergens* were observed walking around only 9% and not moving 91% of the time in

habitat domain bioassays when present with lycosids compared to walking 22% and not moving 78% of the time when other predator species were not present. While we did not measure predator location or movement in the environmental chamber bioassays, it is possible that *H. convergens* exhibited similar behavior in these cages. It is possible that in the *H. convergens* + Lycosidae threat cages, *H. convergens* had reduced movement and spent less time actively hunting, leaving fewer cues on the collard leaves, resulting in less response from the larvae. In *H. convergens* + Lycosidae predator present cages, since there was significant consumption of *P. rapae* larvae it is likely that *H. convergens* were present and hunting on the collards. However, it is possible that *H. convergens* still had reduced movement, and instead of more continuous hunting, spent more time stationary on collard leaves. If this were the case, *H. convergens* may have been stationary on leaves near *P. rapae* for longer periods of time than other predator cages, and larvae may have changed their behavior to feed less and become more inconspicuous while in the presence of *H. convergens*. However, since we did not observe predator activity over time in these cages, it is unclear why larvae responded differently in *H. convergens* + Lycosidae cages compared to other predator assemblages. Additionally, our observations of predator habitat domains and activity constituted only a snapshot of their behavior over a 24 hour time period.

While the literature suggests that increased habitat complexity can lead to decreased intraguild predation (Finke and Denno 2002) and increased prey suppression (Warfe and Barmuta 2004, Grabowski et al. 2008, Sanders et al. 2008), we found no effect of cover crop mulch or plant nitrogen on *P. rapae* larval consumption or weight in our field experiments. This may be due to the fact that our experiments were set up in cages where we manipulated predator communities instead of allowing natural enemies to colonize the plants. It is possible that if we relied on natural predator communities we may have observed different results. Additionally,

cold and variable weather over the length of the experiment may have resulted in reduced insect activity, and reduced *P. rapae* consumption or growth. We also found no impact of mulch or plant nitrogen combinations on predator community diversity, which has been observed in other studies (Lundgren and Fergen 2010). However, we observed an impact of different mulch and nitrogen treatments on wild natural enemy populations.

The majority of observed natural enemies were found in field plots containing a cover crop mulch (ground beetles, spiders, damsel bugs (n.s.)), although lady beetles preferred plots without any cover crop mulch but with high plant nitrogen (about 50% of observed lady beetles). However, when there was no cover crop mulch and no added plant nitrogen, lady beetles were observed the same number of times as in plots containing cover crop mulch with plant nitrogen (both 19% of observed lady beetles), suggesting that added plant nitrogen influences lady beetles. Parasitoid wasps were observed most often (8 times) in field settings with no cover crop mulch but with high plant nitrogen levels, and observed the least (2 times) in plots with no plant nitrogen regardless of cover crop mulch. However, these differences were not significant, likely due to a low number of observations. This suggests that different habitat management techniques can influence natural enemies differently, and that since predator identity has a strong effect on MPEs, understanding how different predators respond to different habitat management techniques could aid in structuring a field to achieve optimum pest management levels. For example, in this study lycosids reduced biocontrol of *P. rapae* while *H. convergens* provided good biocontrol. Therefore, according to this study, if trying to increase predators to control *P. rapae* larvae, it could be beneficial to have fields without cover crop mulches and with added plant nitrogen, as this will support higher numbers of lady beetles and lower numbers of spiders. However, optimal nutrient and cover crop management may change depending on the prey

species that needs to be controlled, and is therefore likely specific to individual study systems (Björkman et al. 2010).

These results support a growing understanding that species identity plays an important role in multiple predator assemblages and pest suppression (Finke and Denno 2002, Straub and Snyder 2006, Woodcock and Heard 2011, Long and Finke 2014). In addition, multiple studies have found a significant impact of lady beetles on prey suppression (Straub and Snyder 2006, Long and Finke 2014) which was also observed in this study. While both of these previous studies were observing impacts on aphid suppression, we found that in our system *H. convergens* led to greater pest suppression across predator assemblages than *P. maculiventris* or lycosids, likely due to behavioral changes of *H. convergens* when threatened by intraguild predators resulting in increased prey suppression by *H. convergens*. While we did not detect an effect of cover crop mulch or plant nitrogen on predator consumptive or non-consumptive effects in caged bioassays, we did find an impact on natural enemy abundance in field plots. Therefore, it may be possible to enhance pest management in a field setting with an understanding of predator species identity effects on predator-prey interactions, and associated habitat management preferences of these key predator species.

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CHAPTER 5: Conclusions and future directions

In this dissertation, I have investigated how on-farm habitat management practices (cover crop mulches and nitrogen addition) affect *Pieris rapae* host choice preference and performance, as well as biological control of *P. rapae* via single and multiple predator communities. Our general understanding of predator consumptive and non-consumptive effects has grown in recent years (Priesser et al. 2007, Buchanan et al. 2017, Ingerslew and Finke 2018), although there has been a gap in our understanding of how habitat management practices may influence these predator effects. The research presented in this dissertation has started to fill that gap, and provides implications for cole crop growers who may want to utilize these strategies to aid in pest management of *P. rapae*.

In Chapter 2, my research focused on determining a hierarchy of long- and short-distance cues used for *P. rapae* host plant selection in order to better understand which habitat, host plant, or predator cues are most influential on oviposition. Much of the current literature on insect oviposition has focused on responses to single cues (Minkenberg and Ottenheim 1990, Laymen and Lundgren 2015) or host species identity (Courtney et al. 1989), although, while under natural circumstances, insects encounter and evaluate many cues during host plant selection. This research demonstrated that host plant selection is complex, and *P. rapae* evaluate many cues when selecting a host plant. Additionally, I determined that *P. rapae* response to a single cue can be altered when present with other cues, suggesting that specific cues have a higher level of influence on host plant choice than others. Specifically, in this research, long-distance cues (habitat structure and plant size) were assessed before short-distance and contact cues (plant nitrogen and predator cues), with plant size having the largest influence on *P. rapae*. Long- and

short-distance cues also had synergistic effects when together in certain combination, with large sized plants containing high nitrogen levels accruing more eggs than either cue alone.

These results have interesting implications for pest management of *P. rapae*. During host plant finding, *P. rapae* did not respond to predator cues, but did show large responses to habitat and plant cues, suggesting that within-field habitat management could be an effective strategy for deterring *P. rapae* adults from ovipositing on a cash crop. This could include using cover crop mulches in fields, or utilizing trap crops to draw *P. rapae* adults away from the cash crop. Past studies have explored using trap crops or intercrops of different species or cultivars on *P. rapae* (Theunissen and Den Ouden 1980, Latheef and Oritz 1983, Bender et al. 1999, Jankowska et al. 2009), but with mixed results. However, there has been relatively little exploration of using a trap crop that is planted prior to a cash crop, and therefore larger in size. This could be studied in the future, as my research suggests that large sized plants have the greatest impact on oviposition in *P. rapae*, and therefore a trap crop that is older and larger in size than a cash crop may provide good management of this pest.

In Chapter 3, my research focused on the consumptive and non-consumptive effects of two different predator species on *P. rapae* adults and larvae. This research determined that both predator (top-down) factors and host plant (bottom-up) factors impact predator-prey interactions, but that predator species identity drives how *P. rapae* responds to bottom-up factors. Most of our current understanding of predator consumptive and non-consumptive effects on pest species arises from studies observing these effects from a single predator species (Trussell et al. 2006, Thaler et al. 2012, Kaplan et al. 2014, Xiong et al. 2015). Therefore, the research in this chapter is novel in that it provides a deeper understanding of these predator effects by observing the

impacts of multiple predator species, and proving that pest species do not respond in the same way to threats by all predator species.

This research opens a door for more studies observing non-consumptive effects of different predator species on prey. My research observed differences between predators of differing life stages of *P. rapae*, but more research should be conducted in order to better understand differences in prey response to different predator species. In addition, this research provides implications for pest management. As I found in this chapter, *P. rapae* responded differently to predation threat as well as bottom-up factors depending on the predator species. This implies that it may be more beneficial to manage a field to enhance specific natural enemy presence to obtain a greater level of pest management. However, in field settings, pest species often encounter many different predator species (Sih et al. 1998), therefore my remaining research observed the impacts of multiple predator communities on *P. rapae*.

In Chapter 4, I focused on single predator and multiple predator community consumptive and non-consumptive effects on *P. rapae* larvae, predator species habitat domains, as well as the impact of habitat management on these predator effects and on predator abundance. This research provides insight into how different in-field habitat management practices can influence predator communities, and how well different predator communities can control *P. rapae* survival and impact behavior. Through this, I determined that predator species identity is a driving factor in *P. rapae* pest management over species richness. Some predator species will change their behavior in the presence of other predator species, making certain predator combinations less successful at controlling *P. rapae*. Therefore, specific predator community composition is needed to obtain the highest levels of control for this pest.

In this chapter, I also found that while habitat management in the form of cover crop mulches and nitrogen addition did not impact predator consumptive or non-consumptive effects on *P. rapae*, it did affect abundance of many predator species. This is important, because as the research in both Chapter 3 and 4 shows, predator species identity is the most important factor for control of *P. rapae* larvae, and therefore understanding what management strategies attract species that are beneficial for control of this pest can help increase pest population regulation in a field setting. This research can be enhanced with further studies addressing the impacts of different management strategies on different predator communities and their consumptive and non-consumptive effects on different pest species.

Overall, this dissertation was the first to explore the impacts of cover crop mulches and nitrogen on predator consumptive and non-consumptive effects, and provides many insights into pest management of *P. rapae*. According to my research in these chapters, host plant size has the largest impact on *P. rapae* adults while egg-laying, suggesting that utilizing a trap crop might be an effective early management strategy. For control of larval life stages, *H. convergens* and *P. maculiventris* had a synergistic effect on pest management of *P. rapae*, and in the field a higher number of coccinellids were found in plots without cover crop mulches and with added nitrogen, while a different Hemipteran, damsel bugs, showed no preference for field management strategy. Additionally, wolf spiders negatively impacted biological control of *P. rapae*, and spiders overall were found more often in field plots containing cover crop mulches compared to those without. Therefore, fields without cover crop mulches may actually be more beneficial for biological control of *P. rapae* as they draw in higher numbers of coccinellid beetles and lower numbers of spiders. Overall, future research in this field should further explore the impacts of habitat management on consumptive and non-consumptive effects of predators on prey species. The

research in this dissertation was performed using caged bioassays, and can be enhanced in the future through experiments observing these interactions in more natural field settings.

Additionally, observing these interactions between numerous predators and prey species will help improve our understanding of predator non-consumptive effects.

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: 2019-01

Author: Margaret Lund

Title of thesis: Habitat management and biological control influence *Pieris rapae* (Lepidoptera: Pieridae) host plant choice and performance

Museum(s) where deposited:

Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

<u>Family</u>	<u>Genus-Species</u>	<u>Life Stage</u>	<u>Quantity</u>	<u>Preservation</u>
Pieridae	<i>Pieris rapae</i>	adult	10	pinned
Pieridae	<i>Pieris rapae</i>	larval	20	in alcohol
Coccinellidae	<i>Hippodamia convergens</i>	adult	20	pinned
Pentatomidae	<i>Podisus maculiventris</i>	nymph	11	in alcohol
Lycosidae		adult	10	in alcohol
Nabidae		adult	3	pinned
Carabidae		adult	2	pinned

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