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Oecologia

ISSN 0029-8549

Oecologia

DOI 10.1007/s00442-020-04686-w



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Predation threat modifies *Pieris rapae* performance and response to host plant quality

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Abstract

Predators impact prey populations through both consumptive and non-consumptive effects, such as behavioral and physiological changes by prey in response to a predation threat. Additionally, various top-down (e.g. predator characteristics) and bottom-up factors (e.g. plant nutrients) may impact non-consumptive effects, yet little is understood about how these interact. We studied how host-plant choice, leaf consumption, and growth of an herbivore, *Pieris rapae*, were impacted by different levels of plant nitrogen (N) and two predator species representing varying degrees of threat, *Hippodamia convergens* (predator of early-instars) and *Podisus maculiventris* (predator of all-instars). We found that *P. rapae* adults and larvae made similar choices about bottom-up and top-down factors when threatened by two different predator species. Adults and larvae preferred high N plants when threatened by *H. convergens*, but plant N did not influence their host plant choice when threatened by *P. 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. These results indicate that top-down factors such as predator identity may determine how *P. rapae* modulate their responses to bottom-up factors such as host plant quality when utilizing anti-predator behaviors.

Keywords Non-consumptive effects · Predator–prey interactions · Top-down · Plant quality

Introduction

The impact of predators on prey are often due to both consumptive and non-consumptive effects, indicating that predators can have strong effects on prey even if they are not

eating them (Lima 1998; Murdoch et al. 2003; Werner and Peacor 2003; Preisser et al. 2005; Thaler and Griffin 2008). Non-consumptive effects are costly defensive strategies induced in prey by predators, leading to decreased 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). The importance of non-consumptive effects have been well established in terrestrial arthropods, where prey often rely on plants as a food source (reviewed in Buchanan et al. 2017; Hermann and Landis 2017). Thus, in these systems both predators (top-down) and host plant quality (bottom-up) interact to impact herbivore populations (Price et al. 1980; Poelman et al. 2008).

Host plant quality and the amount of plant consumed by herbivores is an important factor that determine the overall impact of predators on herbivore performance (Ode 2006; Kersch-Becker and Thaler 2015). Poor plant quality leads to decreased larval growth rate, subsequently leaving them vulnerable to predation for a longer period of time (slow growth high mortality hypothesis; Clancy and Price 1987;

Communicated by Jennifer Thaler.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00442-020-04686-w>) contains supplementary material, which is available to authorized users.

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Loader and Damman 1991) and also reducing energy for evading predators (Anholt and Werner 1998; Hawlena and Schmitz 2010; Kaplan and Thaler 2010). Variability in host plant quality can be caused by nutrient amendments (Aqueel and Leather 2012), for example, changes in nitrogen application influence glucosinolate content in brassicas (Rosen et al. 2005) which in turn impact *Pieris* sp. host plant choice and larval performance (Chew 1977; Huang and Renwick 1994). Thus, fertilization can influence plant defenses that indirectly impact predators through causing changes in prey anti-predator defenses.

Herbivores are making choices throughout their development to navigate the tradeoff between finding high quality host plants and fending off predators. Holometabolous insects, such as Lepidoptera, could deploy different anti-predator behaviors and change responses to bottom-up and top-down factors as adults compared to immature stages. Caterpillars are influenced directly by host plant quality through ingestion and are often confined to a single plant chosen by the female during oviposition, while butterflies are not consuming the host plants, but evaluate them to select the highest quality food source and enemy-free space for their offspring (Chew 1977; Valladares and Lawton 1991). These different experiences may cause adults and larvae to be differentially affected by predation threat and plant nutrient levels. Since adult butterflies are making choices among a variety of factors for the well-being of their offspring, they must evaluate multiple plant and predator cues to determine what oviposition strategy will be most successful. We may expect to see bottom-up factors like plant nutrient levels have a greater influence on oviposition than top-down factors, since good plant nutrition is vital for larval survival (Lund et al. 2019). However, since larval stages are often confined to a plant chosen by their mother, they cannot choose among plants with different nutrient levels, therefore, larvae may show a stronger response to top-down factors. On the other hand, the availability of food quality could impact a larva's ability to respond to predation threat (Loader and Damman 1991). Larvae that have high quality food available may be able to take more risks and respond more often to predation threat, while those with low quality food sources may not be able to respond as frequently to predators because reducing food intake could be fatal.

While it is recognized that insects utilize anti-predator behaviors to avoid predation and maximize survival (e.g. Hermann and Thaler 2018), it is less clear if they are able to distinguish among cues from different predator species when using such responses. Herbivorous insects are usually exposed to multiple predator species during their lifetime, and the ability to discriminate among predators could be valuable for herbivores to avoid using antipredator responses that are costly or are not useful in preventing consumption (Relyea 2001). For example, if ovipositing females

discriminate between cues from predator species that feed on larvae throughout the full larval growth period versus just early growth stages and determine a heightened threat to their offspring, they could spread their egg load among more plants instead of targeting higher quality plants to increase the chance of larval survival. Additionally, if larvae can discriminate among cues of different predators, such as ones of only early life stages versus all life stages, they could change their responses depending on the magnitude of threat posed to their current life stage. A better understanding of whether herbivores are able to differentiate cues from multiple predator species, and if so how that impacts overall herbivory, could help develop more efficient and targeted biological control strategies for herbivorous pests. For example, in an agricultural system, if a pest responds to a specific predator by reducing feeding to become more inconspicuous (Lima and Dill 1990) encouraging higher numbers of this predator species could be beneficial. However, if the herbivore increases feeding in response to predation threat by a second predator species (Xiong et al. 2015) encouraging higher numbers of this predator could have negative outcomes in a cropping system.

Here, we evaluated the responses of adult and larval imported cabbage worm (*Pieris rapae* L., Lepidoptera: Pieridae) to varying plant nitrogen (N) levels and predation threat by two arthropod predators representing different types 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). *Pieris rapae* larvae are only vulnerable to predation by *H. convergens* during early instar stages (Cook and Webb 1995; Evans 2009), but vulnerable to predation by *P. maculiventris* during all larval stages (Mukerji and LeRoux 1969). Specifically, we wanted to better understand how these different predator types interact with plant nutritional status to influence host plant choice, how different predator cue types might alter non-consumptive effects in prey life stages that are confined to a host plant, and if these predator cue types impact prey in a field setting. To do this, we observed both adult and larval *P. rapae* host choice and behavior in greenhouse and environmental chamber bioassays, and followed-up the bioassays in a field experiment to observe the impacts of different types of N applications in cabbage. Because adult *P. rapae* make choices about where to oviposit eggs while larvae are most often confined to a single host during all larval stages, we performed a variety of both choice and no-choice experiments to observe the effects of top-down and bottom-up factors.

Methods

Insect and plant maintenance

Pieris rapae were reared in a greenhouse colony at Michigan State University. Insects originated from Michigan State University's Farms and were continuously kept in colony since 2015; field caught adults were added yearly to the colony to maintain genetic diversity. Larvae were reared on collard greens (*Brassica oleracea* cv. "Georgia"; W. Atlee Burpee & Co., Warminster, PA) ad libitum and moved to a separate cage with no collards upon pupation. Adults were fed a honey or sugar water solution and offered collard plants for oviposition. Greenhouse temperatures were maintained at 25–30 °C with a 16:8 h L:D photoperiod.

Two different predator species 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 control supplier (Rincon-Vitova Insectaries, Ventura, CA), fed a 10% honey water solution upon arrival, then kept at 10 °C until used in experiments. Prior to being used in experiments, *H. convergens* were allowed to warm up at room temperature for 24 h without food. Third instar *P. maculiventris* were obtained from a colony and reared according to a protocol described in Coudron and Wittmeyer 2002. Prior to use in experiments, *P. maculiventris* were kept in individual containers with a wet cotton ball and a dead mealworm larva, in an environmental chamber (25 °C, 16:8 h L:D). Third instar *P. maculiventris* were used in experiments.

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 h L:D) received either no N ("low N") or organic blood meal ("high N"; one application of 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 into 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, to help reduce odors created by the blood meal and maintain similar soil appearances in both high and low N treatments, all of which could influence *P. rapae* behavior. All collards used in greenhouse and environmental chamber experiments were 4–5 weeks old.

Influence of plant nitrogen and predator cues on *P. rapae* oviposition (Fig. 1a)

Four-choice oviposition experiments were conducted in a greenhouse (25–30 °C, 16:8 h L:D) at Michigan State

University from 22 May 2017–31 March 2018 to determine how bottom-up and top-down factors interact to influence adult female *P. rapae* oviposition preference. Two experiments were conducted using two different predator species to determine how female *P. rapae* respond to plant N and predator cues during oviposition, and to better understand how *P. rapae* ovipositional strategy changes when presented simultaneously with fixed bottom-up factors (both high and low N plants), but different predator cues.

The first experiment 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 × 70 × 70 cm, Nasco, Fort Atkinson, WI), with a total of 22 cages set up over a series of weeks. Plants were arranged in two rows down the length of the cage, with pots evenly spaced apart. This created a square of four pots in both cage halves, each of which contained all of the four treatments in randomly selected placements. 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 h 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 h to oviposit on the collards. After 24 h, the *P. rapae* adult was removed, and the number of eggs on each plant in the cage was counted and recorded.

The second experiment 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 experiment, 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 h, *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 set up over a series of weeks.

Difference in proportions of eggs laid in four-choice oviposition experiments were analyzed using a generalized linear model with a quasibinomial distribution, with plant N and predator presence as fixed factors and cage as a random factor ($\alpha = 0.05$; package = 'stats'). We analyzed the experiments with *H. convergens* and *P. maculiventris* independently.

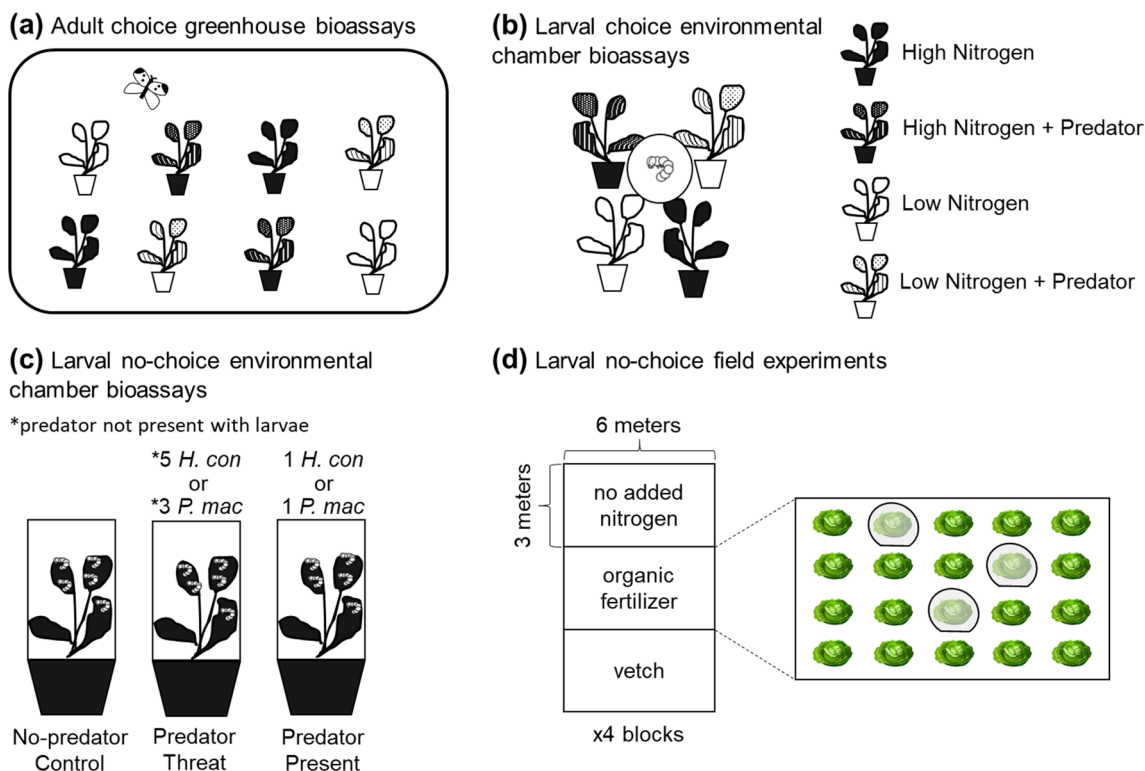


Fig. 1 Overview of experimental set up used in the study. **a** Adult *P. rapae* choice tests in the greenhouse to evaluate effect of plant nitrogen (N) and predator threat on oviposition. **b** Larval *P. rapae* choice in an environmental chamber to evaluate effects of plant N and predator threat on larval host plant choice. **c** Larval *P. rapae* no-choice bio-

assays 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

Influence of plant nitrogen and predator cues on larval *P. rapae* host plant choice (Fig. 1b)

Pieris rapae neonate larval choice tests were conducted in an environmental chamber (25 °C, 16:8 h L:D) to determine if bottom-up and top-down factors interact and how it differs from *P. rapae* adult host plant choices. Since young larvae have low mobility, they are often confined to the plant chosen by the butterfly. Here, we tested whether choices made by adults during the previously described oviposition choice tests match larval preferences when presented with the same fixed bottom-up factors and predation threats. These larval choice tests allowed us to better understand if oviposition strategies overlap with offspring preference, or if larvae that are experiencing predation threat attempt to modify their host plant choices.

The first experiment was set up between 20 May 2017 and 1 Jun 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*. Treatments with predators had five *H. convergens* bagged to one collard leaf with a mesh bag for 24 h prior to the start of the

experiment. After 24 h, *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. The four treatments were arranged in a square, spaced about 0.3 m away from each other, with one fully extended true leaf facing inward. A piece of tape was added to the underside of the top 1.5 cm of the fully extended true leaf for each treatment (in predator treatments this was the leaf that *H. convergens* had walked on for 24 h), and each leaf was taped to a piece of Whatman filter paper (7 cm diam.), so that all inward facing fully expanded leaves from the four plants were attached to one filter paper, creating a bridge among the four leaves (Supplementary Material Appendix 1, Fig. A1). Plants were placed to ensure that bridges remained level, and the placement of the four treatments were randomized in each replication. One neonate *P. rapae* larva was placed in the center of the filter paper with a paintbrush, and left for 24 h. Due to the low mobility of *P. rapae* larvae, they are not likely to leave their host plants, therefore plants were not bagged or caged for this experiment. After 24 h, each plant was checked for the larva, and larval choice was recorded. Larval choice tests with *H. convergens* were replicated a total of 28 times, over several days.

A second experiment was set up from 14 Feb 2018 to 2 Mar 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 h. Larval choice tests with *P. maculiventris* were replicated a total of 28 times, over several days.

Differences in choice counts among the four treatments in larval choice bioassays were analyzed using a generalized linear model with a binomial distribution and logit link function, with plant N and predator presence as fixed factors, and time as a random factor. We analyzed experiments using *H. convergens* and *P. maculiventris* separately.

Predator cue impacts on larval *P. rapae* survival, activity, and growth (Fig. 1c)

Because larvae are often confined to a single host plant, we conducted no-choice bioassays to measure the consumptive and non-consumptive effects of two predator species without varying bottom-up effects. We measured larval feeding response and growth to three levels of predation cues to better understand the response of larvae to predation threat by the two different predator species. Because both adults and larvae preferred high over low N plants in the previous choice tests, and N application is common in agriculture, we decided to use plants that received high N in this experiment.

Bioassays were conducted in an environmental chamber (25 °C, 16:8 h L:D) to determine *H. convergens* consumptive and non-consumptive effects on *P. rapae* larval survival, growth, and leaf consumption from 28 Aug 2017 to 1 Sep 2017. 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. 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 collar, pushed 1–2 cm into the soil and covered with a mesh lid. In predator threat treatments, 5 adult *H. convergens* were added to cages 24 h 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 *H. convergens* was added to the cage with larvae. No predators were added to control cages. One predator was used in predator present cages, because higher numbers tested in preliminary experiments resulted in almost complete consumption of *P. rapae* larvae, and therefore, a lack of data on *P. rapae* response (data not shown). Five predators were added in predator threat cages since larvae were not present with predators. 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 on paper with a 1 × 1 cm grid to standardize each picture, and Image J (Version 1.50i; National Institutes of Health, MD) was used to calculate the leaf area consumed.

A second set of bioassays was set up to determine *P. maculiventris* consumptive and non-consumptive effects on *P. rapae* larvae from 20 Apr 2018 to 1 May 2018. Cages were set up the same way as for *H. convergens* experiments, except three *P. maculiventris* were used in predator threat cages and one *P. maculiventris* in predator present cages. Treatments were replicated 25 times total, split into 2 groups that were set up 7 days apart due to predator availability.

Larval survival was analyzed with a linear mixed-effects model, using the 'lmer' function in the 'lme4' package (Bates et al. 2015), 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 *P. rapae* in all treatments were 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 were not 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.

Field nutrient application and predator cue impacts on larval *P. rapae* survival and growth (Fig. 1d)

To determine if bottom-up and top-down factors interact to influence larval response, we conducted no-choice bioassays similar to our environmental chamber experiments, but with three different types of nutrient application methods in a field setting (no N, added N, hairy vetch cover crop). This allowed us to determine if *P. rapae* larval response to two different predators would be affected under field conditions, and how plant N may impact these larval responses. 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. Due to feeding on plants from naturally occurring

insect pests prior to experimental set-up, we were unable to measure leaf consumption in these experiments.

Cabbage seedlings were grown in a greenhouse (25–20 °C; 16:8 h L:D) in 98 cell plug trays for 4 weeks and transplanted in the field on 6 Jul 2016, 29 Jun 2017, and 5 Jul 2018 in a randomized complete block design with four blocks and three treatments. Plots in each block measured 3 × 6 m, and blocks were spaced 4.5 m apart. Each plot consisted of 4 rows of cabbage with 76 cm between-row and 36 cm in-row spacing. In each block, three levels of nutrient treatments and three levels of predator treatments were fully crossed. The three levels of nutrient treatments consisted of: (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* cv. “VNS”, Albert Lea Seed, Albert Lea, MN) 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 area between future cabbage rows by blocking drop tubes on the grain drill. In hairy vetch treatments, vetch was sown (23 kg/ha) at the same time as rye, but only in the in-row zone. 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 2 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 cabbage rows, and vetch incorporated into the soil in cabbage rows as a N source. Additional details of yearly field and crop management activities are provided in Supplementary Material Appendix 1, Table A1.

Experiments with insects were set up between 1 and 20 August 2016 (11 replications), 12 and 29 August 2017 (12 replications), and 5 and 26 August 2018 (10 replications). Predation treatments consisted of three levels: 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 × 55 cm white mesh bags (Hummert International, Earth City, MO), and either five (*H. convergens*) or three (*P. maculiventris*) predators were added. Bags 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 h. After 48 h, 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’ treatments 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 were analyzed separately for each field season. Larval survival and weight 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 were not transformed for any year. Weight data were ln transformed to meet 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. The N content of collards used in all of our bioassays are reported in Lund et al. (2019).

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 Tukey's HSD 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

Hippodamia convergens consumptive and non-consumptive effects

Influence of plant nitrogen and predator cues on *P. rapae* oviposition

Overall, females laid a higher proportion of eggs on high N plants than low N plants ($t = 3.62$, $df = 21$, $P < 0.01$), but the presence of *H. convergens* had no overall effect on oviposition ($t = 0.80$, $df = 21$, $P = 0.42$), and there was no significant interaction between N and predator effects ($t = 0.08$, $df = 21$, $P = 0.94$). Females laid about 2.5 times more eggs on both high N plants and high N plants with *H. convergens* compared to low N plants with predators, and almost 3.5 times more eggs on both high N plants

and high N plants with *H. convergens* than low N plants without predators (Fig. 2a).

Influence of plant nitrogen and predator cues on larval *P. rapae* host plant choice

Larvae chose high N plants significantly more than low N plants ($z = 2.63$, $df = 27$, $P = 0.01$), but *H. convergens* presence did not influence larval choice ($z = 0.54$, $df = 27$, $P = 0.59$), and there was no significant interaction ($z = 0.67$, $df = 27$, $P = 0.50$). In total, 46% of larvae chose high N plants with predators, 39% high N plants, 11% low N plants with predators, and 4% low N plants (Fig. 2b).

Predator cue impacts on larval *P. rapae* survival, activity, and growth

The presence and threat of *H. convergens* did not impact survival of *P. rapae* larvae (Fig. 3a; $F = 1.47$, $df = 2, 38$,

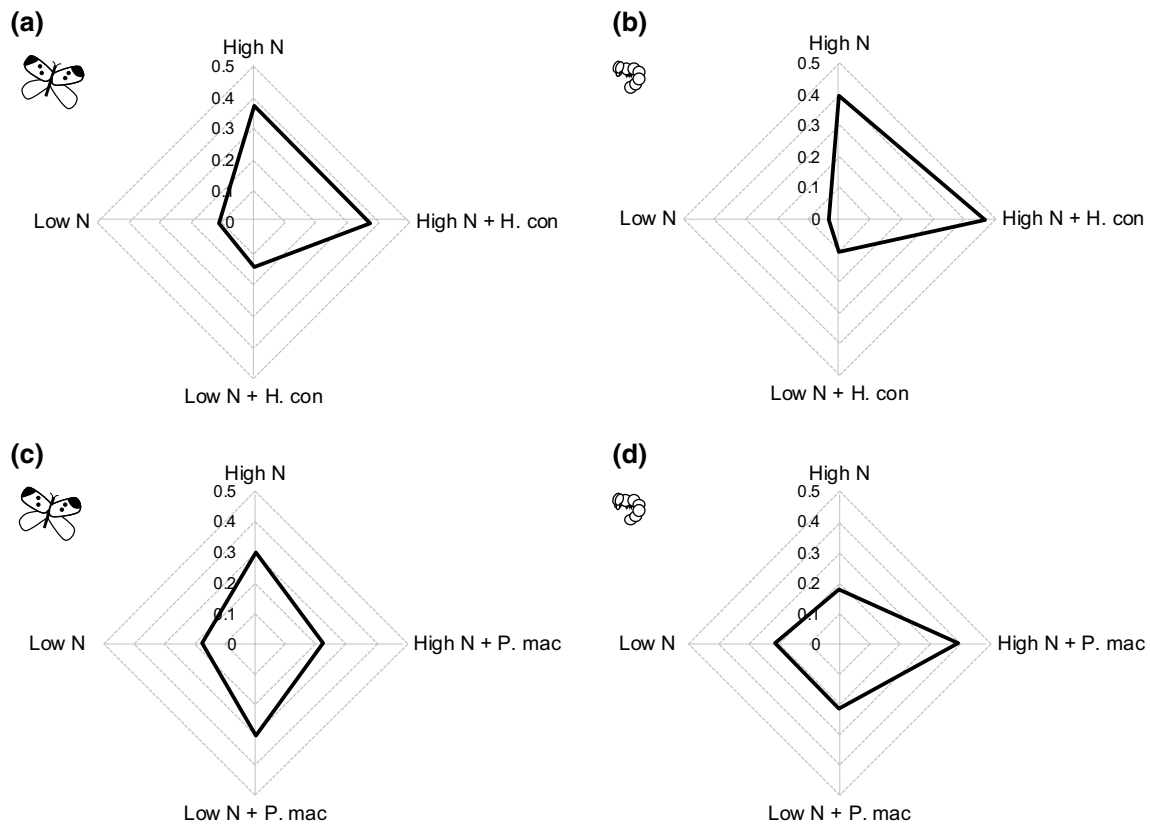


Fig. 2 Results of four-way *P. rapae* choice tests with potted plants in the greenhouse using high N collard, low N collard, high N collard with predator threat (either *H. convergens* or *P. maculiventris*) and low N collard with predator threat. Adult *P. rapae* choice tests with *H. convergens* (a), *P. rapae* larval choice tests with *H. convergens* (b), adult *P. rapae* choice test with *P. maculiventris* (c), and *P. rapae* larval choice test with *P. maculiventris* (d). In predator threat treatments, predators were bagged on one collard leaf immediately before

butterflies were added in choice tests, and 24 h 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 proportion of total number of eggs laid on each treatment (a and c) or proportion of number of larval choices per treatment (b and d) along each axis for the four choices presented ($\alpha = 0.05$). *H. con.*, – *Hippodamia convergens*; *P. mac*, *Podisus maculiventris*; N, nitrogen

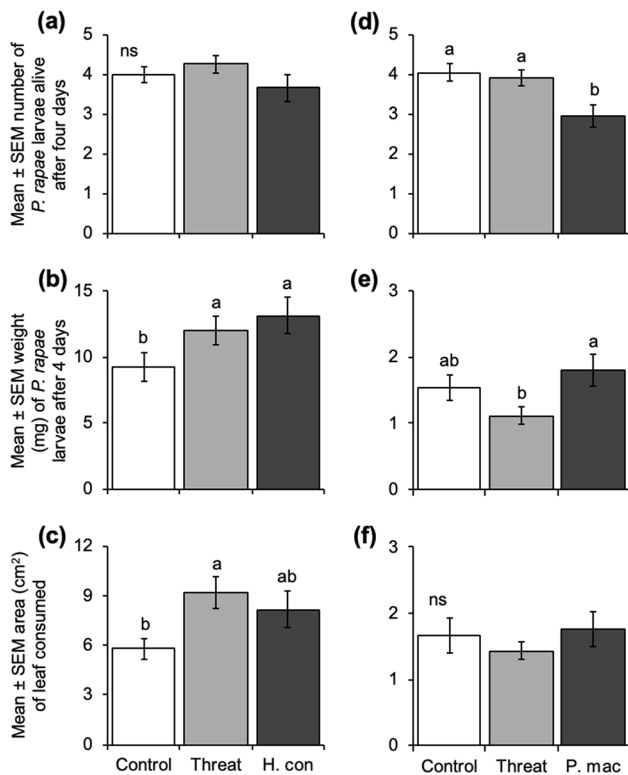


Fig. 3 Results of environmental chamber bioassays observing the effects of *H. convergens* and *P. maculiventris* on *P. rapae* larval survival (**a**, **d**), weight (**b**, **e**), and collard leaf consumption (**c**, **f**) after 4 days. Effects were observed across three predation treatments: no-predator 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 ($\alpha=0.05$)

$P=0.24$). Larval weight was significantly affected by *H. convergens* presence (Fig. 3b; $F=3.66$, $df=2$, 217, $P=0.03$), with a 42% weight increase 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 amount of leaf tissue consumed by larvae was impacted by treatment (Fig. 3c; $F=4.74$, $df=2$, 38 $P=0.01$), with 58% more tissue consumed by larvae in *H. convergens* threat treatments compared to no-predator control ($t=3.00$, $df=38$, $P=0.01$), and 40% more in predator present treatments compared to controls ($t=2.13$, $df=38$, $P=0.06$).

Field nutrient application and predator cue impacts on larval *P. rapae* survival and growth

There were significant differences in larval survival ($t=4.49$, $df=197$, $P<0.01$) and weight ($t=19.22$, $df=315$, $P<0.01$) between 2016 and 2017, so data from the 2 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 1). Additionally, there were no significant effects of the interaction between predator and nutrient treatment on larval survival (Table 1; $F_{4,80}=0.59$, $P=0.67$; Fig. 4a). However, nutrient treatment alone 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 1). When observing the interaction of predator and nutrient treatment on larval weight, in no-predator control cages there was a similar result, with a significant increase in larval weight in vetch treatments compared to both no N (60% increase; Fig. 4b; $t=3.53$, $df=286$, $P<0.01$) and organic fertilizer (30% increase; Fig. 4b; $t=2.22$, $df=285$, $P=0.04$) treatments. However, these weight differences were only observed in cages where predator cues were not present, and weights of caterpillars collected from both predator threat and predator present cages were not different among the three nutrient treatments.

In 2017, larval weight was influenced by the interactive effects of predator and nutrient factors. In particular, compared to larvae in cages without predators present, larval weight increased by 70% in predator present cages (Fig. 4d; $t=2.74$, $df=392$, $P=0.01$), and by 50% in predator threat cages (Fig. 4d; $t=2.62$, $df=392$, $P=0.01$), but only in plots that were treated with organic fertilizer.

Podisus maculiventris consumptive and non-consumptive effects

Influence of plant nitrogen and predator cues on *P. rapae* oviposition

There was a significant interaction between plant N and *P. maculiventris* presence on the proportion of eggs laid by *P. rapae* ($t=2.38$, $df=21$, $P=0.02$), with females laying more eggs on high N plants than low N plants when no predators were present, but when *P. maculiventris* were present laying a higher proportion of eggs on low over high N plants. In total, 30% of eggs were laid on both high N plants with no predators and low N plants with *P. maculiventris*, 22% on high N plants with *P. maculiventris*, and 18% on low N plants with no predators (Fig. 2c).

Influence of plant nitrogen and predator cues on larval *P. rapae* host plant choice

In *P. rapae* larval choice tests, neither plant N ($z=0.34$, $df=27$, $P=0.74$) nor *P. maculiventris* cues ($z=1.74$, $df=27$, $P=0.08$) influenced larval choice, and there was no significant interaction ($z=1.21$, $df=27$, $P=0.23$). Overall, 40% of larvae chose high N plants with *P. maculiventris*, low N with predators and low N without predators were both chosen

Table 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

	Main effects			Treatment means comparison				
	<i>F</i>	<i>df</i>	<i>P</i> -value	P: no. predator N: no. N	Threat Fertilizer	Present Vetch	<i>t</i>	<i>P</i> -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 × 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 × 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 × 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 × 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 × 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 × N	0.76	4, 339	0.55					

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, nitrogen (*N*) nutrient main effect

21% of the time, and high N plants were chosen 18% of the time (Fig. 2d).

Predator cue impacts on larval *P. rapae* survival, activity, and growth

In no-choice bioassays using *P. maculiventris*, predator presence significantly affected *P. rapae* larval survival (Fig. 3d; $F=6.59$, $df=2, 48$, $P<0.01$), with an average of about three out of five larvae surviving after 4 days in treatments 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 where in each case four larvae survived on average. Larval weight also varied across *P. maculiventris* predator treatments (Fig. 3e; $F=3.80$, $df=2, 254$, $P=0.02$). 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 (Fig. 3f; $F=0.19$, $df=2, 40$, $P=0.83$).

Field nutrient application and predator cue impacts on larval *P. rapae* survival and growth

During the 2018 field season, *P. maculiventris* predator treatment had a significant impact on *P. rapae* larval survival (Table 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 1; $F_{2,75}=1.06$, $P=0.35$). While nutrient treatment did not significantly affect larval survival (Table 1; $F_{2,333}=0.68$,

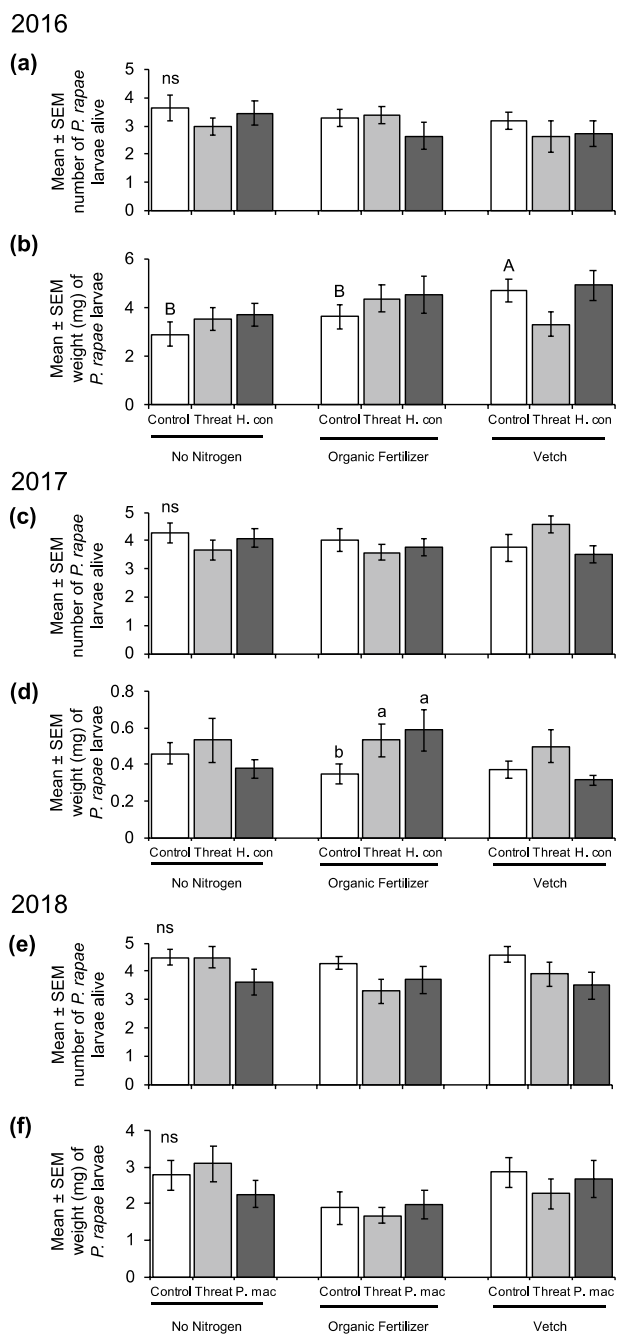


Fig. 4 *Pieris rapae* larval survival (a, c, e) and weight (b, d, f) in an experimental cabbage field in 2016 (a, b), 2017 (c, d), and 2018 (e, f) after 4 days. Larval survival and weight were observed across three predation treatments: no-predator control, predator threat, and predator present (either *H. con*—*H. convergens* or *P. mac*—*P. maculiventris*), each replicated within three nutrient treatments: no nitrogen (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 treatments within a nutrient treatment, while ‘ns’ indicates that treatments were not significantly different ($\alpha=0.05$)

$P=0.51$), there were differences in larval weight among nutrient treatments (Table 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 there was no significant effect on either larval survival (Table 1; $F_{4,78}=0.85$, $P=0.50$; Fig. 4e) or larval weight (Table 1; $F_{4,339}=0.76$, $P=0.55$; Fig. 4f).

Plant nitrogen content

Cabbage N content in the field was affected by 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 *P. rapae* adults and larvae make similar choices about bottom-up factors such as plant N, and top-down factors such as predation threat when selecting a host plant. In laboratory choice tests, *P. rapae* adults and first instars did not differentiate plants with *H. convergens* cues, but both life stages preferred high over low N plants. These results support the ‘mother knows best’ hypothesis (Thompson 1988; Jaenike 1990; Renwick and Chew 1994), as both butterflies and larvae showed the same preference for host plant quality, and suggests that *P. rapae* are influenced by bottom-up factors (plant N) when in the presence of an early-instar predator (*H. convergens*) (Evans 2009; Lund et al. 2019). In no-choice bioassays, *H. convergens* predation threat (chemical cues only) caused larvae to consume more leaf tissue and grow larger than larvae that were not exposed to predation threat. When *H. convergens* were present with larvae in bioassays, larvae again weighed more than those without predation threat, and consumed 40% more (although this was not statistically significant) leaf tissue than larvae in control cages. These results suggest that under the threat of a predator of early life stages only, it might be more beneficial for *P. rapae* to consume more leaf tissue to outgrow vulnerable life stages. In a study observing non-consumptive effects

of a different lady beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae), on *Helicoverpa armigera* (Lepidoptera: Noctuidae), 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*. This further indicates that gaining weight faster to evade predation may be an important strategy when threatened by an early-instar predator. The fact that larvae were consuming the most leaf tissue in predator threat cages, but weighed the most in predator present cages suggests that larvae may be responding differently when predators are visually present, and there may be physiological changes occurring in larvae, not directly related to their leaf consumption, when *H. convergens* are present in the environment (Hawlena and Schmitz 2010).

These results were mirrored in the field in 2017, where there was a similar increase in larval weight in the presence of *H. convergens* in the organic fertilizer treatment compared to our no-choice environmental chamber bioassays (larvae gained weight in predator threat and predator present cages compared to controls; compare Fig. 3b to Fig. 4d). Of the three field nutrient treatments used, the cabbage plants in the organic fertilizer field treatments were treated most similarly 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 *H. convergens* when a higher quality diet is available. The adult and larval preference for high N plants when *H. convergens* cues are present on host plants could allow larvae to evade predation by growing faster on these plants. It is important to note that cabbages grown in the organic fertilizer treatments in 2017 were numerically, though not statistically, higher in N content than cabbages grown in the other two treatments, suggesting that this nutrient treatment may have effects on the plant that impacts the larvae other than N content alone. Additionally, the results from our field experiments were variable, and measured responses were generally weaker than in our no-choice environmental chamber bioassays. 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); for example, in this experiment we observed differences that could have been due to abiotic environmental effects as well as differences in the plant varieties used. In 2017, cooler temperatures and consistent rain during our experiments may have contributed to slow larval development, but patterns in larval weight 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.

When experiments were set up with a different predator species, *P. maculiventris*, we also found that *P. rapae* adults and larvae were making similar host choices when observing the influence of plant N and predator cues on host plant choice, though with different outcomes than with *H. convergens*. In laboratory choice tests with *P. maculiventris*, adults exhibited a risk-spreading strategy (Levins 1962). In this strategy, adults spread their eggs among all host plants regardless of quality to increase chances of some offspring surviving. Unlike the strategy we observed when *H. convergens* cues were present, where adults laid eggs predominantly on high N plants, here *P. rapae* oviposited evenly among high and low N plants and plants with and without predator cues. Interestingly, *P. rapae* larvae also chose evenly among the four choices. In a previous study, we found that in the absence of predator cues, *P. rapae* adults preferred high over low N plants (Lund et al. 2019), suggesting that in these choice tests top-down effects by *P. maculiventris* cause a risk-spreading response in *P. rapae*. When observing predator impact on larval survival, activity and growth, *P. rapae* larvae did not consume or weigh more when a *P. maculiventris* threat or predator was present compared to control cages without predator cues. We observed similar results in our 2018 field experiments in the organic fertilizer and vetch treatments for *P. maculiventris* (compare Fig. 3e to Fig. 4f). Since *P. maculiventris* feeds on all life stages of *P. rapae*, changes in activity such as increased feeding may not be effective at increasing chances of survival. It is possible that *P. rapae* reacted physiologically to the threat by *P. maculiventris*, but we did not measure these responses in this study. Because larvae do not appear to alter their consumption or growth in response to cues from a predator that feeds on all life stages such as *P. maculiventris* (Mukerji and LeRoux 1969), depositing eggs on plants without being selective of plant quality will likely increase larval chances of survival since eggs are spread over more plants, and may explain why a risk-spreading strategy is exhibited. 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 Griffin 2008; Thaler et al. 2012; Kaplan et al. 2014). 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.

Throughout this study, we expected to see a higher response and preference by *P. rapae* for enemy-free space (Jeffries and Lawton 1984; Thompson 1988; Denno et al. 1990), but instead *P. rapae* made decisions based on host plant quality over enemy-free space when cues from either

predators were present. It is possible that predator visual and chemical cues were not strong enough in experiments where adults and larvae were making host plant choices for *P. rapae* to differentiate between enemy-free space and plants where the enemies were present. However, since *P. rapae* larvae are less mobile compared to the predator species used in these experiments, predators can move in and out of spaces occupied by *P. rapae* easily throughout their early life stages. In this case, *P. rapae* may not respond to predator location (i.e. avoiding plants where predator cues are present), but instead to predator species present (i.e. making plant choices based on species specific cues in the environment), which aligns with the results that we saw when observing the impacts of predator cues and plant N on adult and larval host plant choice. We also found these similar responses to plant N and predator cues between both adult and larval *P. rapae* among experiments with differing methodology (i.e. in some experiments, predator visual and chemical cues were present, while others were just chemical). Since we found similar responses in choice tests by both adults and larvae when either predator cue was present, as well as similar results between lab and field experiments when testing larval activity and growth, it is likely that predator cues were strong enough to detect, and choices made by *P. rapae* in these experiments were in response to predator cues.

Although our experiments observing the impacts of *H. convergens* and *P. maculiventris* on *P. rapae* choice, leaf consumption, and development were not performed concurrently, it is interesting to note that we observed different responses from *P. rapae* across the same series of experiments to the two different predators. This indicates that *P. rapae* response to the interaction of plant quality and predator cues can alter depending on predator species identity, and therefore, *P. rapae* may be capable of differentiating between predator species. Additionally, it appears from this study that differentiating between predator species early, such as during oviposition, can help adults make decisions about host plant quality for their offspring that may lead to the highest chances of survival. However, since experiments were set up at separate times from one another, it is difficult to determine if it is truly a response to predator identity, or if there are other confounding factors affecting our outcomes. If *P. rapae* is responding based on predator identity, this could have implications for pest management strategies in that supporting specific predator species could lead to better or worse pest control. For example, while *H. convergens* and *P. maculiventris* are both predators of *P. rapae*, in this study *P. maculiventris* showed greater consumption of *P. rapae* larvae, and larvae were feeding less on leaf tissue in the presence of *P. maculiventris* than *H. convergens*, leading to less plant damage. In this case, promoting *P.*

maculiventris in an agricultural landscape could lead to better pest control and less pest damage on the crop.

Future studies should look further into comparing how predator identity, not just presence overall, could be an important factor for prey anti-predator responses. This is significant, because as we start 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. Here, we only observed impacts on prey through changes in activity, without measuring physiological changes in prey or changes in plant defenses. We suggest future studies take predator identity into consideration in the context of bottom-up and top-down effects on prey species to obtain a clearer understanding of the interaction between predator non-consumptive effects and other environmental factors, as well as how the interaction of plant quality and predator identity may change physiological responses in prey species and their ability to respond with anti-predator behaviors.

Acknowledgements This research is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2014-51300-222244. Thanks to Patrick Mucci, Joseph Lonchar, Jackie Kirouac, Sarah Galley, and Grace Nagle for help in maintaining colonies and setting up experiments. We also thank members of the MSU Vegetable Entomology Lab for feedback on earlier versions of this manuscript.

Author contribution statement ML and ZS conceived and designed the experiments. ML performed the experiments and analyzed the data. ML, ZS, DB and TC wrote the manuscript; ZS, DB and TC provided material and financial support to perform the experiments.

References

- Anholt B, Werner EE (1998) Predictable changes in predation mortality as a consequence of changes in food availability and predation risk. *Evol Ecol* 12:729–738
- Aqueel MA, Leather SR (2012) Nitrogen fertiliser affects the functional response and prey consumption of *Harmonia axyridis* (Coleoptera: Coccinellidae) feeding on cereal aphids. *Ann Appl Biol* 160(1):6–15. <https://doi.org/10.1111/j.1744-7348.2011.00514.x>
- Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48. <https://doi.org/10.18637/jss.v067.i01>
- Buchanan AL, Hermann SL, Lund M, Szendrei Z (2017) A meta-analysis of non-consumptive predator effects in arthropods: the influence of organismal and environmental characteristics. *Oikos* 126:1233–1240. <https://doi.org/10.1111/oik.04384>

- Calisi RM, Bentley GE (2009) Lab and field experiments: are they the same animal? *Horm Behav* 56:1–10. <https://doi.org/10.1016/j.yhbeh.2009.02.010>
- Chew FS (1977) Coevolution of pierid butterflies and their cruciferous foodplants. II. The distribution of eggs on potential foodplants. *Evolution* 31(3):568–579
- Clancy KM, Price PW (1987) Rapid herbivore growth enhances enemy attack: sublethal plant defenses remain a paradox. *Ecology* 68:733–737
- Cook SP, Webb RE (1995) Predation of early-instar gypsy moth larvae by a generalist predator, *Anatis labiculata* (Say) (Coleoptera: Cicinellidae). *J Entomol Sci* 30:258–261
- Coudron TA, Wittmeyer J (2002) Life history and cost analysis for continuous rearing of *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) on a zoophytophagous artificial diet. *Biol Microb Control* 95:1159–1168
- Denno RF, Larsson S, Olmstead KL (1990) Role of enemy-free space and plant quality in host-plant selection by willow beetles. *Ecology* 71:124–137
- Evans EW (2009) Lady beetles as predators of insects other than Hemiptera. *Biol Control* 51:255–267. <https://doi.org/10.1016/j.biocontrol.2009.05.011>
- Hawlena D, Schmitz OJ (2010) Herbivore physiological response to predation risk and implications for ecosystem nutrient dynamics. *PNAS* 107:15503–15507. <https://doi.org/10.1073/pnas.1009300107>
- Hermann SL, Landis DA (2017) Scaling up our understanding of non-consumptive effects in insect systems. *Curr Opin Insect Sci* 20:54–60. <https://doi.org/10.1016/j.cois.2017.03.010>
- Hermann SL, Thaler JS (2014) Prey perception of predation risk: volatile chemical cues mediate non-consumptive effects of a predator on a herbivorous insect. *Oecologia* 176:669–676. <https://doi.org/10.1007/s00442-014-3069-5>
- Hermann SL, Thaler JS (2018) The effect of predator presence on the behavioral sequence from host selection to reproduction in an invulnerable stage of insect prey. *Oecologia* 188:945–952. <https://doi.org/10.1007/s00442-018-4202-7>
- Huang X, Renwick JA (1994) Relative activities of glucosinolates as oviposition stimulants for *Pieris rapae* and *P. napi oleracea*. *J Chem Ecol* 20:1025–1037. <https://doi.org/10.1007/BF02059739>
- Jaenike J (1990) Host specialization in phytophagous insects. *Annu Rev Ecol Syst* 21:243–273
- Jeffries MJ, Lawton JH (1984) Enemy free space and the structure of ecological communities. *Biol J Linn Soc* 23:269–286. <https://doi.org/10.1111/j.1095-8312.1984.tb00145.x>
- Kaplan I, Thaler JS (2010) Plant resistance attenuates the consumptive and non-consumptive impacts of predators on prey. *Oikos* 119:1105–1113. <https://doi.org/10.1111/j.1600-0706.2009.18311.x>
- Kaplan I, McArt SH, Thaler JS (2014) Plant defenses and predation risk differentially shape patterns of consumption, growth, and digestive efficiency in a guild of leaf-chewing insects. *PLoS ONE* 9:1–8. <https://doi.org/10.1371/journal.pone.0093714>
- Kersch-Becker F, Thaler JS (2015) Plant resistance reduces the strength of consumptive and non-consumptive effects of predators on aphids. *J Anim Ecol* 84:1222–1232. <https://doi.org/10.1111/1365-2656.12371>
- Levins R (1962) Theory of fitness in a heterogeneous environment. I. The fitness set and adaptive function. *Am Nat* 96:361–373
- Lima S (1998) Non-lethal effects in the ecology of predator–prey interactions. *Bioscience* 48:25–34. <https://doi.org/10.2307/1313225>
- Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. *Can J Zool* 68:619–640. <https://doi.org/10.1139/z90-092>
- Loader C, Damman H (1991) Nitrogen content of food plants and vulnerability of *Pieris rapae* to natural enemies. *Ecology* 72:1586–1590
- Lowry CJ, Brainard DC (2017) Organic farmer perceptions of reduced tillage: a Michigan farmer survey. *Renew Agric Food Syst* 34:103–115. <https://doi.org/10.1017/S1742170517000357>
- Lund M, Brainard DC, Szendrei Z (2019) Cue hierarchy for host plant selection in *Pieris rapae*. *Entomol Exp Appl* 167(4):1–11
- Mukerji K, LeRoux EJ (1969) The effect of predator age on the functional response of *Podisus maculiventris* to the prey size of *Galleria mellonella*. *Can Entomol* 101:314–327
- Murdoch W, Briggs C, Nisbet R (2003) *Consumer-resource dynamics*. Princeton University Press, Princeton
- Ode PJ (2006) Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy interactions. *Annu Rev Entomol* 51:163–185. <https://doi.org/10.1146/annurev.ento.51.110104.151110>
- Peckarsky BL, Cowan CA, Penton MA et al (1993) Sublethal consequences of stream-dwelling predatory stoneflies on mayfly growth and fecundity. *Ecology* 74:1836–1846
- Poelman EH, van Loon JJA, Dicke M (2008) Consequences of variation in plant defense for biodiversity at higher trophic levels. *Trends Plant Sci* 13:534–541. <https://doi.org/10.1016/j.tplants.2008.08.003>
- Preisser EL, Bolnick DI, Bernard MF (2005) Scared to death? The effects of intimidation and consumption in predator–prey interactions. *Ecology* 86:501–509
- Price PW, Bouton CE, Gross P et al (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annu Rev Ecol Syst* 11:41–65
- Relyea RA (2001) Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology* 82(2):523–540
- Renwick JAA, Chew FS (1994) Oviposition behavior in Lepidoptera. *Annu Rev Entomol* 39:377–400. <https://doi.org/10.1146/annurev.ento.39.1.377>
- Rosen CJ, Fritz VA, Gardner GM et al (2005) Cabbage yield and glucosinolate concentrations as affected by nitrogen and sulfur fertility. *HortScience* 40:1493–1498. <https://doi.org/10.3390/molecules200915827>
- Schoener TW (1989) Food webs from the small to the large: the Robert H. MacArthur award lecture. *Ecology* 70:1559–1589
- Siepielski AM, Wang J, Prince G (2014) Nonconsumptive predator-driven mortality causes natural selection on prey. *Evolution (NY)* 68:696–704. <https://doi.org/10.1111/evo.12294>
- Sih A, Englund G, Wooster D (1998) Emergent impacts of multiple predators on prey. *Trends Ecol Evol* 13:350–355. [https://doi.org/10.1016/S0169-5347\(98\)01437-2](https://doi.org/10.1016/S0169-5347(98)01437-2)
- Thaler JS, Griffin CAM (2008) Relative importance of consumptive and non-consumptive effects of predators on prey and plant damage: the influence of herbivore ontogeny. *Entomol Exp Appl* 128:34–40. <https://doi.org/10.1111/j.1570-7458.2008.00737.x>
- Thaler JS, Mcart SH, Kaplan I (2012) Compensatory mechanisms for ameliorating the fundamental trade-off between predator avoidance and foraging. *PNAS* 109:12075–12080. <https://doi.org/10.1073/pnas.1208070109>
- Thompson JN (1988) Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomol Exp Appl* 47:3–14
- Valladares G, Lawton JH (1991) Host-plant selection in the holly leaf-miner: does mother know best? *J Anim Ecol* 60:227–240
- Werner EE, Peacor SD (2003) A review of trait-mediated indirect interactions in ecological communities. *Ecology* 84:1083–1100. [https://doi.org/10.1890/0012-9658\(2003\)084%5b1083::arotii%5d2.0.co;2](https://doi.org/10.1890/0012-9658(2003)084%5b1083::arotii%5d2.0.co;2)
- Xiong X, Zhen JPM, Pengxiang L et al (2015) Chronic, predator-induced stress alters development and reproductive performance of the cotton bollworm, *Helicoverpa armigera*. *Biocontrol* 60:827–837. <https://doi.org/10.1007/s10526-015-9689-9>