



## Antipredator behavior of Colorado potato beetle larvae differs by instar and attacking predator

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### ABSTRACT

To avoid capture by predators, herbivores often deploy predator avoidance or deterrence behaviors. Chemical defenses of Colorado potato beetles (CPB, *Leptinotarsa decemlineata*) have been described, but little is known about antipredator behaviors of CPB larvae in response to attack by generalist predators. We examined the behavioral responses of second, third, and fourth instar CPB larvae when contacted by predatory lady beetles (*Hippodamia convergens*) or damsel bugs (*Nabis alternatus*). Of these predators, only the damsel bug readily captures CPB larvae. First, in simple Petri-dish arenas we touched single CPB larvae with either a lady beetle or damsel bug adult attached to the end of a wooden dowel, or a bare dowel as a control. Larvae responded to predator contact by walking away, rearing up, regurgitating onto their ventral surface, wiggling their bodies, and/or defecating. The number of behavioral responses increased significantly when larvae were touched with a predator compared to a bare dowel, when larvae were in later instars, and when larvae were contacted by a damsel bug rather than a lady beetle. Next, we observed natural encounters between CPB larvae and predators on potato plants. We again found that fourth instar larvae were most likely to initiate antipredator behaviors, and that all larval stages reacted more intensely to damsel bugs than lady beetles. In summary, we found that CPB larvae deployed a complex suite of behaviors when accosted by generalist predators. The frequency of behavioral response varied by larval instar, and appeared to be scaled to the risk posed by the predator encountered.

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### 1. Introduction

Predators indirectly protect plants by killing herbivores in a wide variety of agricultural and natural systems (Schmitz et al., 2000; Halaj and Wise, 2001). This occurs through a chain of trophic interactions known as trophic cascades, with predators consuming herbivores that otherwise would have consumed plants (Hairston et al., 1960). However, ecologists have grown increasingly aware that predators also impact prey, and thus indirectly protect plants, through non-trophic channels (e.g., Schmitz et al., 1997). Oftentimes, in the presence of predators, herbivores leave preferred feeding sites, or leave plants altogether, in an attempt to reduce their predation risk (Lima and Dill, 1990). For example, aphids and caterpillars often drop from plants to escape from predators in the foliage (Losey and Denno, 1998; Wang and Keller, 2002; Castellanos and Barbosa, 2006). Even when herbivores do not leave plants, they may feed less often to escape detection by predators that hunt using vibrational signals (Griffin and Thaler, 2006). There is growing evidence that the effects of predators on prey through

non-trophic channels, mediated by antipredator behaviors in herbivore prey, may benefit plants as much as those due to actual predation (Werner and Peacor, 2003; Schmitz et al., 2004; Preisser et al., 2005). For example, Nelson et al. (2004) found that predatory damsel bugs (*Nabis* spp.) rendered incapable of killing by snipping the mouthparts, reduced pea aphid population growth nearly as much as did un-manipulated, lethal predators. This occurs because non-lethal predators still chase aphids from their feeding locations, which disrupts aphid feeding as much as actual predation (Nelson et al., 2004; Nelson and Rosenheim, 2006). Furthermore, the strength of both lethal and non-lethal predator effects may change through the course of prey development, as prey move among stages that are more or less vulnerable to being preyed upon (Rowe and Ludwig, 1991; Abrams and Rowe, 1996; Ovadia and Schmitz, 2002). At all stages, the intensity with which antipredator behaviors are deployed is expected to reflect a balance between the risk of being killed by predators versus potential gains from foraging or other risky behaviors (Lima and Dill, 1990).

The Colorado potato beetle (“CPB”, *Leptinotarsa decemlineata*) is a major pest of potato (*Solanum tuberosum*) and other solanaceous crops (Hare, 1990; Weber, 2003). Unchecked, potato beetles can entirely defoliate the crop with catastrophic consequences for yields (Hare, 1980). CPB rapidly develop resistance to most insecticides

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(Alyokhin et al., 2008), and this along with emerging adoption of reduced-spray and organic pest management schemes in potato has re-invigorated interest in CPB biocontrol (Hilbeck and Kennedy, 1996; Ruffle and Miller, 2002; Koss et al., 2005). Potato fields under organic or selective-chemical pest management often contain dramatically higher predator densities than fields receiving broad-spectrum insecticides (Hilbeck and Kennedy, 1996; Hamilton and Lashomb, 1996; Hilbeck et al., 1997; Koss et al., 2005). In the Columbia Basin of Washington State, a major production area for potatoes grown under irrigation, CPB is attacked by a diverse community of generalist predators (Koss et al., 2005). Key among these are the lady beetle *Hippodamia convergens*, which is a major egg predator, and the damsel bug *Nabis alternatus*, which attacks both eggs and small larvae (Chang and Snyder, 2004; Koss and Snyder, 2005; Koss et al., 2005; Ramirez and Snyder, 2009). In the absence of disruptive broad-spectrum insecticides, these predators have the potential to dramatically lower potato beetle densities (Koss and Snyder, 2005; Koss et al., 2005).

Chemical defenses of CPB have been relatively well-studied. When attacked, adult CPB exude a dipeptide compound from the pronotum and elytra (Daloze et al., 1986). This chemical is toxic to ants and presumably other predators (Daloze et al., 1986), and discourages attack by birds (Hough-Goldstein et al., 1993). The same toxic dipeptide is in the hemolymph of CPB larvae, likely performing a similar defensive function (Armer, 2004). A second toxin in the hemolymph, leptinotarsin, appears to be important primarily in immune defense (Hsiao and Fraenkel, 1969; Armer, 2004; Armer et al., 2004). However, in contrast to the chemical arsenal of these beetles, behavioral defenses have been described only for CPB larvae facing attack by *Myiopharus* spp., which are parasitoid tachinid flies (Lopez et al., 1997). In other herbivorous beetles anti-predator behavior may be as important as chemical defense in avoiding attack by generalist predators (Snyder and Wise, 2000; Williams et al., 2001; Hlivko and Rypstra, 2003; Williams and Wise, 2003), suggesting that antipredator behavioral defenses could also be important for CPB.

Here, we report the behavioral responses of CPB larvae when contacted by predatory lady beetles (*H. convergens*) or damsel bugs (*N. alternatus*). First, we qualitatively described the repertoire of behaviors exhibited by larvae following predator contact. Next, first in small Petri-dish arenas and then again on potato plants, we observed encounters between CPB larvae of different instars and these predators, and quantified the nature and frequency of behavioral responses by the CPB larvae. Finally, in simple feeding trials we determined which of the two predators were able to successfully capture which of the CPB larval instars, so that we could relate the intensity of behavioral responses by the larvae to their true predation risk.

## 2. Materials and methods

### 2.1. The insects used in our experiments

The CPB larvae used in our experiments were collected as eggs from potato plants at Washington State University's Research and Education Center in Othello, WA. Eggs were housed in Petri dishes in an incubator (23 °C; 24 h light) until hatching, and then under the same conditions larvae were provided with a moist cotton wick and *ad libitum* potato foliage until reaching the desired instar. Staggered cohorts of CPB larvae were initiated every few days so that CPB larvae of all instars were simultaneously available when our experiments began.

Adult *H. convergens* and *N. alternatus* were hand-collected from an alfalfa field located at the Washington State University's Tukey Research and Education Center in Pullman, WA. These predators were housed no longer than 48 h in a dark incubator at 5 °C before

they were used in the experiment. This method of storage slowed predator feeding and movement, which helped reduce cannibalism and made it easier to capture predators for use in our experiments without causing them harm.

### 2.2. Qualitative observation of antipredator behavior by CPB larvae

In a series of untimed observations of predator-herbivore encounters on potato plants in the greenhouse, and observations of similar encounters in the field during a series of field experiments (e.g., Ramirez and Snyder, 2009), we developed a list of behaviors that CPB initiate following predator contact. We found these to include walking away, dropping from the plant, rearing up which may or may not be followed by regurgitating down the ventral surface of the body, wiggling (head held down, dorsal side arched up and forward, and movement of the body side-to-side), and defecation (see also Lopez et al., 1997). Walking away and dropping from a plant are obvious, and often effective, ways to reduce predation risk by escaping immediate contact with a predator (e.g., Losey and Denno, 1998). CPB larvae are bright orange, presumably aposematically colored, and rearing up and wiggling behaviors may highlight this larval coloration. Wiggling also may make it more difficult for predators to grasp hold of larvae and/or to insert their mouthparts to begin feeding (Lopez et al., 1997). CPB do not sequester plant toxins (Armer, 2004), but regurgitation of plant material itself rich in toxic glycoalkaloids may bring these compounds into contact with approaching predators (as suggested by Lopez et al. (1997)). We scored these behaviors in the observational studies described below, and found that they fully captured CPB responses to predator contact.

### 2.3. Predator-on-a-stick experiment

At the start of each trial we placed 30 each of second, third and fourth instar CPB larvae, individually, into 10-cm-diameter Petri dishes containing a potato leaf and a moistened cotton wick. These larvae were given 3 h to acclimate to their surroundings, and to assume feeding positions. During this time we affixed the posterior end of 10 adult *H. convergens*, and 10 adult *N. alternatus*, to the tip of a wooden dowel using a small droplet of wax (from a lit tea light candle). This held the predator in place, allowing us to initiate standardized, although artificial, predator-herbivore encounters. These encounters consisted of holding the predator in contact with each larva's head and pronotum, or touching the larva with a bare dowel as a control for contact in the absence of predator-specific cues (i.e., chemical, vibrational, etc.), for 30 s. Each larva was randomly assigned to one of three "contact" treatments: *H. convergens*, *N. alternatus*, or Control, resulting in 10 replicates of each contact-instar pairing. We then initiated bouts of contact as described above, and observed response behavior both during contact and for 60 s after contact was ended. Each CPB larva, and each predator-on-a-stick, was used in just a single encounter. Note that, because the leaf was sitting in a Petri dish, the response "drop from plant" was not possible in this experiment, and it also would have been impossible for a larva to walk to the underside of the leaf to escape from a predator.

We then repeated this experiment with a second set of 30 larvae of each instar, with 10 individuals of each instar assigned to each of the three contact treatments, and using the methodology described above. This formed a second temporal block in our analyses, and yielded 20 replicates of each contact-instar pairing across the two blocks.

### 2.4. Encounters between predators and CPB larvae on potato plants

Here, 10 individuals of each of three CPB larval instars (second, third and fourth) were placed singly onto a potted potato plant

(plants were 2 weeks past emergence) and given 1 h to establish a feeding site. Thereafter, one of three contact treatments was initiated: *H. convergens*, *N. alternatus*, or Control. Each larva received the same contact treatment three times with the behavioral response recorded during each encounter, as described in detail below.

For treatments receiving predator exposure, one predator individual was released at the base of the plant and allowed to initiate foraging. We then observed each replicate until the predator and CPB larva contacted one another, and recorded larval behavior for 1 min thereafter. Our recording of behavioral observations began only at contact because we never observed deployment of any antipredator behaviors until a predator had touched the CPB larva. Predators that attempted to fly away were captured and placed back at the base of the plant until on-plant foraging was established. In Control treatments larvae were prodded with the blunt end of a wooden dowel for 1 min, to reproduce physical contact in the absence of any predator-specific cues. After each period of contact the beetle larvae were allowed to re-establish feeding positions, and once feeding was resumed 5 min of feeding was allowed before we initiated a second round of predator exposure. This process was again repeated before the third round of predator contact. Predators (adult *H. convergens* and *N. alternatus*) were taken out of cold storage (described above) and given 1 h to become active just before use in a trial. Predator individuals and wooden dowels (control) were used for just one contact with each larva, and then were replaced.

### 2.5. Predation trial

In Petri-dish arenas we examined the risk of predation posed by *H. convergens* and *N. alternatus* adults on second, third and fourth instar CPB larvae. CPB larvae were placed individually into arenas housing a potato leaf and a moistened cotton wick, and then allowed 3 h to settle into feeding positions before a single predator was released, with 10 replicates established of each instar–predator combination. An additional 10 individuals of each instar were not paired with a predator, but otherwise treated as just described, serving as no-predator controls. Three days later we scored larvae as either alive or dead.

### 2.6. Analyses

For the predator-on-a-stick experiment, we used logistic regression (PROC GENMOD, SAS Institute, 2002) to determine the effects of potato beetle instar and predator exposure on the likelihood of each antipredator behavior (no response, walk, rear, vomit, wiggle, defecate). Each model included temporal block (1 or 2), potato beetle instar (2nd, 3rd, or 4th), predator exposure treatment (*H. convergens*, *N. alternatus*, or control), and the interaction between instar and predator, as explanatory variables. The significance of main effects was evaluated using likelihood-ratio drop-in-deviance tests (Ramsey and Schafer, 2002), with likelihood-ratio confidence intervals used to compare across levels for each significant effect. We used ANOVA tests with trial, beetle instar, predator, and the interaction between instar and predator to compare the total number of behavioral responses exhibited by beetles. We used LSMeans contrasts to separate means within each model effect for these total-response data.

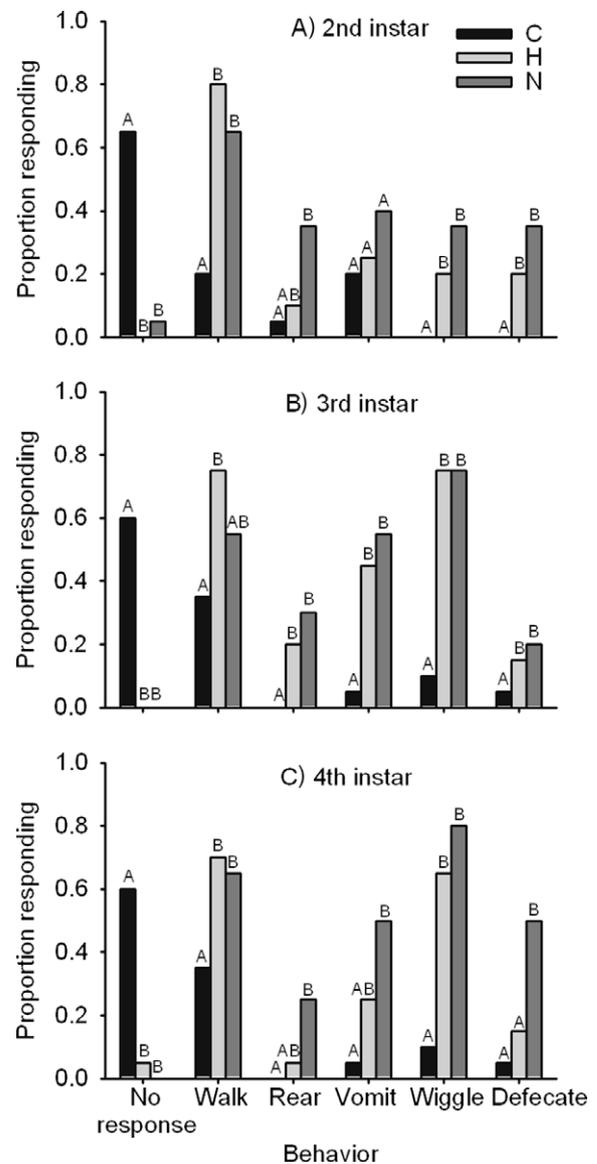
For the on-plant experiment, we used generalized estimating equations (GEE) (Liang and Zeger, 1986; Hardin and Hilbe, 2003) to analyze the likelihood of each antipredator behavior in a repeated measures logistic regression model (PROC GENMOD, SAS Institute, 2002). Each model included trial, potato beetle instar, predator exposure, encounter (repeated measure; 1, 2, or 3), and the interactions between instar and predator, instar and encounter, and predator and encounter, to explain the odds of each behavior

(no response, walk, drop, rear, vomit, wiggle, defecate). Further, we used repeated measures ANOVA with trial, beetle instar, predator, encounter, and the interactions between instar and predator, instar and encounter, and predator and encounter, to explain the total number of behavioral responses exhibited by beetles.

## 3. Results

### 3.1. Predator-on-a-stick experiment

Predator exposure affected all antipredator behaviors (Fig. 1, Table 1). With few exceptions, all beetle instars exposed to *Nabis* performed each antipredator behavior more often than the controls (Fig. 1). The two exceptions to this trend were vomiting for 2nd instar beetles, and walking for 3rd instar beetles. Similarly, beetles exposed to *Hippodamia* were more likely to perform all antipredator behaviors compared to controls across all instars, and were significantly less likely to exhibit no behavioral response (Fig. 1). In this case, the only exceptions were rearing and vomiting



**Fig. 1.** The proportion of Colorado potato beetle (CPB) larvae that exhibited each behavior across both laboratory trials with three predator treatments (Control, C; *Hippodamia convergens*, H; *Nabis alternatus*, N) for the (A) second, (B) third, and (C) fourth larval instars. For each behavior, bars with different letters were significantly different for that instar ( $\alpha = 0.05$ ).

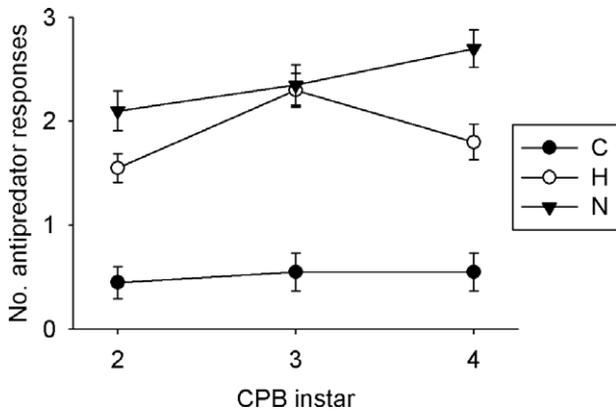
**Table 1**

Logistic regression analysis for likelihood of six potato beetle behaviors for beetles exposed to predators-on-a-stick based on four model effects.

Behavior	Model effect							
	Trial		Instar		Exposure		Instar × Exposure	
	$\chi^2$	P	$\chi^2$	P	$\chi^2$	P	$\chi^2$	P
No response	1.57	0.21	0.00	1.00	92.7	<0.0001	4.23	0.38
Walk	0.11	0.74	0.038	0.98	27.1	<0.0001	2.54	0.64
Rear	0.83	0.36	2.43	0.30	24.1	<0.0001	3.28	0.51
Vomit	0.50	0.48	0.69	0.71	24.4	<0.0001	5.20	0.26
Wiggle	0.60	0.44	18.4	0.0001	53.6	<0.0001	1.29	0.86
Defecate	2.15	0.14	1.51	0.47	21.6	<0.0001	3.71	0.45

for 2nd and 4th instar beetles, and defecating for 4th instar beetles (Fig. 1). Fourth instar CPB defecated more when exposed to *Nabis* than *Hippodamia* (Fig. 1c), but the likelihood of other behaviors did not differ significantly for the other instars exposed to these two predators (Fig. 1). The only behavior that varied between larval instars was wiggling (Fig. 1, Table 1), as both third ( $\chi^2 = 6.09$ ,  $P = 0.014$ ) and fourth instar larvae ( $\chi^2 = 7.59$ ,  $P = 0.0059$ ) were more likely to wiggle than second instar larvae.

The total number of antipredator behaviors performed by beetle larvae depended on predator exposure ( $F = 92.2$ ;  $df = 2, 170$ ;  $P < 0.0001$ ) and larval instar ( $F = 3.90$ ;  $df = 2, 170$ ;  $P = 0.022$ ), but there was no significant effects of trial ( $F = 0.74$ ;  $df = 1, 170$ ;  $P = 0.39$ ) or instar and exposure interactions ( $F = 2.00$ ;  $df = 4, 170$ ;  $P = 0.097$ ) (Fig. 2). Beetles performed more total behaviors when exposed to *Nabis*, followed by *Hippodamia* and controls (Fig. 2, Table 2). We also found that third and fourth instar larvae performed more antipredator behaviors than second instar larvae (Fig. 2, Table 2).



**Fig. 2.** Total number of antipredator responses across both laboratory trials for each CPB instar exposed to Control, C (●), *Hippodamia convergens*, H (○), and *Nabis alternatus*, N (▼).

**Table 2**

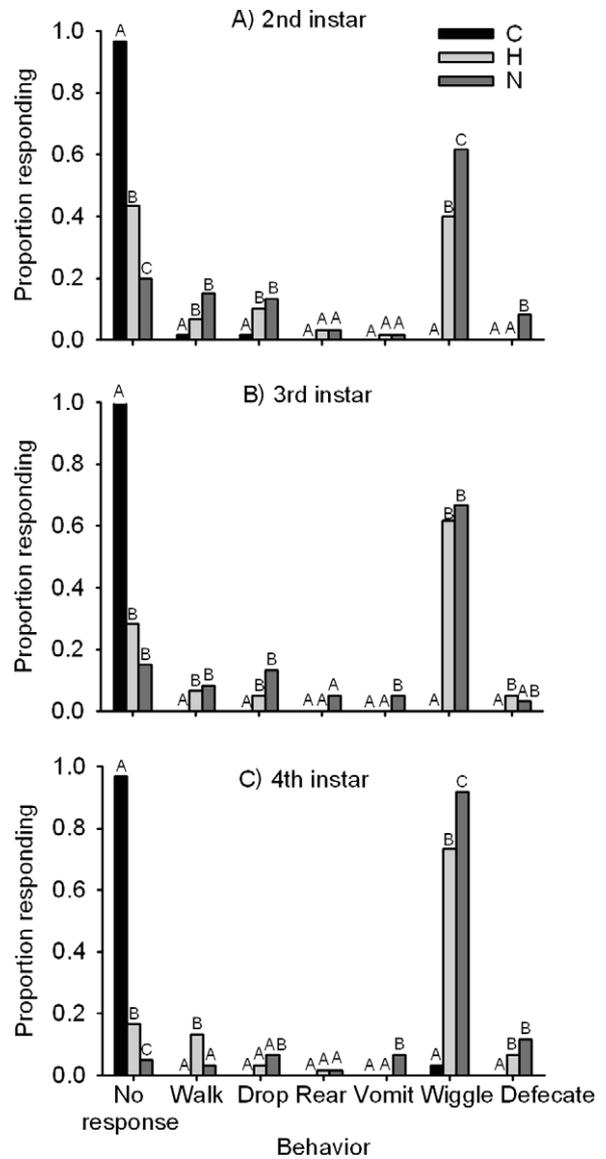
ANOVA for the effects of predator exposure and larval instar on the total number of antipredator responses performed by beetle larvae for beetles exposed to predators-on-a-stick. Each value was averaged across trials.

Model effect <sup>a</sup>	Treatment	Mean number of responses (±SE)
Predator exposure	Control	0.52 ± 0.099 A
	<i>Hippodamia</i>	1.88 ± 0.098 B
	<i>Nabis</i>	2.38 ± 0.11 C
Larval instar	2nd	1.37 ± 0.13 A
	3rd	1.73 ± 0.15 B
	4th	1.68 ± 0.15 B

<sup>a</sup> For treatments within each model effect, values not followed by the same letter were significantly different (LSMeans Contrast,  $\alpha = 0.05$ ).

3.2. Encounters between predators and CPB larvae on potato plants

Our experiments on potato plants indicated that contact with predators significantly increased the likelihood of each defense behavior except rearing compared to the bare-dowel control



**Fig. 3.** The proportion of CPB larvae that exhibited each behavior across both greenhouse trials and multiple encounters with three predator treatments (Control, C; *Hippodamia convergens*, H; *Nabis alternatus*, N) for the (A) second, (B) third, and (C) fourth larval instars. For each behavior, bars with different letters were significantly different for that instar ( $\alpha = 0.05$ ).

(Fig. 3, Table 3). Overall, beetles exposed to *Nabis* were more likely to perform each antipredator behavior except rearing compared to controls, and were less likely to have no response (Fig. 3). However, the difference between the likelihood of walking, dropping, vomiting, and defecating for beetles exposed to *Nabis* also depended on larval instar (Fig. 3). Beetles exposed to *Hippodamia* did not rear or vomit more than controls, but were more likely to perform the other antipredator behaviors we measured, and were less likely to have no response (Fig. 3). However, the difference between the likelihood of dropping and defecating for beetles exposed to *Hippodamia* also depended on larval instar (Fig. 3). CPB was overall more likely to wiggle and vomit, and less likely to exhibit no behavioral response when exposed to *Nabis* compared to *Hippodamia* (Fig. 3). The likelihood of walking and defecating also differed for CPB exposed to *Nabis* versus *Hippodamia* in at least one larval instar (Fig. 3). The only antipredator behavior that varied between instars was wiggling (Fig. 3, Table 3). Fourth instars were more likely to wiggle than second ( $\chi^2 = 7.99$ ,  $P = 0.0047$ ) and third instars ( $\chi^2 = 6.57$ ,  $P = 0.010$ ), and were marginally less likely to have no behavioral response compared to both second ( $\chi^2 = 3.54$ ,  $P = 0.060$ ) and third instar larvae ( $\chi^2 = 3.22$ ,  $P = 0.073$ ). For all models, we detected no significant effects of encounter or any interaction terms ( $P > 0.05$  for all terms), indicating that antipredator behaviors were consistent over time.

The total number of antipredator behaviors performed by beetle larvae on plants depended on predator exposure ( $F = 154.5$ ;  $df = 2$ ,  $170$ ;  $P < 0.0001$ ) and larval instar ( $F = 4.75$ ;  $df = 2$ ,  $170$ ;  $P = 0.0098$ ), but there were no significant effects of trial ( $F = 0.63$ ;  $df = 1$ ,  $170$ ;  $P = 0.43$ ), encounter ( $F = 0.51$ ;  $df = 2$ ,  $170$ ;  $P = 0.60$ ) or any interaction term ( $P > 0.19$  for all terms) (Fig. 4). As in the laboratory experiment, beetles performed more total behaviors when exposed to *Nabis*, followed by *Hippodamia* and controls (Fig. 4, Table 4). Further, fourth instar larvae performed more antipredator behaviors than second or third instar larvae (Fig. 4, Table 4).

### 3.3. Predation trial

*N. alternatus* killed significantly more second instar CPB (6/10) than *H. convergens* (1/10) ( $\chi^2 = 5.50$ ,  $P = 0.019$ ; Fig. 5), whereas no third or fourth instars were killed by either predator (Fig. 5). Control larvae never died during the course of the experiment, suggesting that dead larvae in dishes including predators truly resulted from predation.

## 4. Discussion

Our results indicate that CPB larvae exhibit a diverse array of behaviors when contacted by generalist predators, including walking away, dropping from plants, rearing up and perhaps also vomiting down the ventral surface, wiggling, and defecating. All of

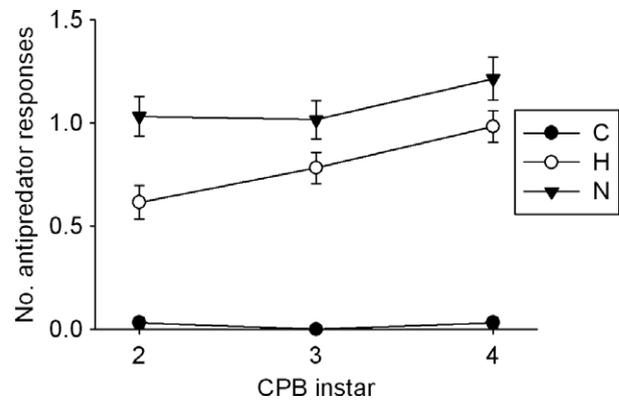


Fig. 4. Total number of antipredator responses across both greenhouse trials and multiple encounters for each CPB instar exposed to Control, C (●), *Hippodamia convergens*, H (○), and *Nabis alternatus*, N (▼).

Table 4

Repeated measures ANOVA for the effects of predator exposure and larval instar on the total number of antipredator responses performed by beetle larvae for beetles exposed to predators on plants. Each value was averaged across trials.

Model effect <sup>a</sup>	Treatment	Mean number of responses ( $\pm$ SE)
Predator exposure	Control	0.022 $\pm$ 0.011 A
	<i>Hippodamia</i>	0.79 $\pm$ 0.046 B
	<i>Nabis</i>	1.09 $\pm$ 0.056 C
Larval instar	2nd	0.56 $\pm$ 0.052 A
	3rd	0.60 $\pm$ 0.051 A
	4th	0.74 $\pm$ 0.058 B

<sup>a</sup> For treatments within each model effect, values not followed by the same letter were significantly different (LSMeans Contrast,  $\alpha = 0.05$ ).

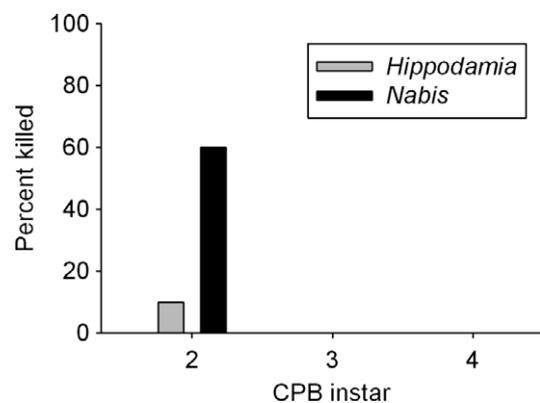


Fig. 5. Mortality over 72 h of second, third and fourth instar potato beetle larvae to *Hippodamia convergens* (*Hippodamia*) or *Nabis alternatus* (*Nabis*) predators.

Table 3

Repeated measures logistic regression analysis for likelihood of seven potato beetle behaviors for beetles exposed to predators on plants based on four main model effects.<sup>a</sup>

Behavior	Model effect <sup>a</sup>							
	Trial		Instar		Exposure		Encounter	
	$\chi^2$	$P$	$\chi^2$	$P$	$\chi^2$	$P$	$\chi^2$	$P$
No response	2.08	0.15	6.39	0.041	365.2	<0.0001	1.62	0.45
Walk	1.70	0.19	3.47	0.18	19.9	<0.0001	1.28	0.53
Drop	28.2	<0.0001	3.65	0.16	22.3	<0.0001	0.68	0.71
Rear	1.10	0.29	0.0005	1.0	7.73	0.021	0.0029	1.0
Vomit	1.08	0.30	0.00	1.0	9.24	0.0099	0.00	1.0
Wiggle	8.69	0.0032	14.4	0.0007	286.1	<0.0001	4.25	0.12
Defecate	0.49	0.48	0.0	1.0	16.1	0.0003	0.00	1.0

<sup>a</sup> Interaction terms (Instar  $\times$  Treatment, Instar  $\times$  Encounter, Exposure  $\times$  Encounter) that were included in the full model are not shown, as none were significant ( $P > 0.05$  for all models).

these behaviors are more likely to occur when larvae contact a real predator than a bare wooden dowel, indicating these responses do not reflect a simple reaction to disturbance. Second, larvae react differently when contacted by *N. alternatus* than *H. convergens* (Figs. 1–4), suggesting CPB possess some ability to distinguish among predator species. Finally, the relative frequency of particular behaviors differ among instars (Figs. 1 and 3) suggesting ontogenetic changes in the repertoire of behavioral defenses CPB larvae deploy toward the most common predators encountered in the field. In most respects, the behavior of CPB larvae touched with a predator fixed to a wooden dowel were similar to those observed in unfettered predator–prey contacts on potato plants, suggesting that our predator-on-a-stick technique may be a useful way to conduct carefully controlled manipulations of the frequency and nature of predator–CPB encounters. However, CPB larvae generally responded more intensely to predators-on-a-stick than real, unfettered predators on potato plants (Figs. 1 and 3), perhaps reflecting the generally longer duration of simulated than real predator encounters. Also, simulated encounters always involved contact with the larva's head, whereas real contacts sometimes involved contact with less-sensitive structures.

Our predation trials suggest *N. alternatus* poses a greater risk to early-instar CPB than *H. convergens* (Fig. 5). This result raises the intriguing possibility that CPB scale the intensity of behavioral defense to risk given our data indicating that larvae consistently respond more strongly to *N. alternatus* than *H. convergens*. At the same time, we also note that third and fourth instar CPB respond to both predators despite an absence of mortality in our predation trials, suggesting in this case a mismatch between predation risk and the intensity of antipredator response. One possibility is that the antipredator behaviors of third and fourth instars are so effective they fully protect older larvae from predation. Relatively few studies have tracked developmental changes in antipredator behaviors used by insect herbivores as they move from relatively vulnerable smaller stages to relatively invulnerable larger stages.

We are aware of only one other study documenting antipredator behavior of CPB larvae. Lopez et al. (1997) observed field encounters between CPB larvae and foraging *Myiopharus* spp. flies, specialist tachinid parasitoids of CPB, in organic potato fields in Massachusetts, USA. Intriguing are the many similarities and differences between our results and this earlier work. Lopez et al. (1997) reported that roughly half of all CPB larvae, across instars, responded to tachinid attack by deploying deterrent behaviors. This is roughly similar to the response rate to generalist predators that we observed (Figs. 1 and 3). Lopez et al. (1997) recorded most of the antipredator behaviors that we found in response to generalist predators, while recording an additional behavioral response, thanatosis (“playing dead”), that we never observed. A second additional antipredator behavior, group flailing, was initiated in the Lopez et al. study by groups of early-instar CPB larvae and so could not have been recorded in our experiments (which examined only single CPB larvae). Likewise, we noted one behavior, rearing, that Lopez et al. (1997) never recorded. Also in contrast to our findings, these authors observed “tensing” (called wiggling in our study) primarily among fourth instar CPB, and regurgitation only among third instar larvae; we commonly observed these behaviors among both third and fourth instar larvae (Figs. 1 and 3). Of course, it is unclear whether these differing results reflect truly different behavioral responses to generalist predators versus specialist parasitoids, versus variation among CPB populations or other differences in methodology between the two studies. For this reason a study of CPB antipredator behavior including both predators and parasitoids would clearly be worthwhile. Also, while no supporting data were reported, Lopez et al. (1997) mention in passing that their CPB larvae never initiated antipredator behaviors when contacted by predatory pentatomid bugs, in sharp contrast to the fre-

quent deployment of such defenses against *Nabis* bugs in our study. Again, it would be worthwhile to further examine these contrasting results.

Aquatic, non-insect systems have produced abundant evidence that prey species often differentiate among predator species and scale their antipredator response to the risk each predator presents (e.g., Turner et al., 1999; Relyea, 2003; Wohlfahrt et al., 2006; Smith and Awan, 2009). However, the relationship between predation risk and the intensity of antipredator response has rarely been examined for insect herbivores. Most examples come from herbivorous beetles, where ability to assess risk is not always perfectly developed. For example, spotted cucumber beetles (*Diabrotica undecimpunctata howardi*) strongly reduced their feeding when exposed to large and dangerous *Hogna helluo* wolf spiders, while reacting relatively weakly to a variety of other spider and ground beetle species almost entirely incapable of killing cucumber beetles (Snyder and Wise, 2000). Still, cucumber beetles somewhat reduced their feeding activity when exposed to domestic crickets (*Acheta domestica*), included as disturbance controls, that posed no predation risk whatsoever (Snyder and Wise, 2000). Similarly, adult Japanese beetles (*Popillia japonica*) dramatically reduced their feeding on soybean plants when exposed to chemical cues left by the deadly wolf spider *H. helluo*, but reacted only slightly to cues left by the small and non-threatening wolf spider *Pardosa milvina* (Hlivko and Rypstra, 2003). However, when facing orb-weaving spiders of two different species, Japanese beetles reacted less strongly to the most dangerous spider (Hlivko and Rypstra, 2003), indicating that their evaluation of true predation risk was not entirely accurate. At least one other non-beetle insect herbivore, the red-legged grasshopper (*Melanoplus femurrubrum*), also appears unable to consistently recognize and avoid all truly dangerous spider predators (Schmitz and Suttle, 2001). Danner and Joern (2003) similarly examined the intensity of antipredator response by nymphs of the grasshopper *Ageneotettix deorum* to *Schizocosa* spp. spiders, and found that behavioral responses to the predators were apparent only in early-stage nymphs. However, true predation risk of the spiders to the different grasshopper stages was inferred, but not directly examined in that study. Taken together our results suggest CPB larvae follow the larger trend that insect herbivores imperfectly tailor the intensity of their antipredator response to reflect the true predation risk. For example, we found that CPB larvae regularly responded to lady beetle and bug predators too small to successfully kill larvae of that stage (i.e., compare Figs. 1 and 3 to Fig. 5). On the other hand, Schmitz (2007) suggests that the physiological costs of continuous vigilance for actively-hunting predators like the lady beetle *Hippodamia*, which may be anywhere in the environment at any given time, leads to a general insensitivity to the risks posed by such predators. In contrast, the stationary location of a sit-and-wait predator like *Nabis* can be identified, and defenses selectively deployed when approaching the predator. In this case, the relative sensitivities of CPB larvae to attack by *Hippodamia* versus *Nabis* may reflect the hunting styles of these two predators more generally, rather than any calibration of the relative risks posed by these two predator species in particular (Schmitz, 2007).

In several other systems, it is clear that beetle herbivores are able to detect predators from a distance using some combination of visual, tactile and chemical cues (Snyder and Wise, 2000; Williams et al., 2001; Hlivko and Rypstra, 2003; Williams and Wise, 2003). However, detection over distance did not seem to be occurring with our CPB larvae. In our experiments we never observed the initiation of behavioral changes until the predator contacted the larva. At the same time, simply bumping a larva with a bare wooden dowel did not initiate the full complement of responses typical when a predator initiated contact, and CPB response differed based on predator species identity. Altogether, these results

suggest a degree of refinement in the predator-detection ability of the CPB larvae, although the precise cue(s) that they are using to identify predators seem to require physical contact with the predator and thus operate only at close range. The situation appears to be quite different when CPB larvae are attacked by tachinid fly parasitoids, which the larvae can detect as much as 0.5 m away (Lopez et al., 1997). This may again reflect the relative risks posed by parasitoids versus generalist predators, with the parasitoid possessing a relatively acute ability to track its host through the environment in comparison to a generalist.

A question left unanswered by our work is whether there is a fitness advantage associated with some of the CPB behavioral responses to predators that we observed. Of course, the advantages of walking away or dropping from plants to escape from predators are obvious and have been demonstrated (e.g., Losey and Denno, 1998), and defecation is perhaps understandable when facing a fearsome predator one's own size. However, the escape benefit of regurgitation, for example, is less obvious. The regurgitant of adult CPB apparently is not toxic or repellent to ants who readily consume it (Deroe and Pasteels, 1977). Toxic glycoalkaloids of the host plant are not sequestered and concentrated within the bodies of CPB larvae, but likely are present in the plant material within the insect's digestive system (Armer, 2004). Thus, regurgitating down the ventral surface may be a way to put toxic plant glycoalkaloids between the larva and its would-be predator (Lopez et al., 1997). Perhaps most difficult to explain is the wiggling behaviors common to all stages, which seems to increase in frequency as the larvae go through development. Presumably such behavior only serves to draw a predator's attention to the aposematically colored larvae. Thus wiggling may make the warning signal more readily apparent to vertebrates or other visually-hunting predators, a possibility that awaits testing. Some lepidoptera larvae use vigorous thrashing behavior to prevent parasitoids from successfully inserting their ovipositor (e.g., Wang and Keller, 2002), and a similar benefit has been suggested for the gentler movements deployed by CPB larvae (Lopez et al., 1997).

Our work has several interesting implications for biological control of CPB that are worthy of further study. First, our results indicate that CPB commonly reacts to predators that may pose little (or no) true risk. In many other studies the initiation of antipredator behavior by herbivores substantially reduces the herbivore's feeding damage to plants (Werner and Peacor, 2003; Schmitz et al., 2004; Preisser et al., 2005). If this is also the case for CPB, then predators incapable of killing particular CPB stages may nonetheless provide a degree of protection to potato plants. Behaviorally-mediated protection of plants by predators also means that simple counts of CPB numbers may underestimate the true biocontrol benefit of some predators. Indeed, the effects of predators that do not kill herbivores but reduce their feeding may only be detected by measuring plant biomass (e.g., Snyder and Wise, 2000). Finally, a recent study (Ramirez and Snyder, 2009) suggests that non-lethal exposure of CPB larvae to predators earlier in development later weakens the ability of CPB pupae to fight entomopathogen infection. One possible explanation for this predator-pathogen synergism is that, by initiating antipredator behavioral defenses, predators drain physiological resources otherwise used to fuel the beetles' immune response (e.g., Rigby and Jokela, 2000; Slos et al., 2009). The work reported here suggests that this mechanism could operate even when CPB face predator species that pose little true risk of killing the beetles. A key gap in our knowledge is just how often non-lethal encounters occur between CPB larvae and their predators under field conditions, as the relative frequencies of lethal and non-lethal encounters can be used to calculate their relative importance for plant protection (Nelson and Rosenheim, 2006). More generally, ecologists are growing increasingly aware of the importance of non-lethal interactions within unmanaged

food webs (Borer et al., 2002; Goudard and Loreau, 2008), and such interactions may also warrant greater attention in biological control systems (Prasad and Snyder, 2006).

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