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Exposure to predators, but not intraspecific competitors, heightens herbivore susceptibility to entomopathogens

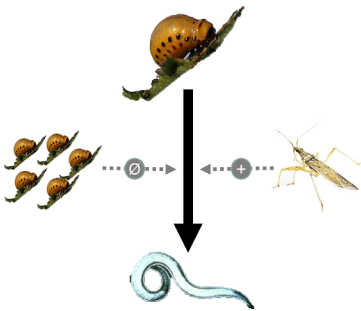
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GRAPHICAL ABSTRACT



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ABSTRACT

Prey commonly must compete with conspecifics for resources while also defending themselves against predators. Both competition and defense can reduce feeding opportunities, or otherwise strain prey energy reserves, even when the prey is not killed. This suggests that stress from competition and anti-predator defense might yield non-lethal harm that differs quantitatively rather than qualitatively. We examined this possibility for an herbivorous prey species, the Colorado potato beetle (*Leptinotarsa decemlineata*), feeding among differing numbers of intraspecific competitors while also avoiding predation by *Nabis alternatus* and *Hippodamia convergens*. In a field cage experiment, predators and intraspecific competitors similarly reduced the herbivores' larval survival. Yet, only exposure to predators heightened the risk of beetles later being killed by entomopathogenic nematodes and fungi, as the beetles pupated in the soil. This suggests that the threat of predation was exerting physiological harm to the prey, although it was also possible that a tradeoff existed where those beetles most resistant to predator attack were most susceptible to pathogen infection. In a second experiment, we found that regular touching of larvae by predatory *Nabis* or *Hippodamia*, in the absence of actual predation, consistently induced predator-avoidance behaviors by the larvae (e.g., rearing, vomiting, wiggling, etc.) that might be energetically costly. Indeed, only being touched by predators, but not by a wooden dowel as a disturbance control, led to greater susceptibility to entomopathogens as pupae. Altogether, our findings suggest that non-lethal interactions with predators might carry a particular cost to beetles in heightened pathogen susceptibility, not seen when facing competitors or simple disturbance. Additional work is needed to determine the specific physiological mechanism underlying this apparent synergism in the effects of predators and entomopathogens, which spans prey life stages.

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1. Introduction

Predators and competitors are two ecological threats that most herbivores must navigate (Relyea and Auld, 2005; Price et al., 2011). At first glance, the degree and immediacy of harm exerted by these two opponents seems to be quite different. After all, successful predation ends an herbivore's life, while competitors drain shared resources and decrease fitness without causing death (Kaplan and Denno, 2007). However, differences may be fewer when considering non-lethal predator effects (Relyea and Auld, 2005). Herbivores often deploy a wide variety of morphological, chemical, and behavioral defenses that successfully block predation, yet carry energetic costs (Lima and Dill, 1990; Beckerman et al., 2007; Preisser et al., 2007; Rinke et al., 2008; Schmitz, 2007, 2009; Buchanan et al., 2017). For example, herbivores may reduce feeding activity or enter refuges when predators are detected, with resulting lost feeding opportunities ultimately reducing growth rate and/or fecundity (e.g., Schmitz et al., 1997; Nelson et al., 2004; Schmitz, 2007, 2009). So, there is reason to think that non-lethal effects of predators might carry broadly similar energetic costs to those resulting from intra- or interspecific competition (Cinel et al., 2020).

A loss of energetic reserves due to competition can trigger tradeoffs with other physiological functions (Rinke et al., 2008; Tigreros et al., 2017). One common route through which this is seen is reduced immune function (Lively et al., 2014). For example, food-limited insects often are more susceptible to infection by viral, bacterial, or fungal pathogens (e.g., Rantala et al., 2000; Cotter et al., 2004; Kapari et al., 2006; Lee et al., 2006; Rantala and Roff, 2007). This is but one example of the many routes through which physiological stress and disease are related (Steinhaus, 1958). Perhaps less appreciated are links between threat of predation and stress-related decline in immune function (Rigby and Jokela, 2000; Cinel et al., 2020). For example, *Daphnia* exposed to cues that fish are present deploy energetically-costly morphological defenses that render them more susceptible to parasites (Yin et al., 2011). Likewise, wood frog (*Rana sylvatica*) tadpoles exposed to caged dragonfly larvae exhibit slower development and weakened cellular immunity (Seiter, 2011). In this way predators might facilitate the infection of shared/prey hosts by pathogens, leading to predator-pathogen synergy and a positive relationship between natural enemy biodiversity and biological control (e.g., Hawkins et al., 1997; Crowder et al., 2010; Duffy et al., 2011; but see Groner and Relyea, 2015). More broadly, it seems that predation and competition might yield roughly equivalent indirect benefits to pathogen transmission, through a similar draining of physiological resources that might otherwise be devoted to immune defenses (Cinel et al., 2020).

Here, we consider the individual and combined impacts of intraspecific competition and threat of predation on the ability of herbivorous Colorado potato beetles (*Leptinotarsa decemlineata*) to resist infection from entomopathogenic fungi and nematodes. The beetles transition between two very different communities of natural enemies as they go through development (Ramirez and Snyder, 2009). Aboveground in plant foliage, potato beetle eggs and larvae face a diverse community of generalist predatory insects (Koss et al., 2005). While specialist tachinid fly (*Myiopharus* spp.) and stink bug (*Perillus* spp.) natural enemies are common threats to potato beetles elsewhere in their range, these specialists are rarely found in eastern Washington state (Koss et al., 2005). Later, after burrowing into the soil to pupate, potato beetles are attacked by entomopathogenic nematodes and fungi (Cantelo and Nickle, 1992; Wraight and Ramos, 2002). Potato beetles approached by predators deploy a diversity of behavioral responses, such as rearing up and vomiting on themselves, that not only deter predators (Deroy and Pasteels, 1977) but which also might carry energetic costs (Ramirez et al., 2010). Indeed, this tradeoff between energy devoted to defense against predation, versus resources needed to mount an effective immune defense, appears to explain why potato beetle larvae that escape predation are later rendered more susceptible to being killed by entomopathogenic nematodes and fungi as pupae

(Ramirez and Snyder, 2009). However, previous work could not reject a competing hypothesis, which is that there is an inherent tradeoff between the ability to escape predation versus the ability to resist pathogen infection (e.g., Kraaijeveld and Godfray, 1997); this could lead to predators selectively killing those individuals that otherwise would be most capable of avoiding being killed by pathogens. Here, we expand upon the earlier work by considering effects of intraspecific competition alongside those of escaping predation, to examine whether interaction with intraspecific competitors or predators will similarly enhance susceptibility to entomopathogens. Further, we work to more carefully link this apparent weakening of immune defense to repeated deployment of defensive behaviors triggered by predators, versus simple interruption of feeding or any tradeoff where individuals best at evading predators are worst at avoiding infection.

2. Methods

Our work had two components. First, we conducted a field cage experiment where we raised Colorado potato beetle larvae among varying densities of intraspecific competitors, in the presence versus absence of generalist predators, before surviving pupae faced infection by entomopathogenic fungi and nematodes. Second, we reared potato beetle larvae in the laboratory while repeatedly challenging them with predators glued to the end of a wooden dowel, versus a bare-dowel disturbance control, to induce anti-predator behaviors before exposing the potato beetle larvae to entomopathogens as pupae. Importantly, because the laboratory experiment did not allow actual predation to occur, any resulting enhancement in the proportion of potato beetles killed by pathogens following predator exposure could not be because the predators had selectively killed those individuals best at escaping pathogen infection (e.g., Kraaijeveld and Godfray, 1997).

2.1. Predation and intraspecific competition manipulation in field cages

The field cage experiment was conducted just outside of Washington State University's Insect Behavior Laboratory and Greenhouse Facility in Pullman, WA, from April to August 2013. Our experimental units were BugDorm Insect Rearing Tents (fine mesh, 60 × 60 × 60-cm cages; <https://shop.bugdorm.com/>), set up in a bare soil and insecticide-free, freshly tilled field. Each cage enclosed two potted potato plants (*Solanum tuberosum*, var. Ranger Russet), reared in a greenhouse (16:8 L:D cycle) for 6 weeks before use in the experiment, with plants watered every other day. We added insects to establish a 2 × 2 factorial cross of two levels of potato beetle density (high, low) and two levels of predation pressure (predators absent, predators present). This yielded four unique treatment combinations: (1) **Low density, no predators**, 40 just-hatched potato beetle larvae per cage but no predators released; (2) **Low density, predators**, 40 potato beetle larvae and also 4 *Nabis alternatus* and 4 *Hippodamia convergens* adults; (3) **High density, no predators**, 80 potato beetle larvae and no predators; and (4) **High density, predators**, 80 potato beetle larvae and also 4 *Nabis* and 4 *Hippodamia* adults. Each experimental block included 8 cages of each of the four treatments (32 cages total), and we conducted two blocks, for a total of 64 replicates. Potato beetle larvae were collected one day after they had hatched and placed evenly on the leaves of the potato plants in each cage, and allowed 24 h to assume feeding positions before predators were released into those cages receiving predators. Potato beetle eggs were collected from commercial potato fields in eastern Washington, then incubated at 23° C, with 24 h light, in an environmental chamber until hatching. In order to establish our potato beetle density treatments, we could not add only larvae hatched from a single clutch into each cage, such that cages did not necessarily contain only full or half siblings as would be the case in the natural situation when a single egg mass hatched on a plant. Adult *H. convergens* and *N. alternatus* were fresh-hand-collected from commercial alfalfa (*Medicago sativum*) fields in eastern Washington State.

After insect release, we then allowed natural development to the fourth larval instar (usually taking 10–20 days). Cages were checked every-other day, during watering, to determine larval instar of the potato beetles, until some beetles began to reach the third instar; thereafter, cages were checked daily. Late-fourth-instar larvae were collected from each cage each day, and a subset of 10, randomly-selected larvae were moved to pupation arenas where they were exposed to the fungal pathogen *Beauveria bassiana* and the entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema feltiae* (see also Jabbour et al., 2011). Our pathogen-exposure arenas were 32-oz plastic deli containers containing 700 g of soil collected from the same field where the experiment was conducted, and housed in a greenhouse (23 °C ± 3, 16 h daylight). We used distilled water to dampen the soil, and then used aqueous suspensions to release approximately 1000 each of the entomopathogenic nematodes *H. bacteriophora* and *S. feltiae*, and a solution of approximately 40 million spores of the fungus *B. bassiana*, strain GHA; these inoculation rates reproduce typical infection levels seen in commercial organic potato fields (Ramirez and Snyder, 2009; Jabbour et al., 2011). The cups were checked daily to record any pupae that emerged as adults. We allowed 5 weeks for the pupae to emerge before destructively searching the soil to verify that no living pupae remained; none were found. It is sometimes possible to differentiate between infection by the different pathogen taxa that we included based on pupal color/condition (e.g., Kaya and Stock, 1997). However, in order not to disturb the natural course of infection and/or pupal development, we did not attempt the regular recovery of infected pupae from the soil that would be needed to visually score the infection status of each individual.

Data were analyzed using logistic regression, because survival was a binary variable, with the factors: potato beetle larval density (High, Low), predators (Present, Absent), and Block (1, 2), and all interactions among these terms (Table 1). We ran separate models to explore effects of intraspecific competition and predation on larval survival and pupal survival. Analyses were performed using the 'glm' function in the package 'lme4' (Bates et al. 2015) in R (R Core Team, 2019).

2.2. Bug-on-a-stick experiment

Potato beetles used in these experiments were collected as eggs as described above, and housed individually in 100 × 15-mm Petri dishes, provided *ad libitum* potato foliage and a moistened cotton wick, until reaching the fourth instar, in an environmental chamber under conditions described above. Predators were collected in alfalfa fields as described above, and stored in an incubator at 5 °C until use in encounter simulations.

Fourth-instar potato beetle larvae were repeatedly exposed to either an *H. convergens* lady beetle, an *N. alternatus* damsel bug, or a no-predator control. Non-lethal predator exposure was achieved by affixing the abdomen of a predator to the end of a wooden dowel with super glue; this allowed the head and legs to move freely (see Ramirez et al., 2010). The no-predator control treatment consisted of a bare dowel. During each contact, the larva was repeatedly, lightly touched, for 30 sec, with the predator's head/thorax or the bare stick; responses from the larvae were recorded for the next 60 sec. Following Ramirez et al. (2010), behaviors scored as an anti-predator response were: walking away, rearing up on hind legs, regurgitating onto ventral surface, wiggling the body, and/or defecating. This contact was executed twice daily, once in the morning between 0800 and 1000 h and once in the afternoon between 1300 and 1500, each day until a larva initiated pupation. We assigned 20 beetle larvae to each of the three treatments per experimental block, with two blocks yielding a total of 180 replicates. These blocks were conducted sequentially from June to July 2013.

We used logistic regression to determine effects of predator exposure on the likelihood of displaying antipredator behavior, with walking, rearing, vomiting, wiggling, and/or defecating in response to

touching. All of the antipredator behaviors described above were summed to determine the total number of anti-predator behaviors deployed (see also Ramirez et al., 2010). Each model included the predator treatment, temporal block, and their interaction. To assess whether potato beetle survival to pupation, and then eclosion to adult, was influenced by exposure to predators, we fit generalized linear models with a binomial distribution and logit link function using the 'glm' function in the 'lme4' package (Bates et al., 2015). Predator treatment, temporal block, and their interaction were designated as fixed effects. Significance of treatments was assessed by Wald Chi Square analysis of deviance (Type II) using the 'Anova' function in the 'car' package (Fox and Weisberg, 2019).

3. Results

3.1. Predation and intraspecific competition manipulation in field cages

The presence of predators did not affect survival of larval potato beetles ($Z = -0.70$, $P = 0.49$; Table 1), but heightened densities of conspecific competitors significantly reduced larval survival ($Z = -5.06$, $P < 0.0001$). There was a significant interaction between predator presence and larval density (Fig. 1A, Table 1, $Z = 3.48$, $P = 0.0005$), suggesting that mortality due to predators versus intraspecific competition was substitutive rather than additive. Although larval survival was significantly higher in Block one compared with Block 2 ($Z = -2.66$, $P = 0.0079$), the effects of predators and larval density did not differ across blocks (all $P > 0.11$; Table 1).

Survival of potato beetles through the pupal stage, during which they were exposed to entomopathogenic nematodes and fungi, was significantly lower for those beetles previously exposed to predators compared with those that were not (Fig. 1B, Table 1, $Z = 2.27$, $P = 0.023$). However, density of conspecific competitors had no effect on pathogen susceptibility ($P > 0.40$ for the main effect and all interactions; Table 1). These results were consistent across blocks (all $P > 0.28$; Table 1) and were qualitatively similar with block held as a random effect (SI Table 1).

3.2. Predator-on-a-stick experiment

Larvae responded (wiggled, walked, etc.) more frequently when exposed to either *Nabis* predatory bugs ($Z = 4.062$, $P < 0.0001$) or *Hippodamia* lady beetles ($Z = 2.033$, $P = 0.042$), than the no-predator control (Fig. 2A). Predator-mediated increases in larval response rates were stronger in the second experimental block, but overall patterns

Table 1

Logistic regression of potato beetle survivorship for an experiment where predators were present versus absent, and where larvae were initially established at lower versus higher densities; the experiment was conducted as two blocks separate in time.

Factor	Estimate	SE	Z	P
<i>Larval survival</i>				
Predator presence	-0.12	0.17	-0.70	0.49
Larval density	-0.89	0.18	-5.06	< 0.0001
Predator × density	0.95	0.27	3.48	0.0005
Block	-0.43	0.16	-2.66	0.0079
Predator × block	0.27	0.23	1.17	0.24
Density × block	0.22	0.23	0.96	0.34
Predator × density × block	-0.58	0.36	-1.60	0.11
<i>Adult survival</i>				
Predator presence	0.81	0.36	2.27	0.023
Larval density	-0.035	0.29	-0.12	0.91
Predator × density	-0.36	0.53	-0.67	0.50
Block	-0.23	0.28	-0.81	0.42
Predator × block	-0.48	0.44	-1.07	0.28
Density × block	-0.0091	0.38	-0.024	0.98
Predator × density × block	0.50	0.66	0.76	0.45

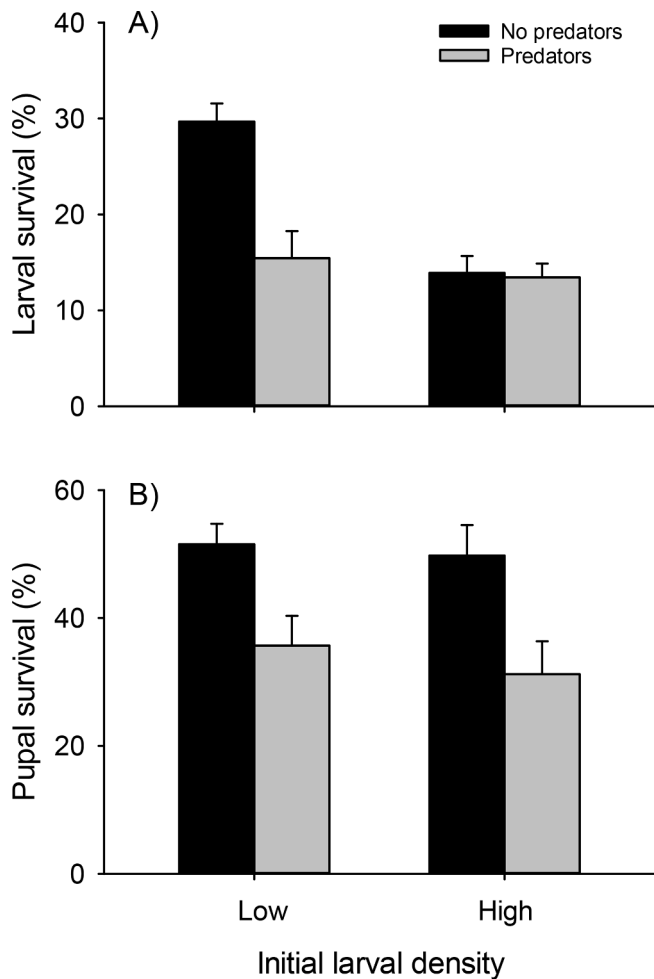


Fig. 1. (A) The proportion of larvae surviving in cages with low (40 larvae) or high (80 larvae) initial larval densities and with two predator treatments (present/absent). (B) The proportion of CPB completing development from larvae to adult across these same treatments. In both panels, results are pooled across blocks, as density and predator effects were similar across blocks ($P > 0.12$ for all analyses).

were similar between blocks ($Nabis \times \text{Block}$: $Z = 2.316$, $P = 0.021$; $Hippodamia \times \text{Block}$: $Z = 4.194$, $P < 0.0001$; Fig. 2A).

Overall, potato beetle larvae were less likely to survive to pupation when touched by a *Nabis* bug than when touched by a *Hippodamia* lady

beetle or a bare wooden stick (Predator: $\chi^2 = 8.3443$, $df = 2$, $P = 0.015$; Fig. 2B). However, this effect varied among experimental blocks, such that there appeared to be minimal effects of predator treatments on larval survival in the first experimental block, but large reductions in larval survival when larvae were touched by damsel bugs in the second block (Predator \times Block: $\chi^2 = 15.35$, $df = 2$, $P = 0.0005$; Fig. 2B). Of those beetles that survived to pupation, beetles were 42% less likely to emerge from pupae after exposure to entomopathogenic fungi and nematodes if touched by either predator (Predator: $\chi^2 = 7.62$, $df = 2$, $P = 0.022$; Fig. 2C). This effect was consistent between experimental blocks, though overall pupal survival was greater in the second block (Block: $\chi^2 = 5.62$, $df = 1$, $P = 0.018$; Fig. 2C).

4. Discussion

Previously, we have shown that facing attack by predators earlier in development, without being killed, renders Colorado potato beetles more susceptible to being killed by entomopathogens as the beetles pupate in the soil (Ramirez and Snyder, 2009). In another study, we found that the anti-predator behaviors that potato beetle larvae have long been known to deploy when contacted by predators (e.g., Deroe and Pasteels, 1977), including vomiting on their ventral surface, likely carry substantial costs in terms of lost feeding opportunities (Ramirez et al., 2010). This suggests a chain of events linking costly predator-avoidance behaviors deployed in the face of possible predation, a draining of physiological resources otherwise devoted to immune defenses, and heightened susceptibility to entomopathogens. Indeed, melanization of a model foreign body implanted into potato beetles is reduced when the beetles have been reared alongside predatory insects, compared to beetles reared alone (Ramirez and Snyder, 2009). Under this model, the physiological cost of predator avoidance is no different in kind from any other drain on energetic reserves, as for example might occur when beetles compete with conspecifics for limiting resources (e.g., Cinel et al., 2020).

We considered this possibility in a field cage experiment, wherein we manipulated predator presence or absence alongside a weakening or intensification of intraspecific competition. We found that interspecific competition significantly reduced potato beetle larval survival, roughly halving beetle survivorship to the penultimate larval instar when predators were not present (Fig. 1A). Predators only appeared to have an effect on larval survival when initial potato beetle larval density was low, but predators seemed to have little effect when intraspecific competition was more intense (i.e., with high initial larval densities) (Fig. 1A); this would be consistent with a decline in potato beetle density following predation leading to relaxed intraspecific

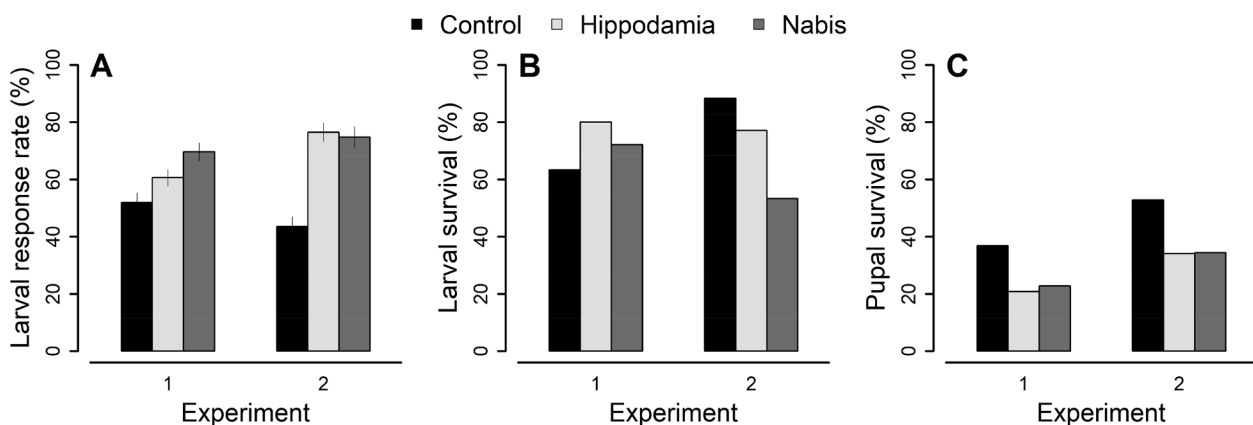


Fig. 2. (A) Response rate of larva to exposure to a no-predator control, *Hippodamia* lady beetle, or a *Nabis* predatory bug. (B) Larval survival to 4th instar with two predator treatments and a no-predator control. (C) Pupal survival to adulthood following exposure to pathogens for pupae that were exposed to one of two predators or a no-predator control in the larval stage.

competition. However, exposure to predators versus intraspecific competitors earlier in development rendered quite different impacts on the beetles' later susceptibility to entomopathogens (Fig. 1B). Only exposure to the possibility of predation as larvae, but not heightened competition, rendered beetles more likely to be killed by entomopathogenic nematodes and fungi during pupation in the soil (Fig. 1B). So, overall, while intraspecific competition yielded apparently greater harm to beetle larval survivorship, heightened later susceptibility to pathogen infection was unique to previous predator exposure.

We also worked to solidify the link between the deployment of defensive behaviors and heightened pathogen susceptibility, by experimentally subjecting potato beetle larvae to repeated contacts with a predator versus a bare wooden stick. We found that beetles more consistently responded to being touched by predators, compared to pokes with a bare-wood stick (Fig. 2A). This suggests that the potato beetles were responding to some cue associated only with real predators. Notably, it was only predator contact, and not poking alone, that heightened susceptibility to pathogen infection (Fig. 2C). Overall, our results further the idea that potato beetles can differentiate contact with predators from other forms of disturbance (see also Ramirez and Snyder, 2009; Ramirez et al., 2010). The specific mode through which this operates remains unclear, but might be some combination of chemical, visual, or tactile cues; further work is needed to identify the specific mechanism at work (see also Cinel et al., 2020).

An alternative explanation for our observation of greater pathogen infection following exposure to predators in field cages (see also Ramirez et al., 2009), is that the potato beetle individuals best able to escape from predators are least able to evade infection by pathogens; selective predation by predators then could leave behind only the most-pathogen-susceptible larvae. This would be analogous to the finding by Kraaijeveld and Godfray (1997) that *Drosophila melanogaster* flies selected for improved resistance to the endoparasitoid *Asobara tabida* necessarily experienced reduced competitive ability. That is, there may be an inherent tradeoff between the traits that facilitate escape from predators versus those that increase resistance to pathogens. However, we now have two lines of evidence that allow us to tentatively reject this possible explanation. First, as noted above, Ramirez et al. (2009) found that potato beetle larvae exposed to non-lethal encounters with predators exhibited a reduced melanization response that suggested reduced immune function in the absence of actual predation. Second, here we have shown that potato beetle larvae repeatedly exposed to predators without any chance of the larva being killed, nonetheless experienced heightened risk of later infection by entomopathogens (Fig. 2).

Our central hypothesis underlying this work was the energetic loss associated with using anti-predator behaviors carried a cost in reduced resistance to pathogens broadly consistent with that resulting from competition, mechanical interruption of feeding, or any other form of energy drain. However, our results present a tentative suggestion that potato beetles may view threat of predation as some uniquely stressful event. Consistently, we found that the presence of predators led to an apparent weakening of resistance to entomopathogens, not seen when facing intraspecific competitors or being physically disturbed by poking (Figs. 1 and 2). We suggest several next steps. First, much additional work is needed to describe a specific physiological mechanism linking non-lethal predator encounters to changes in potato beetles' pathogen resistance. One approach would be to compare expression of immunity-linked genes for beetles experiencing threat of predation through multiple stages of development and under realistic field conditions. Second, it would be valuable to more closely track beetle energetic resources and survivorship throughout each larval instar, rather than only assessing survivorship to pupation as was done here. After all, in the field cage experiment it may be that intraspecific competition drained energy reserves and weakened immune function initially, but as competitors died and resource limitation was relaxed, the beetles

recovered in time for pupation. One approach to better make this link would be to destructively collect, dry, and weigh both larvae and host plants repeatedly through development while facing differing levels of predation and competition, to specifically quantify the degree of food limitation to the larvae and resulting effects on their growth and development. Likewise, it would be helpful to link differences among individual beetles in their likelihood to deploy particularly-draining defenses like vomiting, and fat reserves or other measures of beetle health and condition. Finally, our work would have been strengthened by the inclusion of additional control treatments that were omitted due to logistical constraints. In future field cage experiments, it would be useful to rear single potato beetle larvae alone so that they experienced neither competition nor predation so that both factors were truly absent, exposure to entomopathogens. Second, future "bug on a stick" experiments should include control larvae that are never prodded by even a bare wooden dowel, to see how larvae entirely free of disturbance respond to entomopathogens.

There has been substantial recent interest in how communities of natural enemies might work together to exert complementary harm to herbivores that strengthen biological control (Snyder, 2019). Complementarity is generally envisioned to result from different natural enemy species attacking different subsets of the pest population in space or time, as do the predators and entomopathogens examined here (Wilby and Thomas, 2002; Schmitz, 2007; Preisser et al., 2007). However, here we report additional evidence that the form of predator-pathogen complementarity at work for the enemies of the Colorado potato beetle operates through a novel channel, as stress, in some form, exerted by the predators later indirectly benefits the pathogen (see also Roux et al., 2015). This joins the growing body of examples of facilitation among natural enemies that spans prey life stages (e.g., Peckarsky et al., 1993; McCollum and Van Buskirk, 1996; Vonesh, 2005; Costanzo et al., 2011; Davenport et al., 2014), which can form a particularly powerful means for greater natural enemy biodiversity to strengthen biological control (Sih et al., 1998).

CRediT authorship contribution statement

Tina Miller: Conceptualization, Methodology, Investigation, Writing - original draft. **Michael S. Crossley:** Formal analysis. **Zhen Fu:** Conceptualization, Methodology, Formal analysis. **Amanda R. Meier:** Formal analysis. **David W. Crowder:** Conceptualization, Formal analysis. **William E. Snyder:** Conceptualization, Supervision, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocontrol.2020.104403>.

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