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ORIGINAL ARTICLE

Variation in immune response in the generalist herbivore fall webworm across four common host plants

Audrey M. Mitchell | **Dhaval K. Vyas** | **Shannon M. Murphy**

Department of Biological Sciences, University of Denver, Denver, Colorado, USA

Correspondence
Audrey M. Mitchell, Department of Biological Sciences, University of Denver, 2090 E. Iliff Ave, Denver, CO 80210, USA.
Email: audrey.mitchell@du.edu

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Abstract

Dietary generalist herbivorous insects are widespread and often occur in a variety of environments. Across their geographic range, herbivorous insects may encounter variable plant traits as they feed on high-quality or low-quality plants. Herbivorous insect larvae experience both bottom-up (host plant) and top-down (parasitoid) factors that affect survival. Host plant quality may affect larval growth and survival in that larvae feeding on low-quality plants often suffer reduced fitness. However, herbivores on different host plants are also subject to different levels of parasitism. High-quality plants confer stronger larval performance (higher survival, more offspring), but larvae may also face higher parasitism. In some herbivore species, diet mediates larval immune response. The generalist insect herbivore fall webworm (FW), *Hyphantria cunea* Drury (Lepidoptera: Erebidae), is a moth native to North America, and its larvae have considerable variance in their performance when reared on different host plants. We investigated whether diet affects the immune response in FW larvae when they are reared on different host plant species known to vary in food quality. We measured immune response by melanization of a nylon filament. We found significant differences in immune response across host plants, indicating that diet mediates immune response in FW larvae. Our study helps elucidate the factors that cause variation in immune response in a generalist herbivore.

KEYWORDS
dietary generalism, eco-immunology, Erebidae, fall webworm, herbivorous insect, *Hyphantria cunea*, Lepidoptera, performance, plant–insect interaction

INTRODUCTION

Plants provide herbivorous insects with essential nutrients needed not only for growth and development but also for immune response (Smilanich & Muchoney, 2022). Herbivorous insects that are dietary generalists face an important challenge in that not all plants provide the same quality resources. Host plants vary in their nutritional quality, and the types and varieties of plants eaten may also affect how successful herbivores are at defending themselves against natural enemies, such as pathogens and parasitoids (Vidal & Murphy, 2018). Parasitoids are a particularly potent threat for many insect herbivores (Hawkins et al., 1997), and herbivores have evolved various strategies to defend themselves from these natural enemies including chemical, behavioral, and morphological defenses (Barbosa & Caldas, 2007; Gross, 1993; Smilanich, Dyer, & Gentry, 2009; Veldtman et al., 2007). If these defense strategies fail and a parasitoid successfully oviposits, the host herbivore's cellular and humoral immune responses are then enacted. Notably, diet may affect the innate immune systems of herbivorous insects (Carper et al., 2019; Singer et al., 2014; Smilanich, Dyer, Chambers, et al., 2009).

Eco-immunology is a rapidly emerging field that relates variation in immune function to the surrounding environment and the evolution of a species (Rolff & Siva-Jothy, 2003; Schulenburg et al., 2008). The relationship between immune function and environmental factors (e.g., diet breadth and host plant type) has been evaluated in several studies (Gallon & Smilanich, 2023; Ghosh & Venkatesan, 2019; Muchoney et al., 2022). Immune responses are costly to maintain (Ardia et al., 2012; Freitak et al., 2003; Moret & Schmid-Hempel, 2000), and insects possess a finite pool of resources. A cornerstone of the eco-immunology field focuses on the resource-mediated trade-off between growth and immune function (Ponton et al., 2011, 2013; Sheldon & Verhulst, 1996).

Insect cellular immune responses primarily involve immune cells called hemocytes that attack a foreign entity or invader (e.g., parasitoid or pathogen) and encapsulate that entity (Gillespie et al., 1997; Lavine & Strand, 2002; Schmidt et al., 2001; Strand & Pech, 1995). The hemocyte cells that adhere to foreign surfaces in lepidopteran larvae are granular cells and plasmatocytes, and these cell types make up more than 50% of hemocytes in Lepidoptera (Lackie, 1988; Lavine & Strand, 2002; Strand & Pech, 1995). Many studies have measured the amount of melanin deposited on an artificial object (e.g., glass bead and nylon filament) as a proxy for the immune response an insect is capable of mounting against natural parasitoid attacks (Diamond & Kingsolver, 2011; Honkavaara et al., 2009; Lavine & Beckage, 1996; Nagel et al., 2011; Rantala & Roff, 2007; Smilanich, Dyer, Chambers, et al., 2009). Furthermore, studies have shown that encapsulation responses may be affected by the nutritional quality of the host's diet in various ways. For example, a plant diet that is higher in protein compared with carbohydrates may lead to stronger immune function by increasing encapsulation responses (Lee et al., 2006; Wilson et al., 2019). As diet can affect an insect's cellular immune response, which host plant an herbivorous insect feeds upon may be a crucial factor in determining an insect's resistance to parasitoids.

Fall webworm (FW), *Hyphantria cunea* Drury (Lepidoptera: Erebidae), have considerable variance in their survival and growth performance when reared on host plants of different qualities (Murphy & Loewy, 2015; Vidal et al., 2020). FW larvae reared on low-quality plants suffered reduced fitness in terms of development time and pupal mass (bottom-up effects), but they faced less parasitism (top-down effect) compared with FW reared on high-quality plants (Murphy & Loewy, 2015). Whether this reduced parasitism on low-quality host plants is due to effects on adult parasitoids (e.g., parasitoid preferences for plant volatiles or plant microenvironment) or larval parasitoids (e.g., nutritive value and defense compounds) mediated by plant effects is unknown. Recently, Vyas and Murphy (2022) found that diet affected the cellular immune response of FW larvae attacked by the parasitic wasp, *Therion sassacus* Viereck. However, by using live parasitoids, this study was unable to guarantee a challenge to the immune system in every attack, as some female parasitoids failed to oviposit eggs that would induce an immune response (Vyas & Murphy, 2022). Immune function is crucial to host survival, as an early immune response after an herbivore is parasitized can sometimes kill the parasite and save the herbivore.

We investigated how the immune response of FW is affected when reared on different host plants that vary in dietary quality for FW. Whereas previous research has demonstrated that FW reared on certain plants have higher levels of parasitism, less is known about how host plant quality affects immune response, other than the results of Vyas and Murphy (2022). We aimed to study the relationship between the immune response and the diet of FW using a synthetic object (nylon filament) to approximate an invasion of a foreign body that challenges the immune system. Our goal for this experiment was to determine whether host plant quality affected the immune response (degree of melanization) by FW.

MATERIALS AND METHODS

Study system

Fall webworm is a moth species that is native to North America and a pest in Asia and Europe (Gomi & Takeda, 1996; Yang et al., 2006). Female moths are batch layers that lay hundreds of eggs on a host plant (Loewy et al., 2013); thus, although FW are dietary generalists that feed on many host plant species at the population level, individual FW only feed on a single host plant during their development. Once the larvae hatch, they spin a web and live gregariously with their siblings. FW are noted dietary generalists and can feed on dozens of host plant species from different plant families (Vidal & Murphy, 2018; Warren & Tadic, 1970). There are at least two genetically and morphologically different types of FW that are distinguished by their head capsule color: red-headed and black-headed (Vidal et al., 2020). In Colorado (CO, USA), the red-headed morphotype is the only documented form. Populations of red-headed FW in Colorado eat a generalized diet of different host plants and have been observed feeding on 19 plant species from eight families (Murphy & Loewy, 2015). In 2019, we collected FW from Boulder, Jefferson, and Larimer counties in CO and reared them in our laboratory at the University of Denver, following protocols outlined in Robinson-Castillo et al. (2021) and Loewy et al. (2013). The larvae in this experiment are the offspring of the FW collected in 2019, and we reared these larvae in the laboratory in 2020.

Experimental design

After female moths laid eggs in spring 2020, we monitored the egg clusters until we observed head capsules beneath the chorion of the eggs, and then, we divided each egg cluster into four equal sections. We placed each of the four sections on a different leaf from one of four plant species used in our experiment: thinleaf alder, *Alnus tenuifolia* Nutt. (Betulaceae), apple, *Malus* sp. (Rosaceae), chokecherry, *Prunus virginiana* L. (Rosaceae), or narrowleaf cottonwood, *Populus angustifolia* James (Salicaceae). We classified plants as high-quality (chokecherry and narrowleaf cottonwood) and low-quality (alder and apple) food for FW based on measures of survival, pupal mass, and development time from Murphy and Loewy (2015).

We used FW larvae from 33 matrilines for our experiment trials. We aimed to measure melanization from at least five larvae per maternal line per host plant species in our experiment, choosing to maximize the number of maternal lines rather than the number of full siblings per maternal line. However, because of mortality, the final number of larvae per treatment varied (Table S1), the total number of larvae in our experiment was 335. We reared FW larvae on each of the four plants and inserted nylon filaments to measure melanization and immune response.

We reared FW larvae at a maximum density of 10 larvae per liter rearing container, replenishing leaves as needed. We haphazardly selected larvae from containers that were 23–27 days old; we chose larvae of this age because they were large enough for filament insertion. We measured their head capsule widths by determining the distance between the lateral sides of the head using digital calipers. After head capsule measurements were taken, we inserted into the larva a disinfected (with 70% alcohol) insect pin (size no. 0; pinprick) above the penultimate proleg (Figure 1A). After withdrawing the pin, we inserted one 3-mm-long nylon filament into the hole using fine forceps. The nylon filaments were made of 0.3-mm-diameter nylon fishing line and rubbed with fine sandpaper to create a surface for the hemocytes to melanize. Filaments remained inserted in the larvae for 24 h during which time the larvae were stored individually in 1.5-mL plastic tubes with a piece of the plant on which they were reared. After 24 h, we removed the filament from the larva and placed it on filter paper to dry. We stored filaments individually in microcentrifuge tubes at room temperature (20°C).

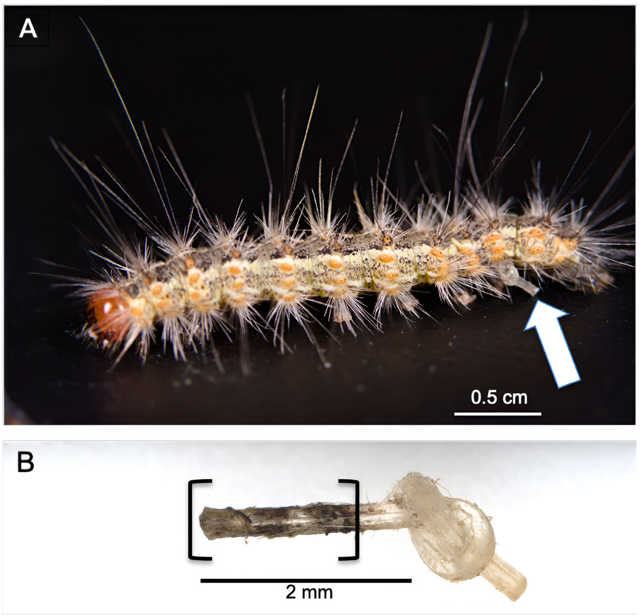

FIGURE 1 (A) Image of a nylon filament inserted above the penultimate proleg (arrow) in a fall webworm (FW) larva. (B) FW immune cells encapsulate and melanize the filament, making it turn black. The brackets indicate the melanized part of the filament.

We used a VHX-7000 Digital Microscope (Keyence, Itasca, IL, USA) to measure the area of melanization of each nylon filament. We analyzed the amount of melanization using GIMP v.2.10.22 software (www.gimp.org). We determined the mean melanization of each filament which corresponded to the number of pixels in the dark area of the image. We ensured white balance was set by adjusting levels with black and white reference before converting the image to grayscale. We cropped the image in the program to only extract data from areas of the filament inserted in the larva (Figure 1B). GIMP then produced a histogram of the data, and we used the mean as our estimate of melanization.

Statistical analysis

All of our data met normality and equality of variance assumptions. We used a mixed model regression to analyze the effects of our independent variables on our response variable of mean melanization. Our fixed effects were *diet* (alder, apple, cherry, and cottonwood) and *head capsule width* (we treated this variable as continuous because although individual larval growth is discontinuous and head capsules have distinct widths in each instar, when considered at a population level, FW head capsule widths are continuous; Tanino-Springsteen et al., 2024). We initially included an interaction between the two fixed effects, but it was not significant, and Akaike's information criterion was lower without it, so we dropped it from the final model. Our random effects were *maternal line* and *days old*. We analyzed differences across host plants using Tukey's post hoc honestly significant difference test. All tests had a significance threshold of 5% and all analyses were conducted in JMP Pro v.15.2.0.

RESULTS

Diet had an effect on mean melanization ($F_{3,316.9} = 3.0234$, $p = 0.029$; Figure 2), but head capsule width had no effect ($F_{1,308.5} = 0.0848$, $p = 0.77$). FW reared on the apple diet had the least melanization of any diet, significantly less than on cherry (Figure 2). The random effect *maternal line* explained 16% of the variation (mixed model regression: $p = 0.016$), whereas the random effect *days old* was not significant and only explained 6.6% of the variation ($p = 0.35$).

DISCUSSION

Host plants can have significant bottom-up effects on herbivore performance, and these effects then influence the herbivore's ability to defend against natural enemies. Previously, we have shown that FW performance is dependent on its host plant species diet (Murphy & Loewy, 2015), and here, we show that FW diets also affect FW immune response. Melanization, which is indicative of a

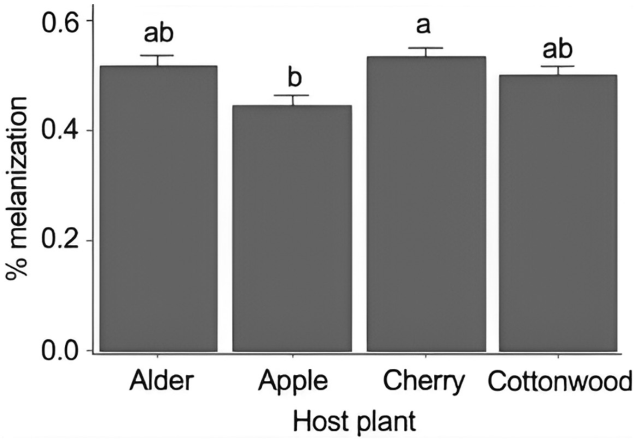

FIGURE 2 Mean (+SE) melanization (%) of filaments that were inserted into fall webworm larvae to simulate a parasitoid attack. Larvae were fed low-quality host plants (alder and apple) or high-quality host plants (chokecherry and cottonwood). Means capped with different letters are significantly different (Tukey's honestly significant difference test: $p < 0.05$).

successful cellular and humoral immune response (Rantala & Roff, 2007; Smilanich, Dyer, & Gentry, 2009), was lowest in FW larvae that fed on apple compared with FW reared on other host plants of both high-quality and low-quality. Apple is a low-quality host plant for FW based on its effects on other larval performance measures (e.g., survival and pupal mass), and we found that feeding on apple impaired FW immune responses compared with other plant options, such as the high-quality host plant chokecherry. However, dietary quality of FW host plants did not consistently predict melanization as alder, which is typically a low-quality host plant for FW similar to apple (Murphy & Loewy, 2015), did not differ in its effect on immune response compared with chokecherry or narrowleaf cottonwood, which have historically been high-quality host plants for FW (Murphy & Loewy, 2015; Vidal et al., 2020; Vyas & Murphy, 2022). Our finding that FW larvae reared on apple have low immune response is consistent with the results of Vyas and Murphy (2022) who found the fewest granulocytes and the highest occurrence of live parasitoid (*T. sassacus*) larvae in FW larvae reared on apple. Our results with nylon filaments suggest that perhaps immature *T. sassacus* experienced a beneficial host environment when FW fed on apple because these host larvae appear to have compromised immune capabilities and are unable to mount a successful immune response against foreign invaders.

Previous research has found that the nutritional quality of a host plant can impact an herbivore's immune response, specifically the protein: carbohydrate ratio (Cotter et al., 2019; Lee et al., 2006, 2008; Povey et al., 2009). For example, Lee et al. (2006) found that larvae fed a high-protein artificial diet had improved immune function compared with larvae fed a high-carbohydrate diet. Povey et al. (2009) found that survival was higher for larvae fed a high-protein diet and that these larvae exhibited high immune response activity and antibacterial activity. In addition to the nutritional quality of the plant, plant chemical defenses may also negatively affect larval growth and immune response. For example, increased dietary levels of iridoid glycosides and glucosinolates have detrimental effects on the immune response of two specialist herbivores (Bukovinszky et al., 2009; Smilanich, Dyer, Chambers, et al., 2009). Plants synthesize secondary metabolites that are often toxic to herbivorous insects (Wittstock & Gershenzon, 2002), and thus, these herbivores face a tradeoff between metabolizing plant secondary compounds or allocating resources to other physiological systems, such as immune function. We are currently in the process of measuring the metabolomics of apple plants and our other host plants; we suspect that the nutritional quality of apple differs in some important aspects from other FW host plants, leading to low FW performance in terms of immune response on this poor-quality host plant.

Our results that FW reared on apple have reduced immune response is intriguing because this would lead us to the prediction that parasitoids should disproportionately attack and survive within FW larvae feeding on apple in the field. Interestingly, we find the opposite pattern in our field-collected data. After over a decade of intensively sampling FW from the field, we have collected very few parasitized FW, from only eight apple trees, and these FW larvae yielded only 11 occurrences of successful parasitoid attacks from the dozens of larvae collected. One explanation for the paucity of parasitoids from apple-fed FW is that apple trees are less abundant in natural areas than many other FW host plants. Murphy and Loewy (2015) found that host plant abundance is positively correlated with parasitism and apple is one of the less abundant host plants at our field sites. Previously, we hypothesized that parasitoids avoided or were unable to emerge successfully from FW feeding on lower-density host plants, such as apple, because FW larvae had high immune function on those plants; however, our results here show that this is not the case as FW reared on apple had low immune response. Apple is the only non-native host plant that we used in this study, and it could be interesting for future research to investigate the exotic status of host plants and the duration of host association in conjunction with FW performance and immune response.

Overall, we found that FW reared on apple had reduced immune response compared with FW reared on other host plants; these results support those found in Vyas and Murphy (2022) who found that FW reared on apple had the greatest odds of yielding a live parasitoid larva. The specific diet-mediated mechanisms by which host plants affect FW immune response remain unclear. Our results suggest that FW host plants may vary in their nutritional quality and whether the metabolomics of these host plants can explain FW performance and immune response should be investigated.

AUTHOR CONTRIBUTIONS

Audrey M. Mitchell: Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); visualization (equal); writing – original draft (equal). **Dhaval K. Vyas:** Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Shannon M. Murphy:** Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); methodology (equal); project administration (equal); resources (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are archived at the Zenodo repository: https://doi.org/10.5281/zenodo.12663681.

ORCID

Audrey M. Mitchell https://orcid.org/0000-0001-5348-1555
Dhaval K. Vyas https://orcid.org/0000-0002-6038-606X
Shannon M. Murphy https://orcid.org/0000-0002-5746-6536

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Numbers of fall webworm larvae and matrilines per host plant.

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