

Examining the performance of an insect generalist reared on unused host plants in Colorado

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Abstract

Dietary generalist insects are important to ecological communities because they are commonly found in many environments and play important roles in ecosystem services like pollination and decomposition. Although dietary generalist herbivores eat a broad range of plant species, regional populations of these species may have significantly narrower or specialized diet breadths. Fall webworm (*Hyphantria cunea*, hereafter FW) is a dietary generalist at the species level, but we do not know if there is dietary generalism at the population level or how generalism varies across populations. In Colorado, FW larvae feed on only a few plant species, but many plant species are available that are used by FW elsewhere and not locally. We investigated if FW may be an example of a species that is a dietary generalist when considered over a large geographic range but is composed of populations with narrower diets regionally. We reared FW larvae from fifteen maternal lines in Colorado on a local high-quality host plant and compared their performance (survival, development time, and pupal mass) with larvae reared on plants that are not used locally. We found that FW performance was significantly reduced on plant species that Colorado FW does not use. Our findings demonstrate that Colorado FW cannot eat the same plants as FW in the eastern United States and thus lack the physiological ability to feed on these plants. Our research also suggests that FW are a generalist species with narrower diets that vary regionally at the population level.

Keywords: diet breadth, dietary generalism, Erebidae, insect herbivore, Lepidoptera

1 INTRODUCTION

There are over half of a million species of described herbivorous insects, the vast majority of which are dietary specialists that typically eat fewer than three plant species¹. However, insects that are dietary generalists are important to ecological communities because they play key roles in ecosystem services, such as pollination and decomposition. Insect dietary generalists are also important because they are often crop pests². Although dietary generalist herbivores eat a broad range of plant species, Fox and Morrow³ suggested that regional populations of these species may have significantly narrower or specialized diet breadths. A generalist population may have a narrow diet breadth and use different plant species for food regionally due to a number of different ecological or evolutionary processes³. For example, herbivore diet can vary depending on genetic variation among herbivores, competition with other herbivores for high-quality plants, or plant availability³.

Thus, species that are dietary generalists can be categorized into two groups: 1) species that are true dietary generalists are composed of populations with generalized diets across their entire range, or 2) species that are regionally composed of populations with narrower diet breadths, but the species is considered a dietary generalist when all populations are considered together over the species' geographic range.

Herbivorous insects may select which plant species to eat because of plant-related traits (interactions between two trophic levels: plant and herbivore). This bi-trophic view on diet breadth considers interactions between herbivore and plant as the most important variables determining the herbivore's food plants. For example, an herbivore may select a food plant because its nutritive value yields a faster development time, or the plant is more abundant than other plants in the environment and thus easier to find. Further, plant quality can vary regionally depending on environmental factors, so bi-

trophic interactions are important to determine the diet breadth of generalist herbivores².

Herbivorous insects may also select which plant species to eat because of traits that only appear when considering their natural enemies: predators, parasitoids, and pathogens (interactions between three trophic levels: plant, herbivore, and natural enemy)⁴. A tri-trophic approach considers these interactions with natural enemies along with interactions with the plant in relation to the herbivore. The presence of predators can decrease herbivore performance (survival, development time, and/or pupal mass) on a host plant even when the plant is a high-quality host. For example, Murphy⁵ found that the Alaskan swallowtail butterfly (*Papilio machaon alaska*) had low performance on high-quality host plants because mortality from natural enemies was higher on these host plants than on low-quality host plants. A tri-trophic approach is important to understand herbivore community structure and population specialization or divergence⁴. However, by conducting a bi-trophic experiment, we can determine the viability of a plant for herbivores to eat depending on plant-related traits.

Fall webworm (*Hyphantria cunea*, hereafter FW) is an insect herbivore that is a dietary generalist, and the larvae feed on more than 400 plant species worldwide^{6,7}. However, in Colorado, FW larvae feed on fewer plant species, as we have only found them on 17 host plants⁸. Many plant species are available to Colorado FW that are used by FW elsewhere but not locally. For example, FW commonly eat box elder (*Acer negundo*) in the eastern United States but have not been found on this plant species in Colorado⁸, even though box elder is relatively common at sites where we collect FW (personal observation). Thus, FW may be an example of a species that is a dietary generalist when considered over a large geographic range but is composed of populations with narrower diets regionally, as described by Fox and Morrow³.

We examined the performance of Colorado FW when reared on plant species that are frequently used as host plants by FW on the East Coast but are not used by FW in Colorado even though these plants can be found in the Front Range of Colorado where FW occur. It is important to consider that there are two morphotypes of FW; red-head FW are the only morphotype found in Colorado, whereas red-head and black-head FW are found sympatrically in the eastern United States⁹. Currently, these morphotypes are considered a single species, but Vidal et al.⁹ showed that genetically they are likely different species. The genetic differences between these morphotypes may influence the regional diet preferences of FW. The goal of our research was to test if the plant species used by eastern FW (black-head) could be physiologically viable host plants for Colorado FW (red-head) even though they are not currently used

as hosts by FW in Colorado. Previously, we have reared eastern, black-head FW on some of these host plants (e.g., box elder) in Colorado, and they performed well on these plants (Murphy and Vidal, unpublished data). Here, we used a bi-trophic approach in this experiment to understand the effects of plant-related traits on herbivore performance. If Colorado FW larvae perform poorly when reared on the available but unused plant species, then this would indicate that FW larvae cannot feed upon the same food plants in all regions and may suggest that FW are a generalist species with narrower diets that vary regionally at the population level. If Colorado FW larvae perform well when reared on the available but unused plant species, then this would indicate that FW can feed upon the same food plants across geographic regions and may suggest that FW is a true dietary generalist.

2 METHODOLOGY

2.1 Study System

FW is a moth that is native to North America and introduced in Asia and Europe¹⁰. FW feeds on over 400 host plant species across its wide geographic range^{6,7}, but how these plant species are used differently by red-head and black-head FW is unclear. As a species, FW feed on a wide range of host plants, but individual larvae feed on the host plant where their mother laid eggs and are thus restricted to a single plant. Although FW is highly polyphagous across its range, populations often have a diet breadth that is limited regionally. In Colorado, red-head FW have been observed on only 17 woody tree species, although many other plant species are available as potential hosts and are used as hosts by FW elsewhere⁸.

2.2 Experimental Design

In June of 2023, we used the egg clusters laid by 15 red-head FW females selected haphazardly from our colony for our experiment. FW in the colony have been reared on multiple host plants, and new individuals are introduced each summer from wild populations. To compare larval performance across host plants for each maternal line, we cut each egg cluster into evenly sized groups and placed each group onto different plant species. We split four of the maternal lines into six groups that were reared on six different plant species. We used black willow (*Salix nigra*, abundant in CO) as a control because it is a high-quality host plant for Colorado FW, so we could compare larval performance on the unused plants with a known high-performance plant. We selected unused plant species for our experiment from a published list of plant species used by black-head FW in the eastern United States⁸ and then selected plants

to use for our experiment based on their availability in Colorado. The five unused plant species were: American linden (*Tilia americana*; abundant in CO), American sycamore (*Plantanus occidentalis*, rare in CO), box elder (*Acer negundo*, abundant in CO), Eastern redbud (*Cercis canadensis*, rare in CO), and tree of heaven (*Ailanthus altissima*, abundant in CO). We then decided to add mulberry (*Morus alba*; rare in CO) into the experiment, so we split the next 11 maternal lines onto seven plant species (the same six listed above plus mulberry). We placed each egg cluster group on a host plant leaf in a 0.5 L deli container along with a piece of damp filter paper to prevent desiccation. We reared the FW larvae in a lab setting with ambient temperature and lighting and fed them leaves from trees located near the University of Denver campus. Once the larvae were large enough to count, we placed five in each container. We fed all larvae a single plant species throughout their development, as wild FW commonly remain on one host plant for the duration of their larval development (personal observation).

Throughout development, we replaced any dry or moldy leaves with fresh foliage three times per week. We reared up to 15 larvae per host-maternal line treatment; some treatments did not have 15 surviving larvae, while other treatments had to be culled to 15. In total, our final sample size was 602 larvae. Thirty days ($\pm$ one day) post-pupation, we recorded pupal mass as a proxy for lifetime performance (to the nearest 0.001 mg; Mettler-Toledo XP6, Columbus, Ohio¹¹). We waited 30 days to gain a more precise pupal mass as FW pupae deplete their fat stores and lose water via evaporation over time¹¹.

2.3 Statistical Analysis

We analyzed FW survival with a Chi-square test, treating host plant and maternal line as fixed effects. Some of the larvae died early in the experiment because their containers were too dry, so we removed these FW from the dataset before analysis. We calculated an individual fitness score by multiplying survival (binary measure, 0 or 1) by pupal mass and dividing by development time. We analyzed FW larval fitness score using a mixed-model ANOVA with host plant as a fixed effect and maternal line as a random effect. To meet assumptions of normality and equality of variance, we square root transformed fitness score for our analyses. We performed all statistical analyses with JMP Pro 15.2.0.

3 RESULTS

For FW survival, we found a significant effect of both host plant species ($\chi^2=186.6$, $df=6$, $P<0.0001$) and maternal line ($\chi^2=43.8$, $df=14$, $P<0.0001$) Figure 1. While both factors were significant ($p<0.05$), host plant had a

stronger effect on FW survival. Notably, no larvae ever survived on box elder. Larvae did eat box elder, so it was not an avoidance of the host plant that caused their mortality, but rather a physiological inability to survive on this plant. For FW fitness score, we found a significant effect of both host plant ($F_{6,545}=69.2$, $P<0.0001$) and maternal line ($F_{14,545}=2.6$, $P=0.0015$) Figure 2. Using Tukey's HSD, we found significant differences in fitness scores across host plants with the highest FW fitness scores on black willow, which is a high-quality host plant used by FW in Colorado, and the lowest on American sycamore and Eastern redbud Figure 2.

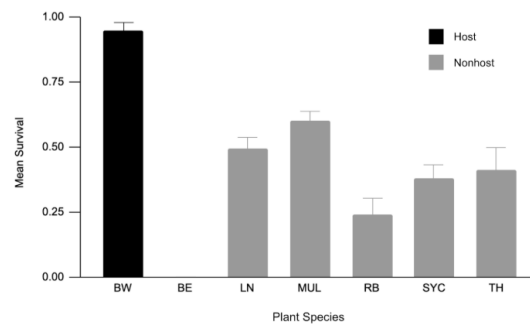


Figure 1. Mean proportion survival ($\pm$ SE) of FW larvae reared on seven plant species in the summer of 2023 in Colorado, USA. The plant species we used were black willow (BW), which is a commonly used and high-quality host plant by FW in Colorado (black bar), along with several nonhost plants (BE = box elder, LN = American linden, MUL = mulberry, RB = Eastern redbud, SYC = American sycamore, TH = tree of heaven; gray bars). FW commonly use nonhost species in the Eastern United States but not in Colorado.

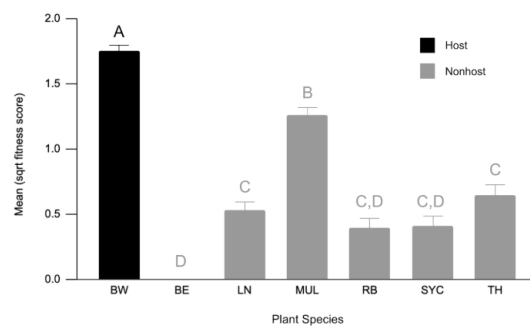


Figure 2. Mean fitness scores (fitness score is square root transformed; $\pm$ SE) of FW larvae reared on seven plant species in the summer of 2023 in Colorado, USA. The plant species we used were black willow (BW), which is a commonly used and high-quality host plant by FW in Colorado (black bar), along with several nonhost plants (BE = box elder, LN = American linden, MUL = mulberry, RB = Eastern redbud, SYC = American sycamore, TH = tree of heaven; gray bars). The nonhost species are commonly used by FW in the Eastern United States but not by FW in Colorado. The letters above the bars indicate significant differences between plant treatments (Tukey's HSD).

4 DISCUSSION

We found that Colorado FW do not perform well on host plants that FW commonly use on the East Coast, which supports the hypothesis that FW larvae cannot feed upon the same food plants in all regions. Our results suggest that FW may be a generalist species composed of populations with narrower diets that vary regionally, as suggested by Fox and Morrow³. While tri-trophic interactions are important to understand how a species interacts with its environment^{4,12}, for our research, bi-trophic interactions are able to explain why FW do not use certain host plant species. Notably, even in a controlled environment without top-down pressure from natural enemies, we found that all FW larvae die on some plant species such as box elder; this finding suggests that bi-trophic interactions are solely responsible for FW not using box elder in Colorado. However, which components of the bi-trophic interactions cause FW to die on box elder are unknown and could be due to either plant-related traits or FW physiology or microbiota.

FW morphotype likely plays a role in our findings that Colorado FW cannot feed on the same host plants as FW in the eastern United States. Vidal et al.⁹ found that the two morphotypes of FW are likely distinct species, but they have not been formally described as such. Our research supports the suggestion that the red and black morphotypes are distinct species as first reported by Vidal et al.⁹. We found that red-head FW from Colorado cannot feed on box elder plants here, but box elder is a commonly used host plant by black-head FW in the eastern United States. It is possible that Colorado box elder trees are different from box elder trees in the eastern United States, but this is not likely to explain our results. Previously, we have reared black-head FW from the eastern United States on box elder trees in Colorado, and we found that they performed well on these plants. Therefore, any chemical differences between eastern and Colorado box elder plants cannot explain the failure of Colorado FW to survive on these plants because eastern FW are able to survive on these same plants. Our findings demonstrate that red-head FW cannot eat the same plants as the black-head populations in the eastern United States and lack the physiological ability to feed and survive on these plants.

Plant abundance may explain why some plant species that were tested in this experiment (e.g., American sycamore, mulberry, and Eastern redbud) are not used by Colorado FW in nature because they are relatively rare in Colorado at FW field sites (Shannon Murphy, personal observation). Because Colorado FW do not frequently encounter these plant species, there may not be selection for individuals to thrive on them. However, box elder is abundant at our field sites and Colorado FW would encounter it frequently, yet no larvae in this

experiment survived on box elder. The abundance of plant species in Colorado also does not explain why American linden and mulberry yielded moderate survival and performance for FW larvae in this experiment; American linden trees are very abundant, yet FW fitness scores were low, and mulberry trees are rare, yet fitness scores were higher.

5 CONCLUSIONS

We found that Colorado FW physiologically cannot feed on some of the same plant species as populations of FW in the eastern United States. Further research should investigate the physiological mechanisms that inhibit or reduce survival on these plants. Some species are true generalists that can uniformly eat a wide range of foods across their range. Other species, including FW, are likely extensive generalists only when considered at the species level, with narrower diet breadths at the population level. Additional research is required to determine what factors prevent FW from using these plant species, and tri-trophic interactions should also be investigated for further understanding. Furthermore, the ability (or inability) to consume wide diets should be investigated across a greater range of FW populations. The complexity of generalist diets and the factors that shape them could have broad implications for many ecosystems.

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