The Role of Enemy-Mediated Competition in Determining Fitness of a Generalist Herbivore

Authors: Ryan A. Beshai, Elizabeth E. Barnes, and Shannon M. Murphy

The Role of Enemy-mediated Competition in Determining Fitness of a Generalist Herbivore

Ryan A. Beshai, Elizabeth E. Barnes*, and Shannon M. Murphy

Department of Biological Sciences, University of Denver, Olin Hall, Room 102, 2190 E. Iliff Ave., Denver, CO 80210

Abstract. The influence of indirect competition on insect communities is not well understood. We studied how the fitness of a gregarious lepidopteran species, fall webworm, *Hyphantria cunea* (Drury), is affected by another gregarious caterpillar species, western tent caterpillar, *Malacosoma californicum* (Packard). Fall webworm and tent caterpillars both feed on chokecherry, *Prunus virginiana* L., and create silken structures in which they reside until just prior to pupation. Preliminary observations suggested that predators and parasitoids reside in tent caterpillar tents after tents have been abandoned by the caterpillars, thus increasing the presence of enemies near tents. We hypothesized that when reared near tent caterpillar tents, fall webworms are less fit because of increased predation from nearby enemies (enemy-mediated competition). We tested our hypothesis at two field sites by placing fall webworm larvae in the field with or without a tent caterpillar tent nearby. We compared differences in survival rates, parasitism rates, and fitness between the larvae reared with and without tents nearby. At one field site, webworm larvae near tent caterpillar tents disappeared ~30% faster than did larvae without tents nearby. Although survival or fitness did not differ significantly between larvae reared with or without tents nearby, distance separating fall webworm larvae from a tent caterpillar tent might be important. Our results suggested that enemy-mediated competition might affect the fitness of fall webworm larvae and should be investigated further.

Introduction

Competitive interactions negatively affect the fitness of competing species and are well-documented in many plant and animal communities (Kaplan and Denno 2007 and references therein). Most studies of competitive interactions between herbivorous insects focus on competition between species that are dietary specialists instead of generalists (Kaplan and Denno 2007, Barnes and Murphy in preparation). Herbivorous insects are known to compete directly with each other (Sigmon 2015), but most competitive interactions between herbivores are probably indirect (Kaplan and Denno 2007).

Indirect competitive interactions occur through the action of an intermediary species such as a plant or a natural enemy, rather than through direct interaction between competitors (Kaplan and Denno 2007). With plant-mediated competition, herbivore damage activates defenses that affect other herbivores feeding on the same plant (Poelman and Kessler 2016). In contrast, with enemy-mediated

---

*Corresponding author: barnes.elizabeth20@gmail.com

69

competition, a common predator decreases fitness of one or more prey species by predation (consumptive effects) and/or fear-based behavioral (non-consumptive) effects (Sih et al. 1985, 2010). For example, Ramirez and Eubanks (2016) found that lady beetles reduced caterpillar population sizes by predation when near a preferred alternative food source. Beleznai et al. (2015) observed the influence of both consumptive and non-consumptive effects in a spider-leafhopper system; spider presence not only reduced leafhopper abundance directly by predation but also decreased rates of foraging by leafhoppers. Reduced foraging time is a non-consumptive effect that might result in prey population declines because when prey feed less in the presence of predators, the reproductive output of the prey population also decreases (Bourdeau et al. 2016). The role of enemy-mediated competition in insects is not as well understood as that of plant-mediated competition (Kaplan and Denno 2007).

Fall webworm, *Hyphantria cunea* (Drury), larvae feed on more than 650 plant species across their range but feed on fewer plants in Colorado (Loewy et al. 2013, Murphy and Loewy 2015). Western tent caterpillars, *Malacosoma californicum* (Packard), are similarly dietary generalists when considered across their entire range, but feed on fewer plants at a local level (Powell and Opler 2009). In Colorado, fall webworms and tent caterpillars feed on chokecherry, *Prunus virginiana* L. (Murphy and Loewy 2015, Barnes et al. 2016). Both herbivore species are gregarious, building communal webs or tents (Fitzgerald 1995, Loewy et al. 2013). Tent caterpillars create silken tents that expand as they develop (Fitzgerald 1995) and remain intact after being abandoned by the caterpillars (Barnes and Murphy 2018).

We tested if enemy-mediated competition occurs between two generalist herbivores, western tent caterpillar and fall webworm. Preliminary data (Barnes and Murphy in preparation) suggested that tent caterpillar tents abandoned by tent caterpillars might harbor a variety of predator species, such as spiders, that may attack nearby prey species, such as fall webworms. We tested the hypothesis that if early-season tent caterpillars indirectly decreased later-season fall webworm fitness through enemy-mediated competition, then fall webworms reared on shrubs that contained abandoned tent caterpillar tents would suffer increased predation pressure and be less fit compared to fall webworms on shrubs without tents. We measured fitness by measuring both survival and body mass (pupal mass), which are reliable proxies for lifetime fitness in fall webworm (Loewy et al. 2013). The fitness proxies allowed us to assess both consumptive and non-consumptive fitness effects of natural enemies on fall webworm. For consumptive effects of predators, we measured how quickly fall webworms disappeared in the field when webs of larvae disappeared and the number of surviving larvae in each web at the end of the field experiment. For consumptive effects of parasitoids, we measured the parasitism rate of fall webworm larvae. For non-consumptive effects, we weighed fall webworm pupae at the end of the experiment to determine whether fall webworm larvae near tent caterpillar tents were less fit than fall webworm larvae not near tent caterpillar tents in the field (e.g., by less time feeding in the field).

Materials and Methods

We conducted our experiment at two field sites in the foothills of the Colorado Rocky Mountains: Betasso Preserve (40° 0'50.58"N, 105°20'37.06"W) and Boulder Canyon (40° 0'48.18"N, 105°18'35.90"W). Betasso Preserve has a mixture of grasses and shrubs, but transitions to ponderosa pines at higher elevations. Boulder

Canyon has a riparian habitat with a mixture of shrubs and deciduous trees. Chokecherry was plentiful at both sites.

We collected 7 fall webworm webs containing early-instar larvae in the summer (27 June-20 July 2016) that became the 7 maternal lines in the experiment. We collected 5 webs from Boulder Canyon, 1 web from Betasso Preserve, and 1 web from Red Rocks Park (39°39'55.57"N, 105°12'20.44"W). Each adult fall webworm female lays a single mass with >100 eggs, so each web was considered an independent maternal line (Loewy et al. 2013). We reared the 7 maternal lines separately in 0.5-liter deli containers provisioned with chokecherry sprigs in a laboratory, following previously established techniques for rearing fall webworms (Loewy et al. 2013). For each maternal line, we initially reared larvae together in a single group because mortality increases if they are separated during early instars. After 2 weeks of being reared together, we divided each maternal line into multiple 0.5-liter deli containers with ~15 individuals per container and allowed the webworms in each container to build new webs for 5 days, which facilitated later transfer to the field.

We collected abandoned tent caterpillar tents from Boulder Canyon and Betasso Preserve. We placed a plastic bag over the tent to prevent predators from escaping, cut the branch to which the tent was attached, and quickly sealed the bag. We put bags containing tents into a cooler with ice to prevent any organism living in the tent from dying because of heat. We chilled tents (~2-4°C) for 3 days in a refrigerator in the laboratory before being placed in the field.

At each of the Boulder Canyon and Betasso Preserve sites, we located 10 pairs of undamaged chokecherry shrubs (n = 20 pairs total at both field sites, thus 40 shrubs total). In each pair of chokecherry shrubs, we haphazardly assigned one shrub to be a no-tent control shrub and the other to be a tent treatment shrub. We paired no-tent and tent shrubs in similar microenvironments and measured the distance between the shrubs (range = 0.6-8.7 m). All shrubs were not damaged by fall webworms and tent caterpillars for at least 1 year (Barnes personal observation) and we closely examined each shrub to ensure no damage by either herbivore species. On shrubs designated the tent-treatment, we attached a fall webworm web (n = 15 larvae), a tent caterpillar tent, and any predators in the tent. On no-tent control shrubs, we attached only a fall webworm web (n = 15 larvae). We used larvae from the same fall webworm maternal line for the tent and no-tent treatments in a shrub pair; each maternal line was used at both field sites, some multiple times.

We attached the fall webworm webs to the chokecherry shrubs using metal hair clips and attached the tent caterpillar tents using black plastic twist ties. We ensured that the fall webworm web and tent caterpillar tent were fixed in close proximity to each other (~10 cm) on the tent shrubs. For each fall webworm web placed in the field, we measured the mean length of individuals in the web by haphazardly sampling a single individual from the web (measured to the nearest 0.01 mm with calipers).

We left fall webworm larvae in the field for 2 weeks and monitored them every other day for signs of predation or disappearance from the web. If a web seemed to have no surviving larvae in it, we searched the entire shrub and all surrounding shrubs to ensure the fall webworm larvae had not moved to a new site. After 2 weeks, we collected the webworm webs from the no-tent and tent shrubs before the larvae reached the last instar and dispersed. We counted the number of webworm larvae that we retrieved from each shrub and calculated the percentage of survival in the field, excluding webs in which all larvae had disappeared. We continued rearing

71

surviving webworms in 1-liter deli containers until pupation in the laboratory (methods described in Loewy et al. 2013). Exactly 10 days after a larva pupated, we measured its pupal mass using a Mettler-Toledo XP6 microbalance (to the nearest 0.01 mg, Mettler-Toledo, Columbus, OH).

We tested the hypothesis that fall webworm larvae would be less fit when reared near tent caterpillar tents than without tents nearby by measuring differences in rates of all larvae disappearing from a web in the field (web disappearance), percentage of larvae surviving in remaining webs, parasitism, and pupal mass. To test for differences in the rate of web disappearance between tent and no-tent shrubs we used a Kaplan-Meier survival analysis; we tested whether disappearance rates varied by the tent treatment and site. For statistical tests of larval survival, at the end of the field experiment and in the laboratory, we used a Chi-squared test with treatment (tent, no-tent) and maternal line as fixed effects. To compare the number of larvae parasitized when reared on tent or no-tent shrubs, we used a Pearson's Chi-square test. To test for differences in pupal mass for larvae reared on tent and no-tent shrubs, we conducted a mixed model ANOVA with treatment (tent, no tent) and sex (male, female) as fixed effects and maternal line as a random effect. We also performed a two-sample T-test to test for size differences between webworm larvae on tent and no-tent shrubs.

In our experiment, we paired shrubs by microenvironment, but distance between the tent treatment and no-tent control shrubs in each pair varied. In several pairs, the no-tent shrub was <1 m from the tent shrub, but in other pairs, the no-tent shrub was much farther from the tent shrub. While all fall webworm webs on no-tent shrubs lacked a tent caterpillar tent, some no-tent shrubs were closer than others to tent shrubs, potentially leading to differential survival among webs on no-tent shrubs. We therefore tested a post-hoc hypothesis that survival for larvae on no-tent shrubs (for webs that had not disappeared during the 2-week field experiment) was correlated with distance between no-tent control and tent treatment shrubs in the pair. We used a linear regression with larval survival and distance between shrubs in the pair as effects. All shrubs in the pairs were 0.5-4.0 m apart except one pair in which the shrubs were 8.7 m apart; we performed the regression with and without the 8.7-m outlier. All statistical tests used either JMP Pro 13.0.0 or R.

Results

Webworm larvae near tent caterpillar tents disappeared at a significantly greater rate than larvae on no-tent shrubs at Betasso Preserve (Wilcoxon χ^2 = 5.19, df = 1, n = 180, p = 0.02) but not at Boulder Canyon (Wilcoxon χ^2 = 1.32, df = 1, n = 180, p = 0.25) during the 2 weeks of the field experiment (Fig. 1). However, we did not find any impact of the tent treatment on the overall percentage of larval survival at the end of the field experiment for webs that still contained larvae (χ^2 = 0.001, df = 1, n = 587, p = 0.97; Fig. 2) or until pupation in the laboratory (χ^2 = 0.004, df = 1, n = 587, p = 0.95; data not shown). We did find significant differences among maternal lines, however, in determining larval survival in both the field (χ^2 = 130.36, df = 6, n = 587, p < 0.0001) and the laboratory (χ^2 = 97.76, df = 6, n = 587, p < 0.0001; data not shown). Overall, we found no significant difference in the number of parasitized webworm larvae between tent and no-tent shrubs (χ^2 = 0.16, df = 1, n = 214, p = 0.68; data not shown). There were no significant differences in size of fall webworm larvae placed on no-tent (14.05 ± 2.6 mm) or tent-treated (13.67 ± 2.4 mm) shrubs (t = 2.02, n = 37, p = 0.63).
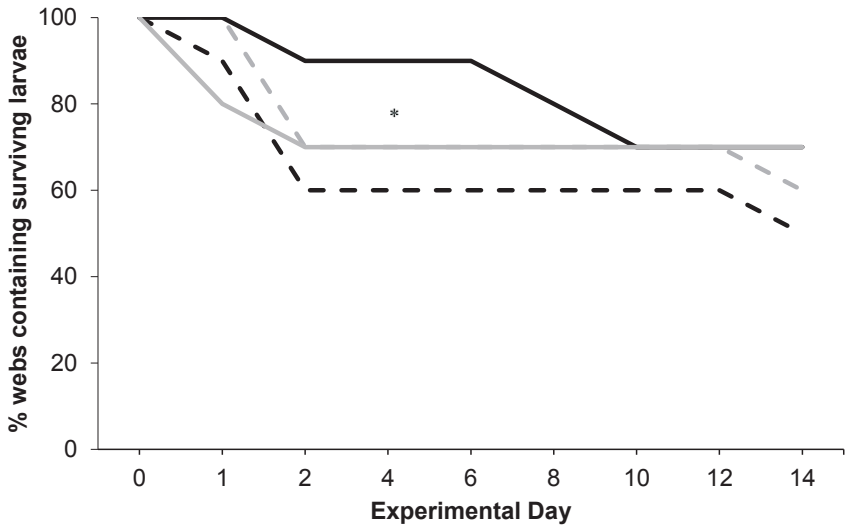
72

Fig. 1. Percentage of webs containing surviving larvae (surviving webs) during the field experiment on tent (gray lines) and no-tent (black lines) shrubs at Betasso Preserve (solid lines) and Boulder Canyon (dashed lines). Significant differences in survival of larvae between tent and no-tent webs at Betasso Preserve (solid black and gray lines) are indicated with an asterisk.
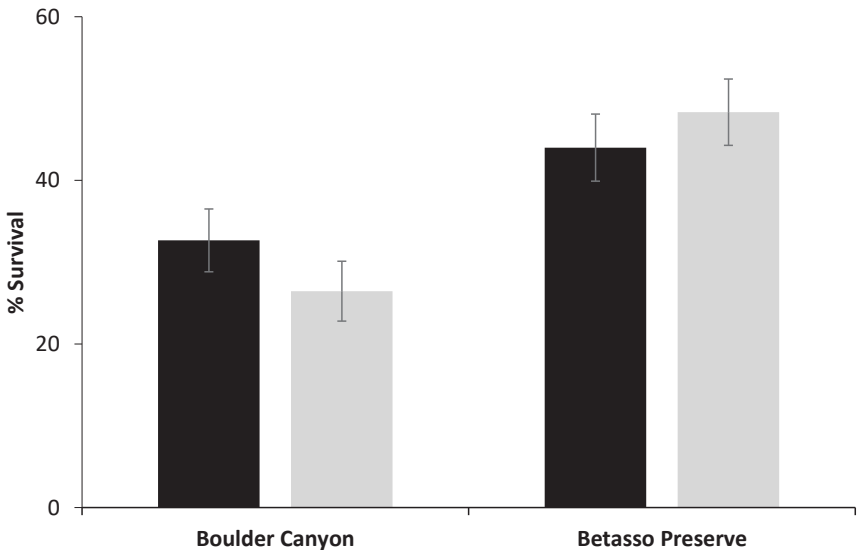

Fig. 2. Percentage of survival of fall webworm larvae reared on shrubs at Boulder Canyon (n = 298 individuals) and Betasso Preserve (n = 301 individuals) at the end of the 2-week experimental period. Larvae at each site were reared for 2 weeks with tents nearby (gray bars) or without tents nearby (black bars). Bar indicates ± 1 SE.

73

For our measure of non-consumptive effects, we found no significant effect of the tent treatment ($F_{1,192}$ = 1.04, p = 0.30) on pupal mass (Fig. 3). Maternal line had a marginal effect on pupal mass ($F_{5,192}$ = 3.79, p = 0.07), and sex (male or female) significantly influenced pupal mass ($F_{1,192}$ = 43.92, p < 0.0001), with female pupae weighing significantly more than males at both sites.
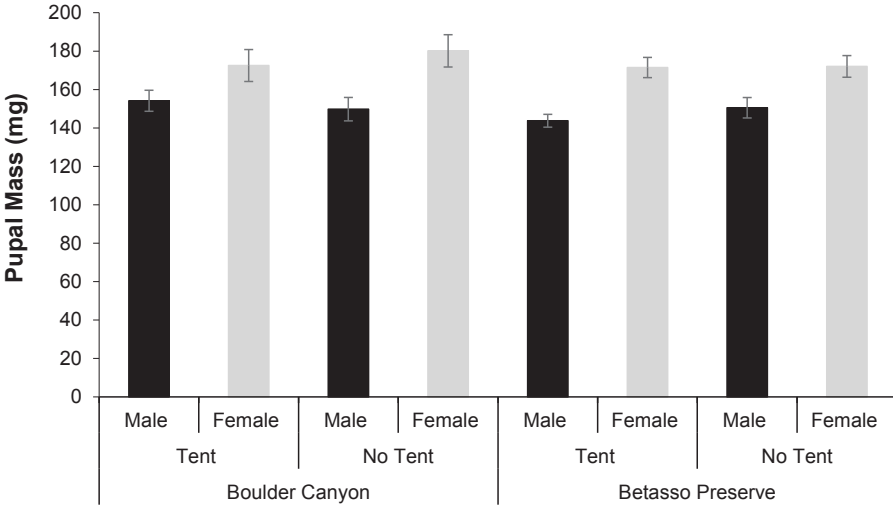

Fig. 3. Mean mass of male (black bars) and female (gray bars) fall webworm pupae reared on chokecherry shrubs with or without tent caterpillar tents at Boulder Canyon and Betasso Preserve sites. Bar indicates ± 1 SE.

Notably, we found an interesting relationship between larval survival in the no-tent control and the distance between paired shrubs; in other words, we found that the absolute distance from the no-tent treatment web to the tent caterpillar tent on the tent-treatment shrub may influence survival. We had one pair of shrubs that was farther apart (8.7 m) than the other pairs, so we tested the relationship with or without the outlier. The relationship between the distance between tent and no-tent shrubs and survival on no-tent shrubs when the 8.7-m point was included (r^2 = 0.29, $F_{1,11}$ = 4.11, p = 0.07, y = -0.05x + 0.74; Fig. 4) or excluded (r^2 = 0.32, $F_{1,10}$ = 4.22, p = 0.07, y = 0.12x + 0.5) was almost significant, but with opposing slopes.
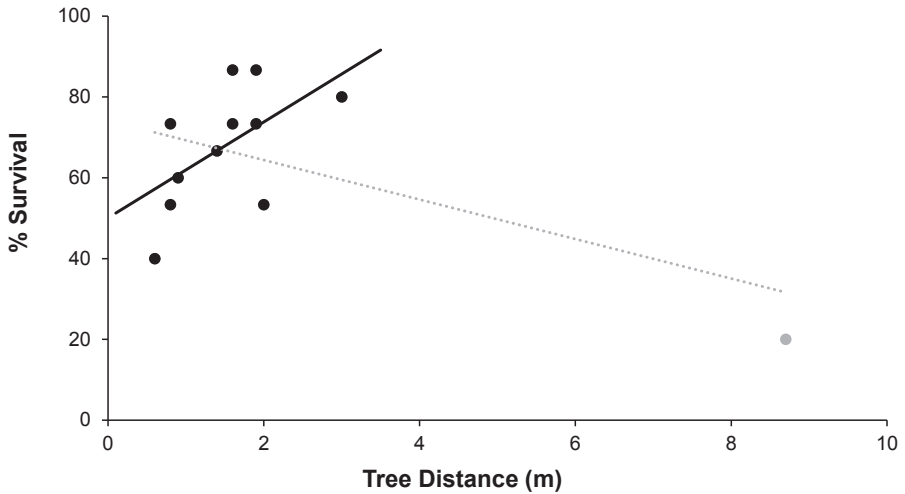
74

Fig. 4. Percentage of survival of fall webworm larvae in no-tent control (n = 12 webs; only webs with larvae surviving at the end of the field experiment are shown) at different distances from tent caterpillar tent treatment shrubs (black points and solid line). The dashed gray line shows the relationship when including all data, but because the shrub pair 8.7 m apart was an outlier, the black solid line shows the relationship for shrub pairs separated 0.5-4.0 m (excluding the outlier).

Discussion

Tent caterpillar tents might negatively affect survival of nearby fall webworm larvae through consumptive effects of predators attracted to tents. Parasitism rate was not affected by the presence of a tent caterpillar tent, so results probably were caused solely by predation. Potential predators of fall webworms include spiders (Araneae, Clerck 1757), paper wasps (Polistes, Latreille 1802), assassin bugs (Reduviidae, Latreille 1807), and stink bugs (Pentatomidae, Leach 1815; S. M. Murphy personal observation). At Betasso Preserve, webworm larvae disappeared at a greater rate when placed near a tent caterpillar tent, which supports the hypothesis that tents increased predation rates on nearby herbivores. Increase in larval disappearance among the webs might be because of greater predator abundance if predators reside in and/or are attracted to abandoned tent caterpillar tents. Muller and Godfray (1997) and Ramirez and Eubanks (2016) found consumptive effects of insect predators and Wesner et al. (2015) found that vertebrate predators reduced abundance of insect prey. While we found that entire webs of larvae disappeared more quickly near tent caterpillar tents at one site, larval survival did not differ in webs that contained larvae at the end of the field experiment, which might suggest that if predators find a web, they consume all of the larvae within it.

Our results also suggested that webworm survival might depend on distance of a web from tent caterpillar tents; we found an almost significant increase in larval

75

survival with distance from a tent (p = 0.07). The effect of distance on larval survival was a post-hoc hypothesis, and the experiment was not designed to test the possibility; future research should specifically test if distance of fall webworm larvae from a tent caterpillar tent affects survival. If predators living in abandoned tent caterpillar tents forage more intensely near tents or if predators are attracted to tents, then fall webworm larvae closer to tents would be more likely to be consumed. Previous research found that predator foraging intensity over distance decreased when prey was abundant and easily accessible (Ramirez and Eubanks 2016), such as our fall webworm webs in our experiment, which might explain increased survival of larvae in more distant webs.

We found that the presence of our tent caterpillar tents did not influence pupal mass, which suggested that predators did not affect webworm larval fitness through non-consumptive effects. We predicted that fall webworm larvae near tent caterpillar tents would reduce time spent foraging due to cues indicating nearby predators, which would decrease growth (e.g., Bourdeau et al. 2016). While Beleznai et al. (2015) and Ramirez and Eubanks (2016) found that prey species reduced foraging in the presence of predators, we did not find a similar response in pupal mass of fall webworms. Butcher et al. (2014) found relatively few prey species altered behavior in the presence of spider chemotactile cues and hypothesized that such predator avoidance techniques were species-specific. More research needs to test how webworm larvae react to predators, especially from different guilds.

Enemy-mediated competition might determine fitness of fall webworm larvae, but future research should directly test whether distance from a tent caterpillar tent affects fall webworm survival. The effect of distance on survival was a post-hoc consideration and thus the distance between tent and no-tent shrubs in each shrub pair varied greatly but did not represent a gradient of distance intervals. Understanding how far predators in tent caterpillar tents forage would be valuable, because the predators probably affect more species than just nearby fall webworms. A meta-analysis by Vidal and Murphy (2018) indicated that top-down forces had a greater effect on the fitness of herbivorous insects than did bottom-up forces. Thus, understanding consumptive effects of predators on prey is important as research continues to address how top-down forces structure communities of herbivorous insects and mediate competitive interactions.

Acknowledgment

References Cited

Barnes, E. E., S. Gosnell, C. Hallagan, K. E. Otten, L. Slayter, and S. M. Murphy. 2016. Performance of western tent caterpillar (*Malacosoma californicum*) on two common host plants, including a new host plant record. J. Lepidopterist's Soc. 70: 227-282.

Barnes, E. E., and S. M. Murphy. 2018. Time-lagged intraspecific competition in temporally separated cohorts of a generalist insect. Oecologia 186: 711-718.

76

Beleznai, O., G. Tholt, Z. Tóth, V. Horváth, Z. Marczali, and F. Samu. 2015. Cool headed individuals are better survivors: non-consumptive and consumptive effects of a generalist predator on a sap feeding insect. PLOS One 10.

Bourdeau, P. E., M. T. Bach, and S. D. Peacor. 2016. Predator presence dramatically reduces copepod abundance through condition-mediated non-consumptive effects. Freshwater Biology 61: 1020-1031.

Butcher, R., H. Binz, F. Menzel, and M. H. Entling. 2014. Effects of spider chemotactile cues on arthropod behavior. J. Insect Beh. 27: 567-580.

Fitzgerald, T. G. 1995. The Tent Caterpillars. Comstock Publishing Associates, Ithaca, NY.

Kaplan, I., and R. F. Denno. 2007. Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. Ecol. Lett. 10: 977-994.

Loewy, K. J, A. L. Flansberg, K. Grenis, M. K. Kjeldgaard, J. McCarty, L. Montesano, J. Vernick, and S. M. Murphy. 2013. Life history traits and rearing techniques for fall webworms (*Hyphantria Cunea* Drury) in Colorado. J. Lepidopterist's Soc. 67: 196-205.

Muller, C. B., and H. C. J. Godfray. 1997. Apparent competition between two aphid species. J. Anim. Ecol. 66: 57-64.

Murphy, S. M., and K. J. Loewy. 2015. Trade-offs in host choice of an herbivorous insect based on parasitism and larval performance. Oecologia 178: 741-751.

Poelman, E. H., and A. Kessler. 2016. Keystone herbivores and the evolution of plant defenses. Trends in Plant Science 21: 477-485.

Powell, J. A., and P. A. Opler. 2009. Moths of Western North America. University of California Press, Berkeley, CA.

Ramirez, R. A., and M. D. Eubanks. 2016. Herbivore density mediates the indirect effect of herbivores on plants via induced resistance and apparent competition. Ecosphere 7.

Sigmon, E. 2015. Interspecific variation in aggressive fighting behavior of shelter-building caterpillars. J. Insect Beh. 28: 403-415.

Sih, A., P. Crowley, M. McPeek, J. Petranka, and H. Strohmeier. 1985. Predation, competition, and prey communities: a review of field experiments. Annu. Rev. Ecol. Ecol. Syst. 16: 269-311.

Sih, A., D. I. Bolnik, B. Luttbeg, J. L. Orrock, S. D. Peacor, L. M. Pintor, E. Pressier, J. S. Rehage, and J. R. Vonesh. 2010. Predator–prey naïveté, antipredator behavior, and the ecology of predator invasions. Oikos 119: 610-621.

Vidal, M. C., and S. M. Murphy. 2018. Bottom-up vs. top-down effects on terrestrial insect herbivores: a meta-analysis. Ecol. Lett. 21: 138-150.

Wesner, J. S., P. Meyers, E. J. Billman, and M. C. Belk. 2015. Habitat consumption and selection across a landscape of multiple predators. Ecol. Evol. 5: 121-129.