Phytohormone Pathway Crosstalk and the Relationship Between Defoliating Herbivores and Phloem-Feeding Insects on White Oak Trees

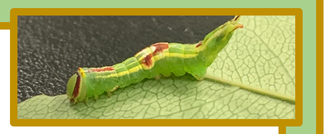
Sarah Timbie, Riley Anderson, Professor Singer
Department of Biology, Wesleyan University

Introduction

Ecosystems consist of complex interactions between species that range from direct (e.g. insects eating a leaf) to indirect, such as the relationship we studied this summer of defoliating herbivores and phloem-feeding insects that share a plant as a food source. Phloem-feeding insects trigger a defensive hormone response pathway in plants: the salicylic acid (SA) pathway. Induction of SA suppresses another defensive pathway: the jasmonic acid (JA) pathway, induced by feeding from leaf-chewers. The JA pathway increases levels of chemical defenses in leaves and suppressive hormone crosstalk can render the plant more susceptible to defoliating herbivores, like caterpillars.

Inhibition of plant resistance to defoliating herbivores caused by hormone crosstalk initiated by phloem-feeders has been noted in many plants. We focused on white oak, manipulating phloem-feeding insects (specifically treehoppers in this experiment) on branches and testing if the presence of these insects altered leaf palatability for caterpillars. We hypothesize that phloem-feeding insects induce plant susceptibility to caterpillars through interactions mediated by plant chemistry and that leaves on branches of white oak with phloem-feeding insects have fewer JA-regulated defenses than those on branches without phloem-feeders. Similar effects should be found when branches are sprayed with defensive phytohormones. We predict that leaves from branches with treehoppers or are exposed to SA will be more palatable to caterpillars than branches devoid of treehoppers or exposed to JA.

Methods



Leaf and caterpillar collection,

- 16 white oak branches with treehoppers from Cockaponset State Forest (CP), Portland Reservoir (PR), and Meshomasic State Forest (MS) were randomly assigned to have treehoppers either manually removed (8) or replaced (8)
- 63 caterpillars of several species were collected from white oak trees at the same forest sites for the treehopper experiment.
- For the hormone experiment, 30 branches of white oak trees lacking treehoppers were used in CP and MS. Five branches at each site were randomly assigned to be sprayed with aqueous solutions of methyl salicylate (100 mg/L), methyl jasmonate (0.5 mM), or with water.
- 73 caterpillars of several abundant species were collected from white oak trees in CP, PR, MS, and Miller's Pond for the hormone experiment. 4 – 6 leaves of treated branches were haphazardly collected 5 – 6 days after field treatments were set up and transported to the lab for caterpillar feeding and growth assays.

Experimental Results

Treehopper Experiment Feeding Assay

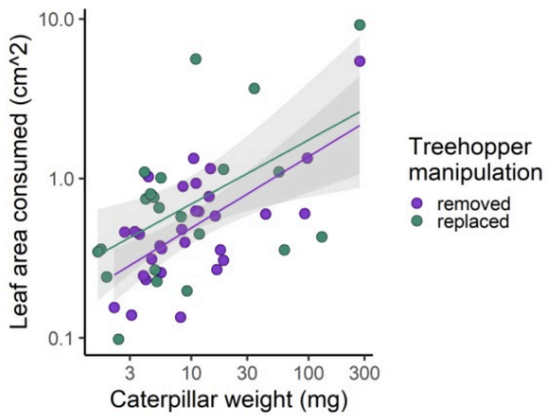


Figure 1. Shows feeding response measured as a function of leaf area consumed and caterpillar weight for each treehopper manipulation. Similar slopes indicate that there was no difference in the amount of leaf consumed due to treehoppers being removed or replaced. The removal of treehoppers did not alter the palatability of the leaves (z = 1.392).

Treehopper Experiment Growth

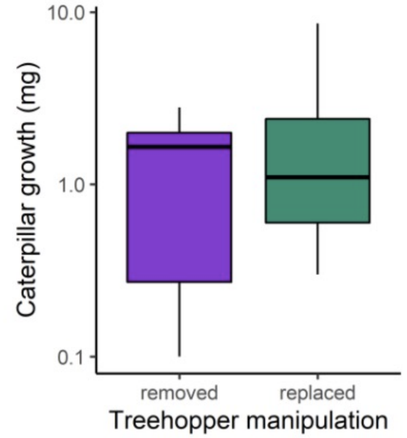


Figure 2. Shows a plot of the distributions of caterpillar growth compared to treehopper manipulation. Interquartile ranges between the two treatments overlap significantly, which is evidence that the removal of treehoppers did not alter the growth of the caterpillars (z = 0.696).

Hormone Experiment Feeding Assay

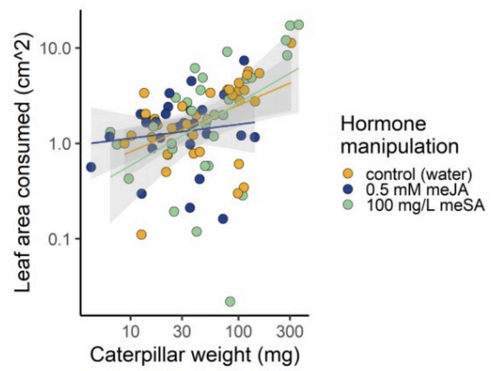


Figure 3. Shows feeding response measured as a function of leaf area consumed and caterpillar weight for each hormone treatment. Datapoints fall far outside of the model's confidence interval. Neither the meJA nor meSA treatment altered the palatability of the leaves (t = -0.962 and t = -1.782).

Hormone Experiment Growth

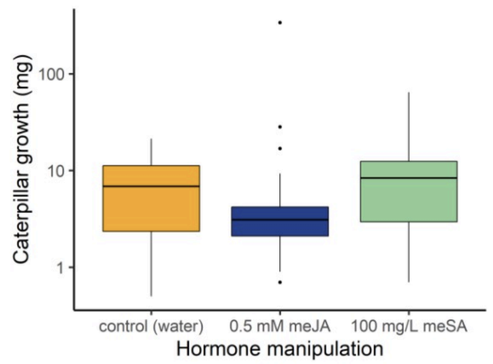


Figure 4. Shows plots of the distributions of data from caterpillar growth in each hormone manipulation. The medians are all relatively close together. Neither the meJA nor meSA treatment altered the growth of the caterpillars (t = 0.525 and t = -0.280).

Conclusions

There was no evidence that phloem-feeding insects induce susceptibility of white oak leaves to feeding from caterpillars.

Similarly, salicylic acid and jasmonic acid treatments did not affect the leaf palatability of white oak for caterpillars.

Discussion

There are many different potential explanations for why the results of this experiment did not support our original hypothesis. It is possible that the hypothesis was wrong all together due to errors in previous observations. This could be because there was a misjudgment in the observation that branches with more phloem-feeders also have more caterpillars, but it could also be that the phytohormone pathway crosstalk does not affect the palatability of leaves for caterpillars. The original hypothesis could also still be true, but the methods we used did not produce supporting results. One option for what could have gone wrong with our methods is that the sample size of caterpillars might have been too small. The caterpillars themselves could also have been too small and the amount that they ate from the leaves was not large enough for our analysis to register trends. The feeding time window for the caterpillars was also rather brief, so allowing the caterpillars more time to feed could alter results.

Acknowledgments

We would like to thank all the Singer lab members, especially Kiran Kowalski for all the help and support this summer, the Wesleyan College of the Environment Research Fellowship Program, and the creators of LeafByte.

Figure 6. Lab setup of caterpillars in 175 mL plastic cups for rearing

Feeding and growth assays:

- Caterpillars were kept in 175 mL plastic cups. Feeding and growth were measured in a 24-hour assay under ambient laboratory conditions.
- Field-collected caterpillars in both experiments were randomly assigned to experimental treatments.
- Each experimental caterpillar was starved for 24 hours then weighed prior to each assay to determine its initial mass. During the assay, each caterpillar was given a single experimental leaf for 24 hours

After the assay, each caterpillar was starved again for 24 hours then weighed to determine its final mass.

- Feeding was measured as leaf area loss. The application LeafByte was used to calculate the area in cm^2 of the treatment leaves before and after caterpillar feeding to determine how much the caterpillars ate.
- Caterpillar growth was measured as the difference between the final and initial mass.

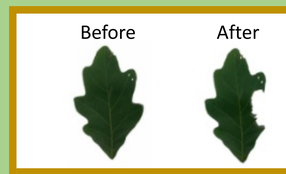


Figure 5. LeafByte images from before and after a caterpillar feeding