

Larval Feeding Ability on a Drought-Stressed Invasive Plant

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Thlaspi arvense

The role of drought-stressed *Thlaspi arvense* on *Pieris macdunnoughii* consumption and its effect on the different instar stages.

Abstract

Native species are threatened by the introduction of non-native and invasive organisms. Invasive plants specifically can change an ecological community at multiple levels within biotic factors by disrupting reproductive, feeding, and other behavioral patterns of the existing native species. Non-native species can interact with native species specifically in plant-herbivore or prey-predator relationships. This can affect insects' oviposition that require host plants to lay their eggs and hatch successfully. We tested the hypothesis that larvae of *Pieris macdunnoughii* (Lepidoptera: Pieridae) would feed on the drought-stressed *Thlaspi arvense* more extensively than non-drought-stressed *Thlaspi arvense* (Brassicaceae). To do this, we created three different groups of *Thlaspi arvense* plants and measured the amount of plant biomass consumed at each instar stage. We then compared the amount of biomass consumed to determine which drought-stressed or non-drought-stressed environment had the most feeding. The results of the experiment showed that the instar, plant treatment, and female preference were insignificant in larval feeding ability. This indicates that the *Pieris macdunnoughii* larvae are deterred from eating *Thlaspi arvense* despite the growth in their mouth parts in the later instar stages.

I. Introduction

Non-native and invasive plant species pose a threat to native plants and animals and can disrupt the balance of an ecosystem. Not only do invasive plants have an effect on producers within the community, but they also impact organisms that rely on native species. Invasive plants can outcompete native species which in turn can disrupt the previously established food chains

and cause other populations to decline. If there is a decrease in native plants, animals must find a replacement for possible food sources, habitat, or new place for oviposition. An invasive species may be the only option and animals will evolve to use them. As the presence of non-native and invasive plants increases due to shifts in species distribution, the possibility of insects interacting with these species increases simultaneously (Morri  en et al., 2010; Rasmann et al., 2014).

Oviposition interactions are essential for insect species to reproduce. Host plants are extremely important for Lepidoptera because butterflies will lay their larvae on a specific plant that can also feed the larvae when they hatch and enter the instar stages. However, when non-native and invasive species outcompete the host plant, there is a possibility the butterfly will oviposit on a plant that can harm the larvae. These non-native and invasive plants have similar chemical cues as native plants; however, they may be unpalatable or toxic (Nakajima et al., 2012). Therefore, when butterflies lay on a non-native or invasive species, there is a chance the larvae will not be able to survive because the plant is toxic. Like other butterflies, the *Pieris macdunnoughii* has a limited number of host plants that can be used for oviposition (Steward et al., 2018 also the Chew papers from the 1970s). In the Gothic, Colorado region, *P. macdunnoughii* oviposits eggs on the native mustard plant, *Cardamine cordifolia*, which is non-toxic to the larvae. However, over approximately the past 140 years, *P. macdunnoughii* have begun ovipositing on the invasive plant, *Thlaspi arvense*. The Eurasian plant, *T. arvense*, was likely introduced to Gothic during the 1800s. *Pieris macdunnoughii* mistakes *T. arvense* for the native hosts, which causes the larvae to ingest the toxic plant. This could have a negative effect on the *P. macdunnoughii* population overtime.

In recent years, climate change and global warming have caused temperatures and climates to change across the earth. In Gothic, Colorado is currently under drought conditions

which means several plants will be experiencing a lack of water. Because *P. macdunnoughii* has been ovipositing on *T. arvense*, we wanted to explore how drought affects the feeding ability of the butterfly. For this experiment, we hypothesized that the *P. macdunnoughii* larvae would feed on the drought-stressed *T. arvense* more extensively than non-drought-stressed *T. arvense*. This is because the chemical composition of drought-stressed and non-drought-stressed plants differs. We predicted that the larval instar and plant treatment would affect the amount of plant biomass consumed. We also hypothesized that female host plant preference and female identity may have significant effects on how much the larva ate.

II. Methods

We collected eight female *Pieris macdunnoughii* butterflies from three different locations: Cement Creek, Quigley Creek, and Copper Creek. These locations had differing elevations and had either both *C. cordifolia* and *T. arvense* or just *C. cordifolia*. This would allow for variation amongst the female preference. We placed each butterfly in a plastic rearing cage with one non-flowering *C. cordifolia* plant and one non-flowering *T. arvense* plant. These plants had been transplanted and were relatively the same size and growth stage. We kept the butterflies in an incubator, fed them twice a day, and counted their eggs. After a butterfly had laid over 50 eggs, we recorded the female's preference, which was determined by how many eggs they had laid on each plant. We removed both the *C. cordifolia* and *T. arvense* plants and put them on a radish which had been grown from seeds earlier in the summer.

We collected *T. arvense* plants throughout the experiment from three different sites: Kettle Ponds, Crystal Cabin, and Gothic Road. We created the three different treatment groups by planting *T. arvense* in four-by-four-inch pots. For the control group, *Thlaspi* Normal (TN), the

plants were planted the day they were collected. However, for Drought-Stressed *Thlaspi* (TDS) and Drought-Stressed Recovered *Thlaspi* (TDR) we kept the plants in a plastic bag until they were physically drought-stressed with wilted leaves. Once drought-stressed, the plants were planted and watered. The *Thlaspi* Normal and Drought-Stressed Recovered *Thlaspi* groups were watered once every day, whereas the Drought-Stressed *Thlaspi* group was watered once a week. We measured the amount of soil moisture present in the soil using the HH2 Moisture Meter. For the drought-stressed treatment, there was five to ten percent of water present. However, for normal and drought-stress recovery, there was 20-25 percent moisture in the soil.

The larvae were kept on the radish plants until they reached a specific instar, at which point they were placed on *T. arvense*. The exception was first instar larvae, which were put directly on one of the plant treatments. Before a larva was placed on a plant, I photographed the *T. arvense* next to a two-by-two-centimeter piece of red paper. This was used as a reference of measure when calculating how much plant biomass had been consumed. I checked on the larvae every two hours, and if they had walked off the plant, I placed them back on it using paintbrushes. If there was evidence the larvae had eaten, I recorded how long they were on the plant and photographed the plant with the red paper again. We calculated how much plant biomass was consumed by using the application, ImageJ 1.53k14. I used the before and after photos to calculate how much plant biomass was missing after the larvae had eaten. I calculated how many minutes the larvae spent on the plants and determined how much of the plant was consumed per minute.

III. Data Analysis

We analyzed the data using R v.3.6.2. We ran multiple general linear mixed models and linear models to determine which factors, larval instar, plant treatment, female preference, or

female number were significant. We ran an AIC test to determine which model best fit the data. However, after analyzing the models, we determined that the generalized linear mixed model where the female number was a random variable fit the data the best. This was due to the fact that we had repeated data in the dataset.

IV. Results

Larval instar, treatment type, female number and female plant preference did not significantly affect the amount of plant biomass the larva consumed (Fig. 1: GLMM). The P-values for the instar level, treatment type, and female preference were 0.08, 0.49, and 0.40 respectively. This was determined when the female number was set as a random variable and the instar, treatment type, and female preference were fixed variables. This contradicted my hypothesis that the different larval instars would consume drought-stressed *T. arvense* more extensively than non-drought-stressed *T. arvense*.

Although the second instar larvae consumed more plant biomass overall, the average amount of plant biomass consumed by each larval instar was relatively similar between the first, second, and third instar larvae (Fig. 2).

V. Discussion

Although the factors in the experiment, larval instar, plant treatment, female number, and female preference had insignificant effects on feeding rate, this provides evidence that the *P. macdunnoughii* larvae have a preference in what plants they consume. As the larvae grow into different instar stages, their heads and mouth parts increase in size which allows them to consume more of a plant. However, the results showed that despite the increase in the size of

mouth parts, the larvae were deterred from consuming *T. arvense*. If the *P. macdunnoughii* larvae preferred *T. arvense* the amount of plant biomass consumed would increase as the instar stage increased. However, the average amount of plant biomass consumed remained relatively the same amongst the instar stages. Also, in several trials, the third, fourth, and fifth instar larvae walked off the *T. arvense* plant and did not return. This suggests that the larvae do not have a preference for *T. arvense*.

There are factors within this study that may be biasing results. Transplantation, the method used to drought stress the plants, and how long they were drought-stressed for may have expedited the flowering process. Flowering produces different chemical cues than non-flowering plants which could have deterred the larvae from eating the *T. arvense*. The method which we used to drought stress the plants may not have been the best way to create a drought-stressed environment. Another factor that may have impacted the results was identifying which instar the larvae had entered. We were able to determine the larval instar stage by looking at the head and body size, however, measuring the head capsules of the larvae would ensure uniformity among the instar data.

It is difficult to draw conclusions from a small sample size, however this research lays the foundation for future experiments to be conducted as there is evidence of plant preference. Future research needs to be conducted in order to determine how consuming *T. arvense* affects each instar stage and which mustard plants the larvae are deterred from. In order to better control these factors throughout the experiment, different methodologies can be used. Growing the *T. arvense* plants from seeds would allow for more uniform control over the age of the plant and generation. Also, using a different method of drought-stressing the plants, such as soaking the plants in water would allow for a more accurate and uniform way of creating the drought-

stressed environment. Measuring the head capsules of the larvae would also ensure the larvae have reached specific instars.

The presence of invasive and non-native species has an impact on ecosystems around the world. Certain insect-plant interactions are affected by their presence, specifically oviposition. This study provides a foundation for understanding how invasive species impact oviposition and larval feeding processes under changing environmental conditions. This experiment also provides a better understanding for how species are interacting and adapting to climate change. We show that drought-stress, as we induced it, has little impact on larval preference.

VI. Acknowledgements

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VII. References

- Cerrilla, C. (2016). *Use of Invasive Mustard *Thlaspi arvense* by Insect Enemies*. 1–5.
- Morrien E, Engelkes T, Macel M, Meisner A & van der Putten WH. (2010). Climate change and invasion by intracontinental range-expanding exotic plants: the role of biotic interactions. *Annals of Botany*, 105: 843–848.
- Nakajima, M., Boggs, C. L., Bailey, S., Reithel, J., & Paape, T. (2012). Fitness costs of butterfly oviposition on a lethal non-native plant in a mixed native and non-native plant community. *Springer-Verlag*, 823–825. <https://doi.org/10.1007/s00442-012-2537-z>
- R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>

Steward, R. A., Fisher, L. M., & Boggs, C. (2018). Pre- and post-ingestive defenses affect larval feeding on a lethal invasive host plant. *Entomologia Experimentalis et Applicata*, 292–294. <https://doi.org/10.1111/eea.12773>

VIII. Figures

Figure 1.

	Estimate St. Error	T. Value	df	Pr(> t)	P-Value
Intercept	9.225e-06	8.570e-06	8.628e+01	1.076	0.28
Instar	4.791e-06	2.722e-06	8.628e+01	1.760	0.08
Plant Treatment (TDS)	-3.608e-06	5.297e-06	8.628e+01	-0.681	0.49
Plant Treatment (TN)	-4.161e-06	4.941e-06	8.628e+01	-0.842	0.40

Figure 2.

