Restoration Efforts for the Oregon silverspot butterfly: How do herbicides impact early blue violet biomass and consumers while exposed to invasive plant competition?

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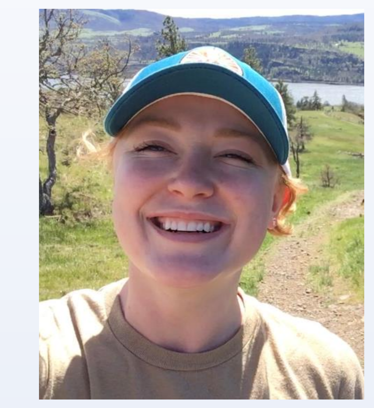
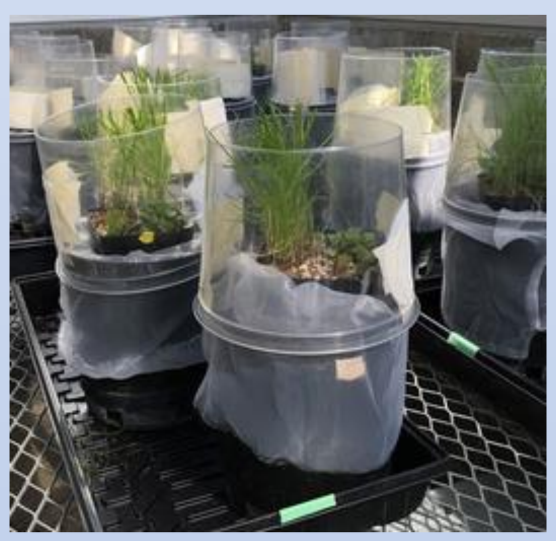
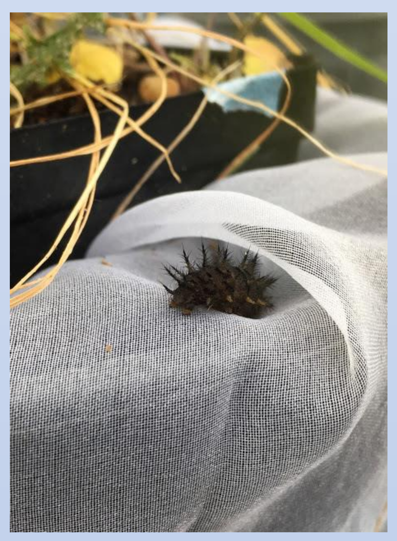


Abstract

Plant resources for at-risk butterflies are increasingly growing in stressful environments due to competition with invasive weeds. Selective herbicides are a sought-after tool by managers to reduce invasive plants, but impacts on butterflies are poorly understood. Although lethal and sub-lethal effects of herbicides have been examined for some species, few studies examine herbicide effects on caterpillars within the context of plant competition. The effects of one grass-specific herbicide (fluazifop-p-butyl) and one broadleaf-specific herbicide (clopyralid) were examined on host-plant violets, *Viola adunca,* grown in competition with tall fescue, *Festuca arundinacea,* and false dandelion, *Hypochaeris radicata*. Caterpillars of a surrogate species related to threatened Oregon silverspot butterfly, *Speyeria zerene zerene,* were raised on treated violets. Differences in violet biomass and *Speyeria z. zerene* pupal and adult biomass were assessed. Grown in competition, violet biomass was positively correlated with use of either herbicide in initial measurements. In final measurements, violet biomass was significantly different between those competitively grown, regardless of herbicide, and those grown without competition. Pupal and adult biomass were positively correlated with use of either herbicide when the violet was grown in competition. Both herbicide treatments produced greater pupal and adult biomass when compared to the untreated procedural control. Significant differences were found between pupae and adults reared on plants grown in absence of competition versus those reared on plants grown in competition and treated with fluazifop-p-butyl. These results imply that stress of competition may alter the quality of host-plants and herbicide use in managing at-risk butterfly habitats requires further examination.





Methods

Host plants were grown in differing conditions to act as a food source for developing caterpillars. Planting protocols were as follows:

VC (Violet Control) - Greenhouse grown violets transferred into 3.5x3.5 inch pots (figure 1).

PC (Procedural Control) - Greenhouse grown violets, greenhouse grown tall fescue grass and harvested false dandelion (figure 2).

CN (Clopyralid Treated) - Violets, tall fescue grass, false dandelion treated with Clopyralid herbicide (broad-leaf specific) (figure 3).

FN (Fluazifop Treated) - Violets, tall fescue grass, false dandelion treated with Fluazifop-p-butyl herbicide (grass specific) (figure 4).

- Plant measurements were to determine aboveground biomass of each experimental group at 2 weeks and 4 weeks post herbicide treatment.
- Caterpillars were removed from diapause in May 2019. Following a period of 1 week, around second instar, the caterpillars were randomly assigned treatment groups, weighed and sprayed with the respective herbicide treatments.
- 1 caterpillar was added to each microcosm (n=15 per treatment).
- Caterpillars were monitored every 24 hours* and transferred to new plants as needed to ensure sufficient plant matter for consumption. *As caterpillar food needs grew, monitoring increased to once every 12 hours.
- ~12 hours following pupation, pupae were removed from their microcosm, weighed and placed into containers to await emergence.
- Upon emergence, butterflies were photographed for morphology purposes and provided melon Gatorade for sustenance until mortality.

Results

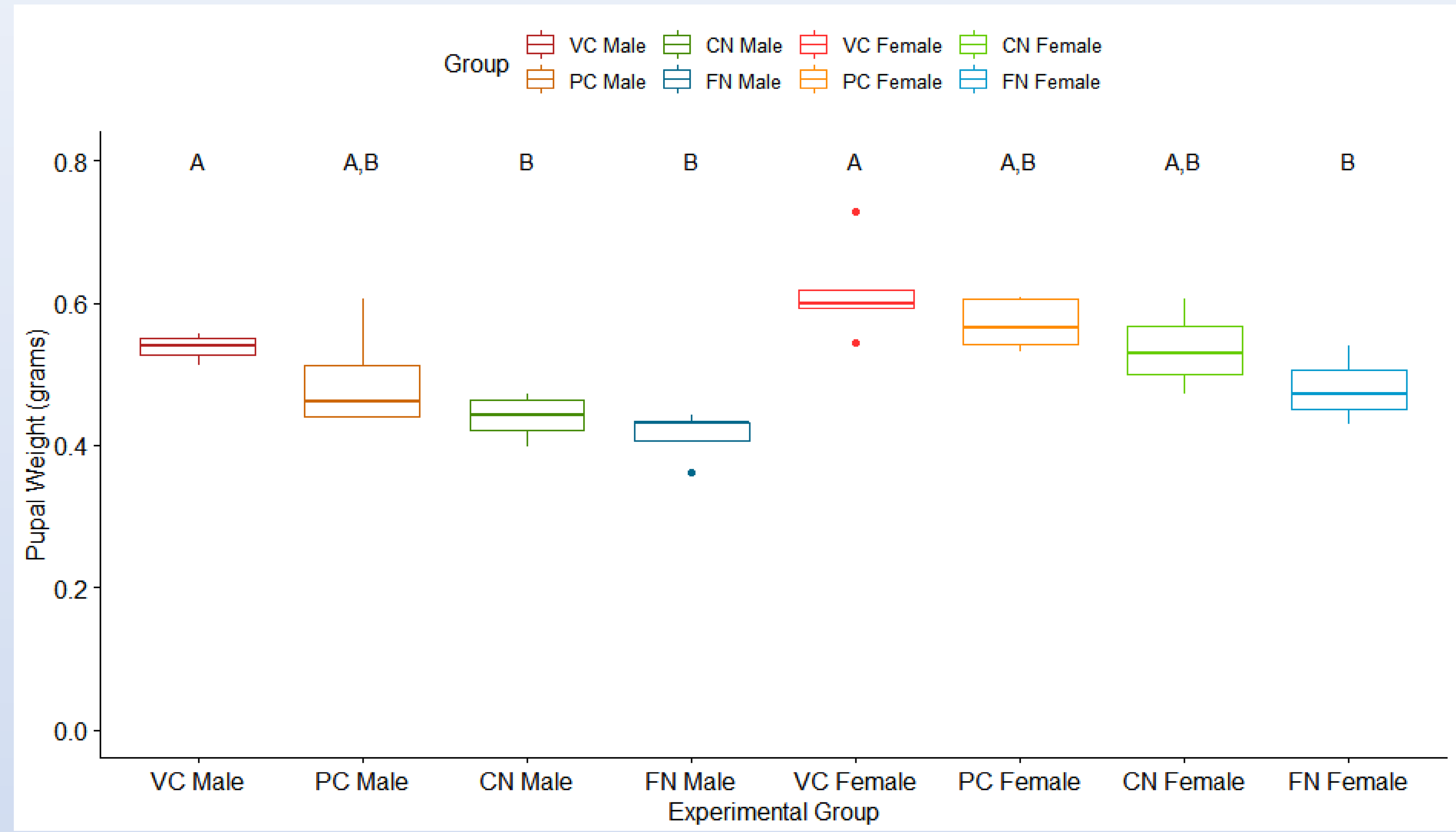


Figure 5. Box plot showing pupal weight, separated by experimental group (n=15) and sex. Statistical analyses included one-way ANOVA and Tukey's Honest Significant Difference Test. Significant values ($p < 0.05$) signified by different letters. It should be noted that males and females were assessed separately.

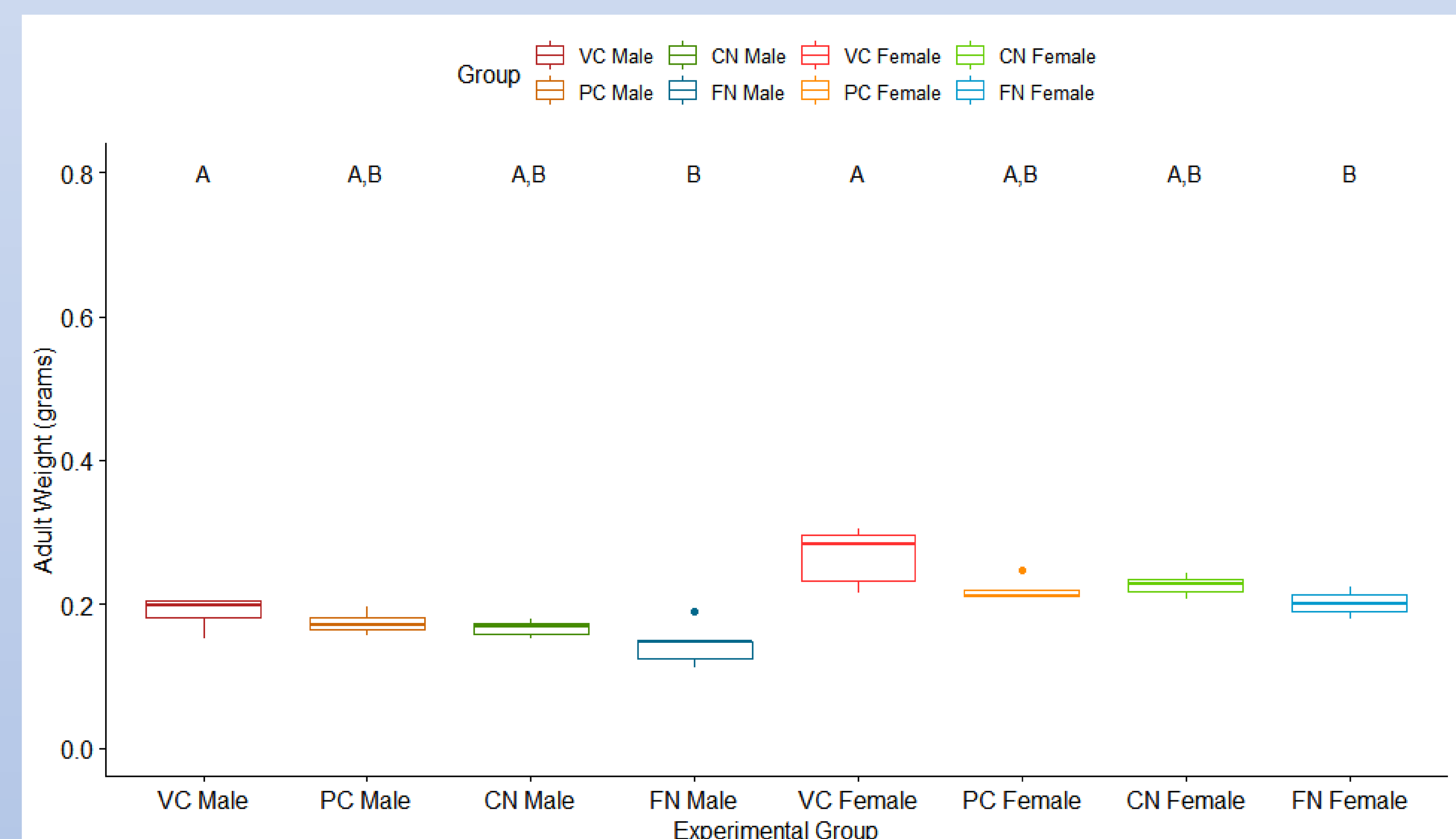


Figure 6. Box plot showing adult weight, separated by experimental group (n=15) and sex. Statistical analyses included one-way ANOVA and Tukey's Honest Significant Difference Test. Significant values ($p < 0.05$) signified by different letters. It should be noted that males and females were assessed separately.

Figure 1. VC (Violet Control) planting protocol.

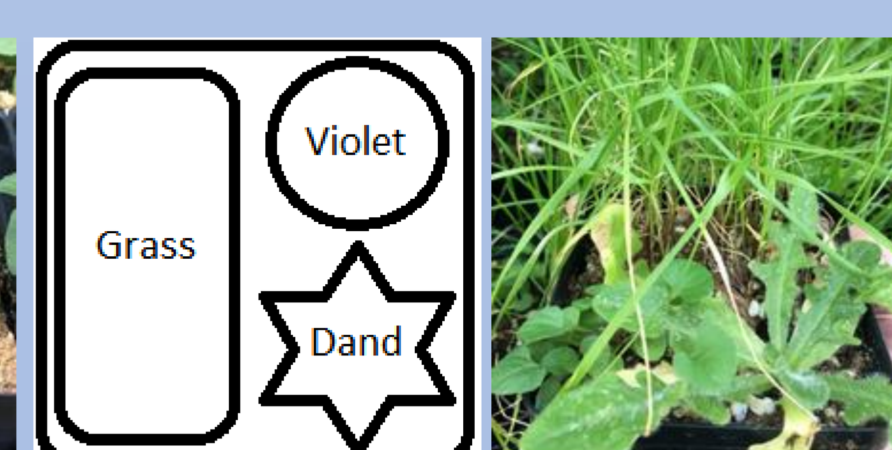


Figure 2. PC (Procedural Control) planting protocol.

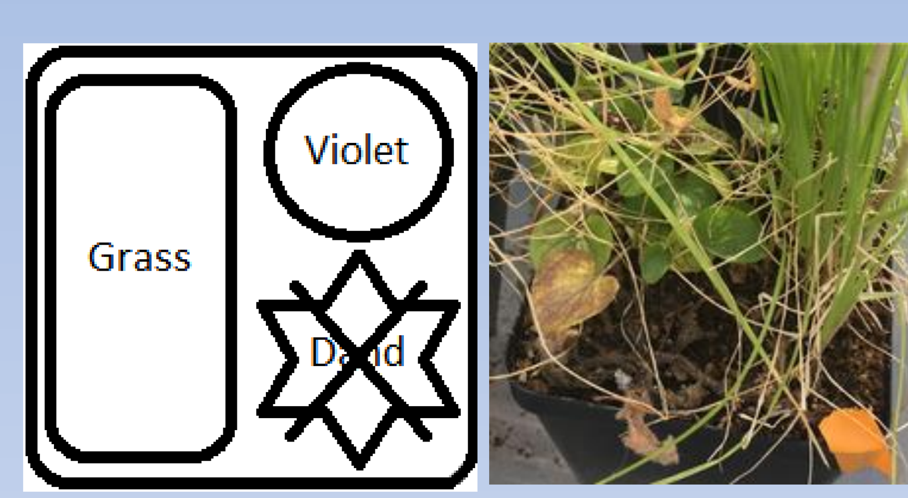


Figure 3. CN (Clopyralid treated) planting protocol, effect on plants.

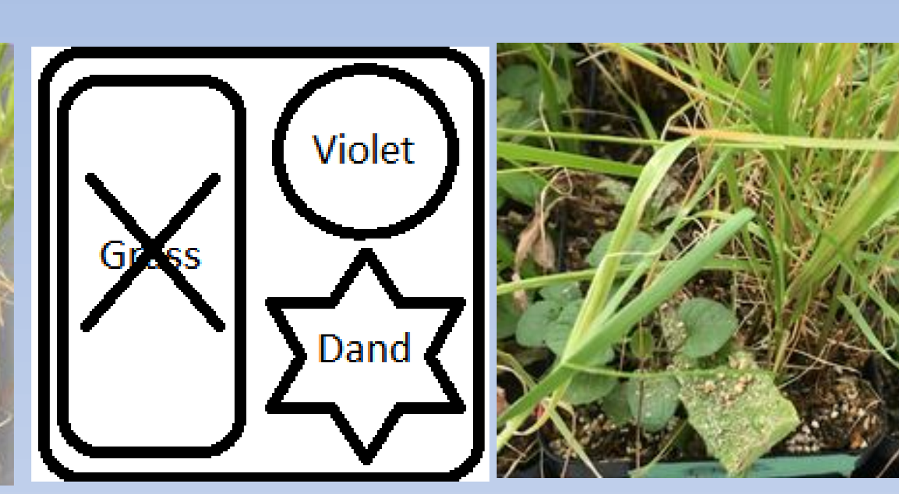


Figure 4. FN (Fluazifop treated) planting protocol, effect on plants.

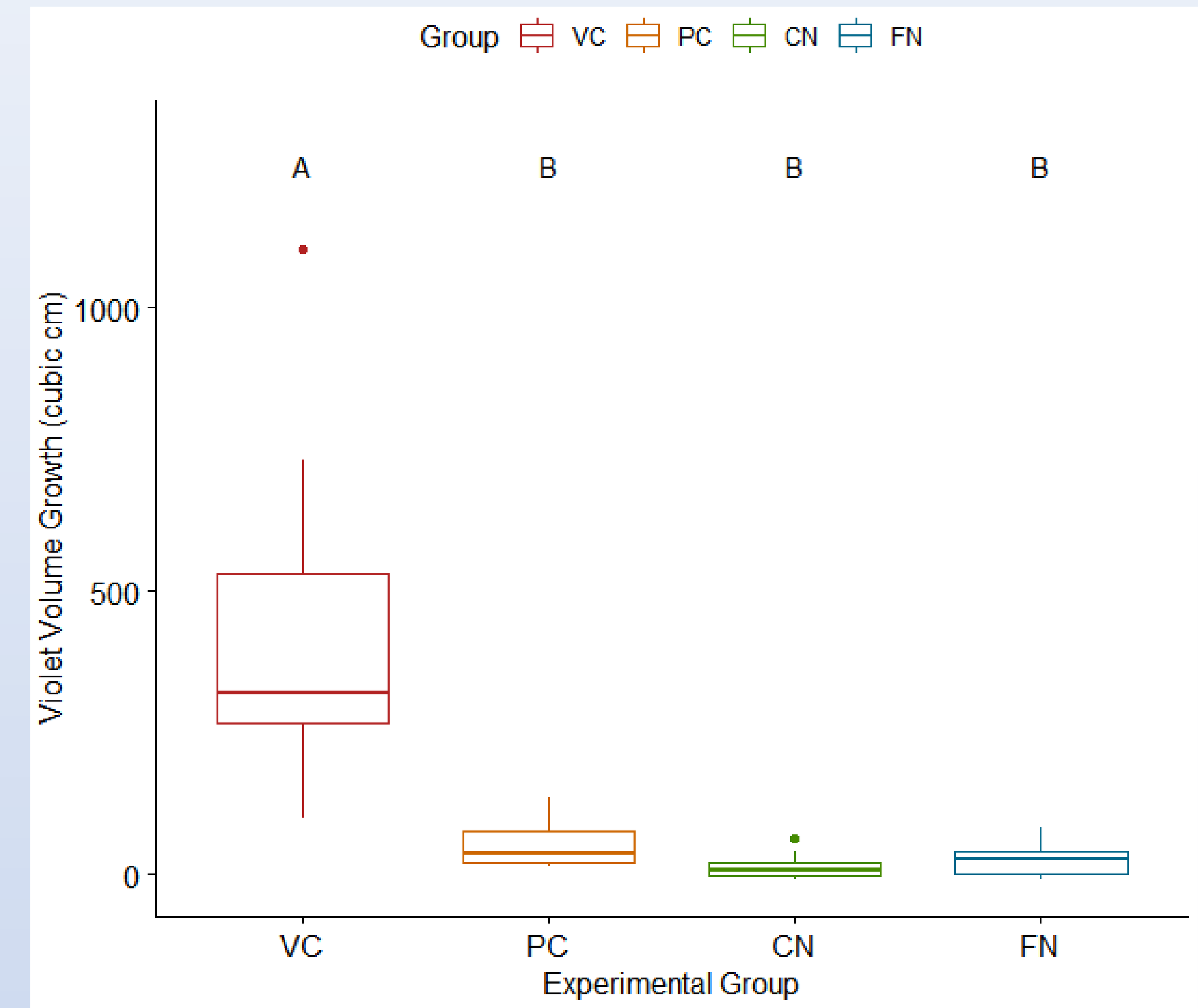
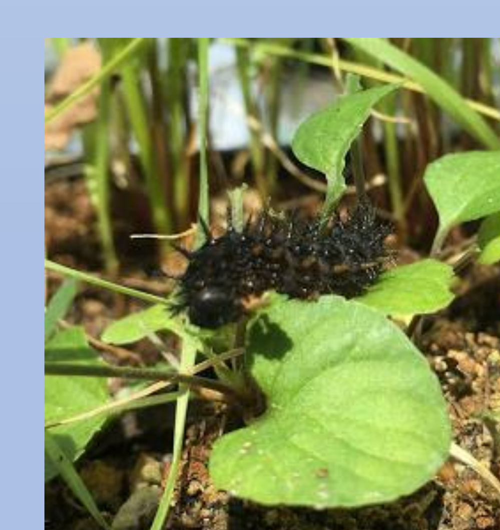
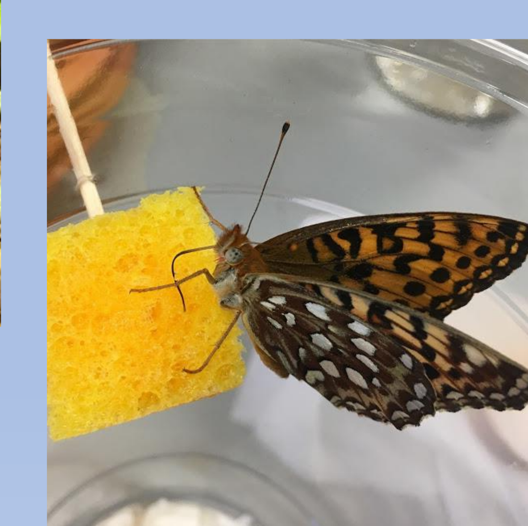


Figure 7. Box plot showing the violet growth between initial and final measurements, separated by experimental group (n=10). Statistical analyses included one-way ANOVA and Tukey's Honest Significant Difference Test. Significant values ($p < 0.05$) signified by different letters.

Conclusions

There is a clear trend in the weight data that the violet control (VC) group pupae and butterflies were larger than all other groups (fig. 5, 6). This implies that there may be a nutritional difference between the violet plants grown in the absence of competition compared to those grown within competition. The one consistently significant difference found in pupal and adult weight was between the violet control (VC) and the fluazifop (FN) treatment, which may imply that the false dandelion growth was more impactful on violet growth than the tall fescue grass. However, there was no significant difference between the violet plant size in those treatments (FN vs. CN), which supports a possible difference in nutritional composition. Violet growth in the VC group was also found to be significantly greater than all other groups, which was not surprising (fig. 7). Chemical analyses of violet leaves from this experiment are currently underway to further our understanding, but these results suggest that reduction in plant-plant competition due to invasive species may overshadow any negative direct effect of herbicides. That is, reducing plant competition via herbicides has the potential to substantially improve habitat quality for focal butterfly hostplants.





Acknowledgments

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