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The Effect of Simulated Herbivory on Pea Plants (Pisum sativum) under Water Stress

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ABSTRACT

Drought can impact various cellular functions in plants, including cell division and elongation; total leaf area; root and stem growth; and number of leaves per plant. Herbivory can also be a detriment to cellular functions, including new growth. There could be a relationship between how drought and herbivory interact. Pea plants express a wide variability in drought responses. In this study, pea plants were germinated and subjected to different amounts of water stress and simulated herbivory. Plant height, number of leaves, count of flowers, and count and size of pea pods were recorded before and after herbivory application. 36 of 60 plants germinated and were administered a control, low, and high stress water treatment for 12 days. Herbivory was simulated on half of the plants. Flowers and pods developed on 11 and 7 plants, respectively. There was no difference in plant height, total number of leaves, number of flowers, and size of pea pods by water and herbivory treatment. However, there were some morphological responses to plants under high water stress treatment, such as chlorosis and malformation, which could have influenced the ability for flowers to develop into pods. This study did not provide sufficient results to link solely these two stressors, herbivory and water deficit, in *Pisum sativum*. However, it was illustrated that there was a great deal of individual variability inside each treatment, suggesting that individuals may have different traits that made them more or less apt to respond to environmental stress.

INTRODUCTION

There are many biotic and abiotic factors that influence plant species and, thus, can have broad implications throughout the entire food web. One predominant abiotic factor that can control plant growth and development is water availability (Osman, 2015; Lopez et al. 1997). Drought severely affects plant growth and development, with consequences such as reduction in the rate of cell division and elongation, leaf area, root and stem growth, interrupted stomatal conductance, and water use efficiency (Farooq et al., 2009). Many cellular functions, including photosynthesis, are very sensitive to water availability. Osman (2015) found that the most critical vegetative parameters affected by water stress are number of leaves/plant and leaf fresh weight, and that drought stress (both long and short term) significantly decreased the number of leaves per plant. Lopez et al. (1997) found that water deficit initially reduced the rate of leaf expansion, followed by an interruption of new leaf production. However, many plant species, including peas, express wide variability in drought response and seem responsive to areas with unpredictable rain patterns, as studied in agriculture in Africa and Latin America (Assefa et al., 2017).

A predominant biotic factor that can control plant growth is herbivory. When plants experience herbivory, a temporary nutrient deficiency is experienced (Luxmore, 1991), which may limit growth more than photosynthesis. After leaf trimming, the plant can respond in many ways, including defensive strategies and limiting new growth. Plant defensive strategies are influenced by the availability of resources, including water, to the plant (Coley et al., 1985).

This study aims to determine (1) if water stress, (2) simulated herbivory, and (3) water stress and herbivory combined cause a difference in pea plant growth, as quantified by total plant length (cm), number of leaves, and flower/pea production. Before the experiment, it was hypothesized that (1) water stress and (2) simulated herbivory will both affect pea plant growth and flowering.

MATERIALS & METHODS

Planting and Treatment Application

Initially, 60 pea (*P. sativum)* seeds were planted 4 cm deep into Miracle-Gro® Moisture Control Potting Mix (The Scotts Company LLC, Marysville, OH) in 1-quart plastic containers. Every container was watered 50 mL every third day for three weeks. After the initial growth period, peas were moved to the Northern Michigan University Greenhouse, where they were kept between 15-30 °C and supplemental light was provided to total 14 hours per day.

36 of the 60 seeds germinated. These plants were broken up into three equal groups of 12 plants for water treatment. From there on, the control, low and high stress received 50, 30, and 15 mL of water, respectively, every other day. After 12 days of differential water treatment, herbivory was simulated on half (six) of the plants under each water treatment. Using scissors, 75% of every leaf was cut back in one slice perpendicular to the madrib (largest vein) and observed the plants for another 11 days.

In order to measure plant growth and development characteristics, plant height at the longest point (cm), number of small (less than 1 cm^2) and large (greater than 1 cm^2) leaves, a count of flowers, and count and size of pea pods was recorded. This was recorded three times before and after herbivory treatment.

Data Analysis

For all six days of recording plant characteristics, the mean height, number of small, large, and total leaves for the three water treatments were compared using a Kruskal-Wallace statistical test. For the three days of recording after the herbivory treatment, the same characteristics were compared between the herbivory treatment and control within each water stress using a t-test. A mean count of flowers per plant was compared between water treatments using a Kruskal-Wallace. Pea pod size was compared between herbivory treatments using a t-test.

RESULTS

Plant Height & Leaf Number

For each of the six days recording plant characteristics, there was no significant difference in mean plant length (cm) between water treatment groups (Kruskal-Wallace, $p>0.05$) (Fig. 1). In addition, there was not a significant difference in the number of small, large, or total leaves by water stress treatment (Kruskal-Wallace, $p>0.05$) (Fig. 2).

After herbivory treatment, the plants under all water stresses of herbivory treatment had a significantly lower number of large leaves (t-test, df=12, $p<0.01$) (Fig. 3). However, there was not a significant difference in plant height and number of small leaves between herbivory and control groups within all three water stress groups (t-test, df=12, $p>0.05$) (Fig. 3).

Flower and Pod Development

Flowers were observed 21 times on 11 plants throughout the study. Plant 27, which was under high water stress and received herbivory treatment, had the highest number of flower observations (five flowers). However, this is possibly because these flowers did not form pods. Of the 11 plants that flowered, six received the herbivory treatment. There was not a significant difference in the mean count of flowers between water stress treatments (Kruskal-Wallace, $p>0.05$) and herbivory treatments within each water stress (t-test, $p>0.05$).

Seven peas were produced on six plants (two on plants with simulated herbivory, five on those without). Only one plant, which was under high water stress and did not receive herbivory treatment, produced two pods. Pea pods were on average 2.04 cm ± 0.77. There was no significant difference in pod size between herbivory treatments (t-test, df=1, $p>0.05$).

DISCUSSION

Water Stress Response

It has been clearly demonstrated in other studies (such as those done by Farooq et al., 2009; Lopez et al., 1997; and Osman 2015) that water stress does influence plant growth, including leaf growth and development and flowering. This is not consistent with the water treatment results from this experiment, where water stress did not cause a difference in plant growth, as quantified by plant height and number of leaves. There was evidence of some qualitative response in plants under high water stress, such as deformed leaves and chlorosis. Further, there was a great deal of variability between individuals of each treatment. This could suggest differential responses to the same environmental stresses, which could be tied to trade-offs, especially those specific to reproductive efforts.

Perhaps, the specific drought responses of peas could vary, as shown in Assefa et al. (2017). However, it is more likely that this study did not effectively provide water stress. This could have been improved by applying water stress treatment earlier in the plant's life, which might have elicited a greater differentiation in responses. By the time the plants were being watered with different, they were on average 20-30 cm tall and had 18-23 total leaves. Additionally, the control treatment was overwatered and caused in-container flooding, which could have correlated with increased leaf die off (Assefa et al. 2017). In future studies, the control treatment should be a lower volume of water, possibly 35 mL every other day, and the other treatments should be adjusted accordingly.

Stimulated Herbivory Response

As was expected, there was a difference in the number of large leaves after herbivory. However, the lack of difference in the mean number of small leaves between the control and herbivory groups suggest that there was a quick response from the plants through regrowth of small leaves. Many of the herbivory-treated plants that were of larger sized at trimming developed entirely novel shoots with many small leaves, seeming to abandon the main stem. This supports the hypothesis of simulated herbivory impacting plant growth, but it is hard to say for certain with such a short time scale. Most other studies, such as those conducted by Coley et al. (1985) and Yoshizuka & Roach (2011) monitored plants for a longer timeframe. Thus, it is hard to compare the results to previous literature, since there is only a lens into the short term. In the future, a longer timeframe and larger sample size would be beneficial to monitor post-herbivory response and allow for a more adequate comparison to previous research.

It must be realized this was a simulation and controlled application and is not necessarily representative of how herbivores actually feed. In many cases, herbivores are selective in their grazing and target specific parts of the plant, such as flowers and fruit (Maldonado et al., 2015). Plants are faced with a dilemma. They must be appealing enough to attract pollinators, but not too much so that they are also targeted by herbivores (Maldonado et al. 2015). The herbivory application was not selective and simply cut off 75% of every leaf. Simulated herbivory may not elicit the same responses as natural herbivory. Lortzing et al. (2017) found that in *Solanum dulcamara*, regulatory genes involved in signaling, defense and water stress were mimicked well by simulated herbivory. However, genes related to photosynthesis, carbohydrate- and lipid metabolism were only regulated by real herbivory (Lortzing et al., 2017). It would also be worthwhile to compare the same simulated herbivory treatment, in the field as well as the greenhouse, such as in Yoshizuka and Roach (2011). This suggests that studies done with just simulated herbivory in the lab setting might not be completely applicable to a natural setting. Since peas are an agricultural crop, there could also be different phenotypic responses than those observed in *Barbarea vulgaris* and *Solanum dulcamara.*

Herbivory can have many implications beyond limiting leaf growth, which was the main parameter used to assess impact. For example, herbivory can influence flower growth, which can in turn influence plant pollination (Herrera, 2000). To address this, future work could look into the impacts of herbivory application during time of maximum reproductive effort, between flowering and pod development.

Finally, it would be interesting to test the effects of different types of stimulated herbivory, such as grinding up or punching holes in leaves, to stimulate different types of herbivores such as insects and mammals. For example, a study by Forkner (2012) simulated herbivory be removing 10% of leaf area on either 25% of or all leaves of *Acer rubrum* using a hole punch.

Conclusions

In conclusion, the conditions did not provide significant enough of results to link solely the two stressors, herbivory and water deficit, in *Pisum sativum*. Other factors, such as nutrient limitation, air and soil temperature, humidity, and light exposure should be investigated to determine the impact on plant growth and reproduction. Additionally, the timing of these factors in the plant's life could have an impact, especially when considering reproductive efforts, such as flowering and pod production. More work, with a larger sample size, over a longer period of time that integrates other factors should be done. Supplementary research could also dig deeper to determine if there is variation in individual responses.

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FIGURES

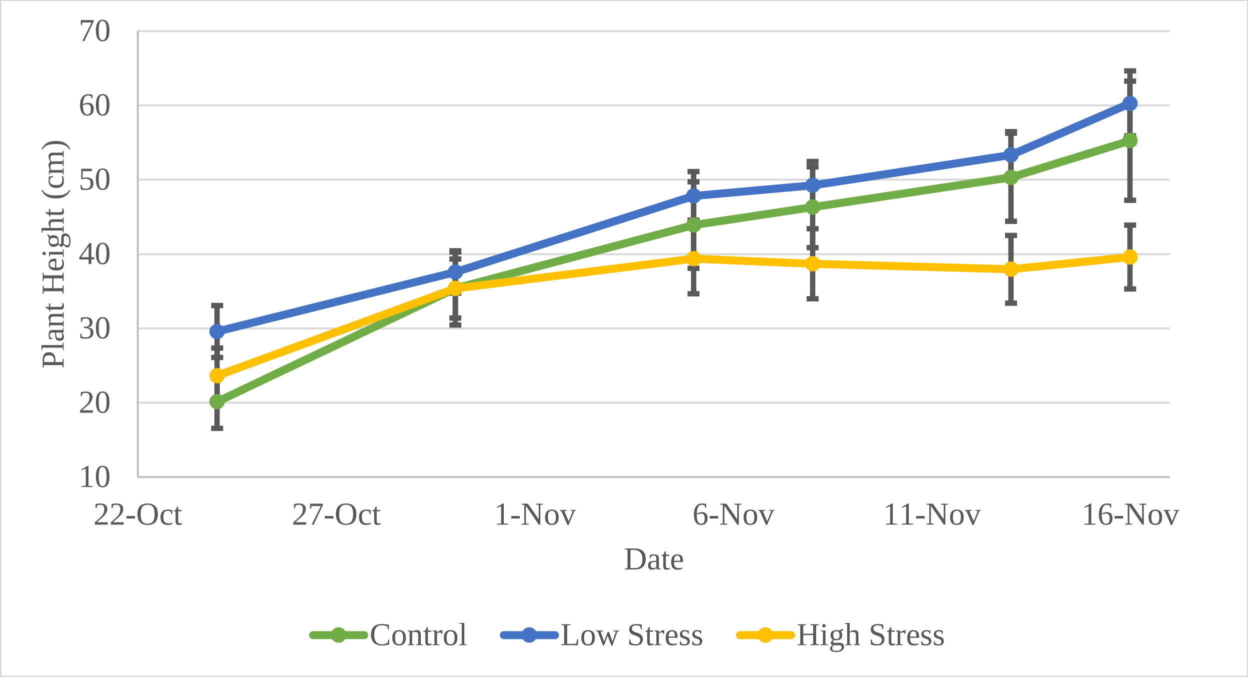

Figure 1. Mean plant height (cm). For all six days that plants were measured, there was not a significant difference between water treatment groups (Kruskal-Wallace, $p>0.05$). Plants under low stress were the tallest.

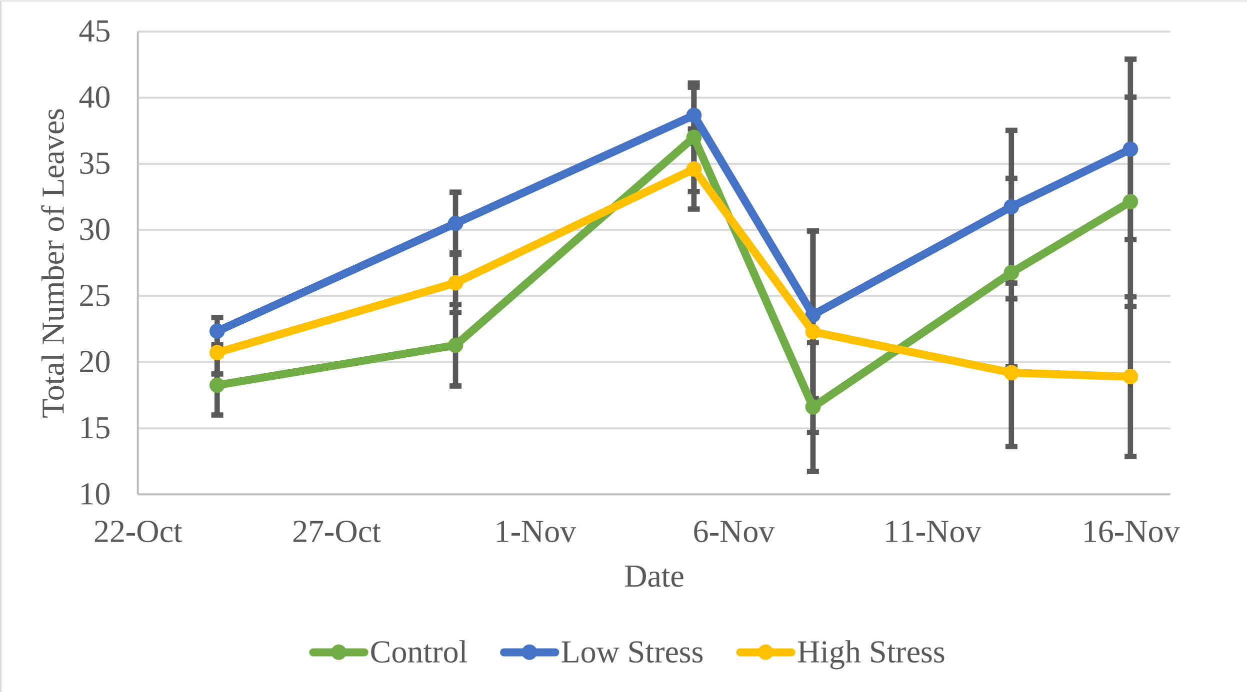

Figure 2. Mean number of total leaves per plant over the six measurements, three of which were before herbivory and three were after. Total number of leaves included both small and large leaves for each plant. There was not a significant difference between water stress groups (Kruskal-Wallace, p>0.05).

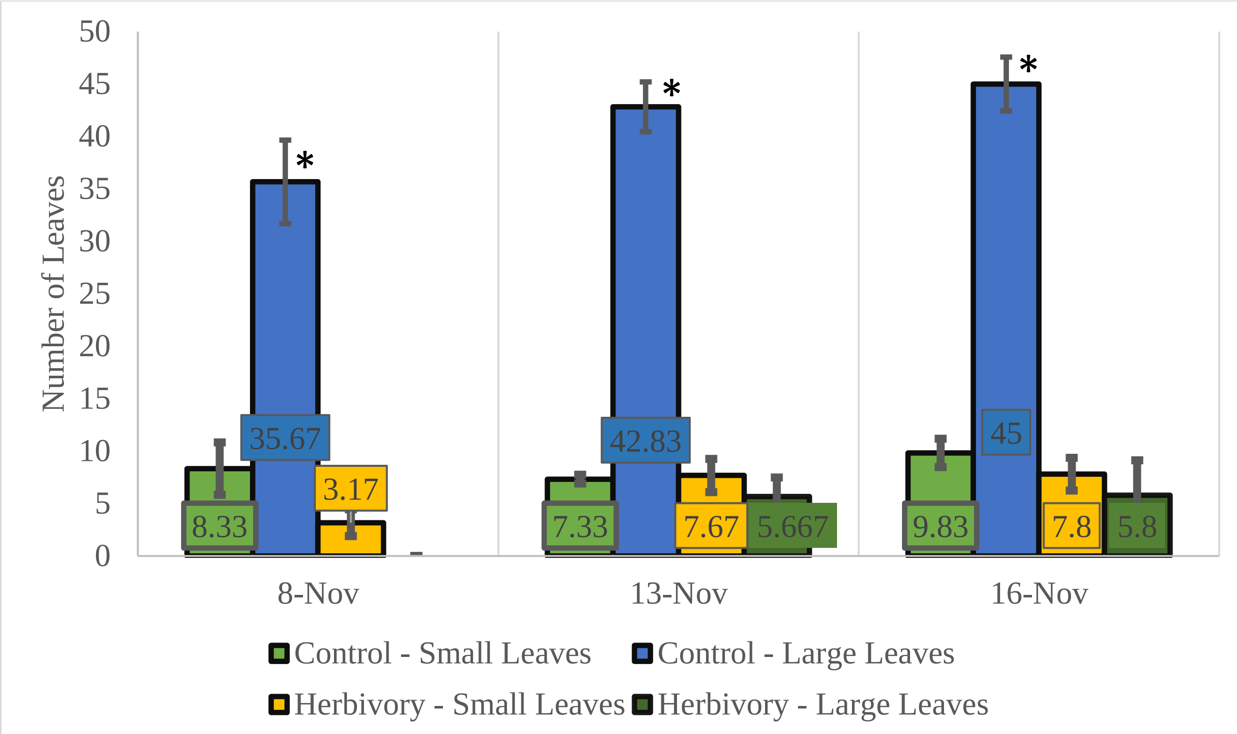

Figure 3. Mean total number of leaves after herbivory treatment. Small leaves were less than 1 cm^2, and large leaves were greater than 1 cm^2. There was not a significant difference in number of small leaves between herbivory and control groups within all three water stress groups (t-test, df=12, p>0.05), but there was a difference in mean number of large leaves, which is designated by an * (Kruskal-Walace, p<0.05).