Drought Effects on *Chromaphis juglandicola* Walnut Aphid Population Dynamics on Walnut Seedlings

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ABSTRACT

Walnuts are an important California crop, and it is crucial for agricultural scientists to understand the biotic and abiotic interactions that affect the production of walnuts. Walnuts are highly susceptible to water stress, and drought conditions may have strong effects on walnut production. Walnuts are also affected by the walnut aphid (*Chromaphis juglandicola),* a prominent sap-sucking insect pest on walnut trees. Through an outdoor cage experiment, I researched the effect of soil water levels on walnut aphid (*Chromaphis juglandicola)* populations on walnut seedlings to determine effects of the California drought on this crop-pest system. The experiment found no significant difference between High, Medium, and Low drought treatments on aphid population composition. However, non-normal data indicates that a larger sample size was needed for conclusive results.

KEYWORDS

California drought, insect ecology, biological control, parasitoid wasp, IPM

INTRODUCTION

Walnuts are an important California crop, generating over $1.3 billion per year in revenue (USDA NASS 2012). This means it is crucial for agricultural scientists to understand the biological complexities of the walnut orchard system, and to understand the ecological interactions that affect the production of walnuts in order to improve pest control and prevent crop losses. This begins by examining how drought affects walnut trees.

Walnut trees (*Juglans sp.)* are highly sensitive to drought conditions, which can lower their ability to be productive agricultural crops (Gauthier and Jacobs 2011). They have a poor ability to photosynthesize under drought stress and experience increased leaf senescence (Gauthier and Jacobs 2011). Black walnuts that were stressed to a low level of -2.5 megapascals (MPa) did not recover from drought, did not regain leaf turgor, and exhibited leaf yellowing and senescence, in addition to leaf abscissions (Davies and Kozlowski 1977, Gauthier and Jacobs 2011). The rate of photosynthesis is affected by drought as well, approaching zero at -2.5 to -2 MPa. (Davies and Kozlowski 1977, Gauthier and Jacobs 2011). Because of this, walnuts exhibit desiccation avoidance mechanisms so that they can maintain high leaf water content and avoid embolism and cavitation (Gauthier and Jacobs 2011). Since water availability in the walnut is a strong physiological factor affecting sap-sucking insects such as aphids (Alsam et al. 2013), there should be a strong effect of drought on walnut aphid population compositions.

Drought could affect the abundance of aphids or the composition of their population. Changes in aphid population composition, such as life stages of the aphid, can lead to a change in aphid parasitoid attack rate and parasitoid population structure, due to changes in size and instar of the aphids (Alsam et al. 2013, Romo and Tylianakis 2013). This potential change in the parasitoid population, and therefore the parasitoid's ability to control the aphid population is why it would be beneficial to study the population composition changes of walnut aphids on walnut plants. Walnut aphids have an economic threshold of 15 aphids per leaflet (Hougardy and Mills 2009) and constitute a key pest in walnut orchards. Studies done in barley plants looking at the effects of drought on cherry-oat aphids might give an indication of the type interaction we may expect to see between walnut aphids and walnut plants. In a study by Alsam et al. (2013), drought did not affect the size of aphid populations or the number of alate aphids. The number of apterous adults increased on drought stressed plants (Alsam et al. 2013). The overall adult aphid populations increased on drought treated plants, and the number of nymphs was higher on irrigated plants. There was a possible decreased development time in response to water stress, to help increase aphid dispersal (Alsam et al. 2013). There may be a similar shift in aphid population towards apterous aphids on walnut plants. Unfortunately, there is currently a gap in knowledge about the effects of drought specifically on walnut aphids.

My research looking at changes in aphid distribution and size will fill the current gap in knowledge about the effects of drought on the walnut aphid populations, and will lay the groundwork for more experiments examining the walnut-aphid-parasitoid interaction. I will research the effect of soil water levels on walnut aphid (*Chromaphis juglandicola)* populations on walnut seedlings. The experiment will expose walnut seedlings and their aphid populations to drought in order to test the hypothesis:

H_{O1}: Drought has no effect on walnut aphid population composition.

H_{A1}: Drought will shift the population composition toward greater apterous adult aphid percentages.

I hypothesize that there will be an increase in apterous adult aphid populations due to the ability of the winged adults to move from droughted to non-droughted plants (Alsam et al. 2013). For this hypothesis, it is assumed that droughted plants have less favorable conditions for the aphids than non-droughted ones; therefore, the aphids in the less favorable environment will attempt to move away from their plants to find a more suitable host plant. This would result in an increase in the number of winged adult aphids. This study will be conducted through an outdoor cage experiment with walnut aphids on walnut seedlings stressed to different soil moisture levels.

METHODS

Study System

In this study I looked at the effects of various soil moisture levels on walnut aphid (*Chromaphis juglandicola)* population dynamics on walnut seedlings (*Juglans regia).* I used English walnut (*Juglans regia*) seedlings, and walnut aphids (*Chromaphis juglandicola).* The walnut seedlings were sourced as nuts from Davis growers. I grew seedlings in individual 1 liter pots using Supersoil potting soil (Scotts Miracle Gro, Marysville, Ohio). The soil contained 14% nitrogen, 9% phosphorous, 2% potassium and 0.25% iron. I grew the seedlings from nuts in a greenhouse at 20-25◦C with an LD 16 : 8 h photocycle, where I watered them three times a week and fertilized them once a week. The aphids were originally collected from Chico, California walnut orchards in June 2009, and the colony was grown on previously grown walnut seedlings and supplemented yearly with aphids from various locations in the Central Valley of California to help maintain genetic diversity. The aphids were kept in cages in the insectary at 20–25 ◦C with an LD 16 : 8 h, grown on potted walnut seedlings.

Data Collection

Table 1. Soil Moisture Levels for each treatment category

Level	High	Medium	Low
% Soil Moisture	70%-80%	45%-55%	30%-40%
Volumetric Water Content (VWC)	44%-75%	15%-23%	8%-13%

I collected the data using a series of outdoor cage trials with walnut seedlings inoculated with aphid populations in order to determine the effects of drought on aphid population composition. I did six 28-day trials of three plants each at high, medium, and low soil moisture levels (Table 1). This resulted in a total of 18 plants per treatment.

When they had grown to at least five full leaves, I moved the walnut seedlings to an outdoor covered bench and droughted them to the initial soil moisture level required for their treatment category. Once at initial drought levels, I kept the plants at their respective levels for 7 days, measuring soil moisture levels with a VWC probe (Vegetronix VG-Meter-200), and watered them to keep them in their moisture ranges. I determined the soil moisture levels by taking four VWC readings of the soil in each pot and averaging them to determine my final reading. On the 8th day, which was day 0 of the experiment, I trimmed the plants to five full leaves and added distribution of aphids to the plant. The distribution consisted of five 1st instars, two 2nd instars, and one 3rd instar, one 4th instar, and one adult winged aphid. I took soil moisture measurements at least every other day and watered the plants to keep them in their soil moisture ranges. On day twenty, I counted the aphids on the plant and recorded them as one of three different categories, 1st to 3rd instar, 4th instar, or adult, and the adult aphids were collected and weighed.

Data Analysis

I used a Shapiro-Wilk test to determine the normality of the data. I analyzed the data using a Kruskal-Wallis one-way analysis of non-normal data using the statistics program R. To determine the power of my statistical analysis, I did a power analysis test.

RESULTS

A Shipiro-Wilk test indicated that none of the variables had normal distributions, which violates one of the initial assumptions for the ANOVA test.

Since the data was not normal as indicated by the Shipiro-Wilk test, I did a Kruskal-Wallis one-way analysis of non-normal data, which showed no significant difference ($p>0.05$) between the treatments of High, Medium, and Low soil moisture levels for the Adults, 4th Instars, 1st-3rd instars, and the total number of aphids **(Figure 1)**.

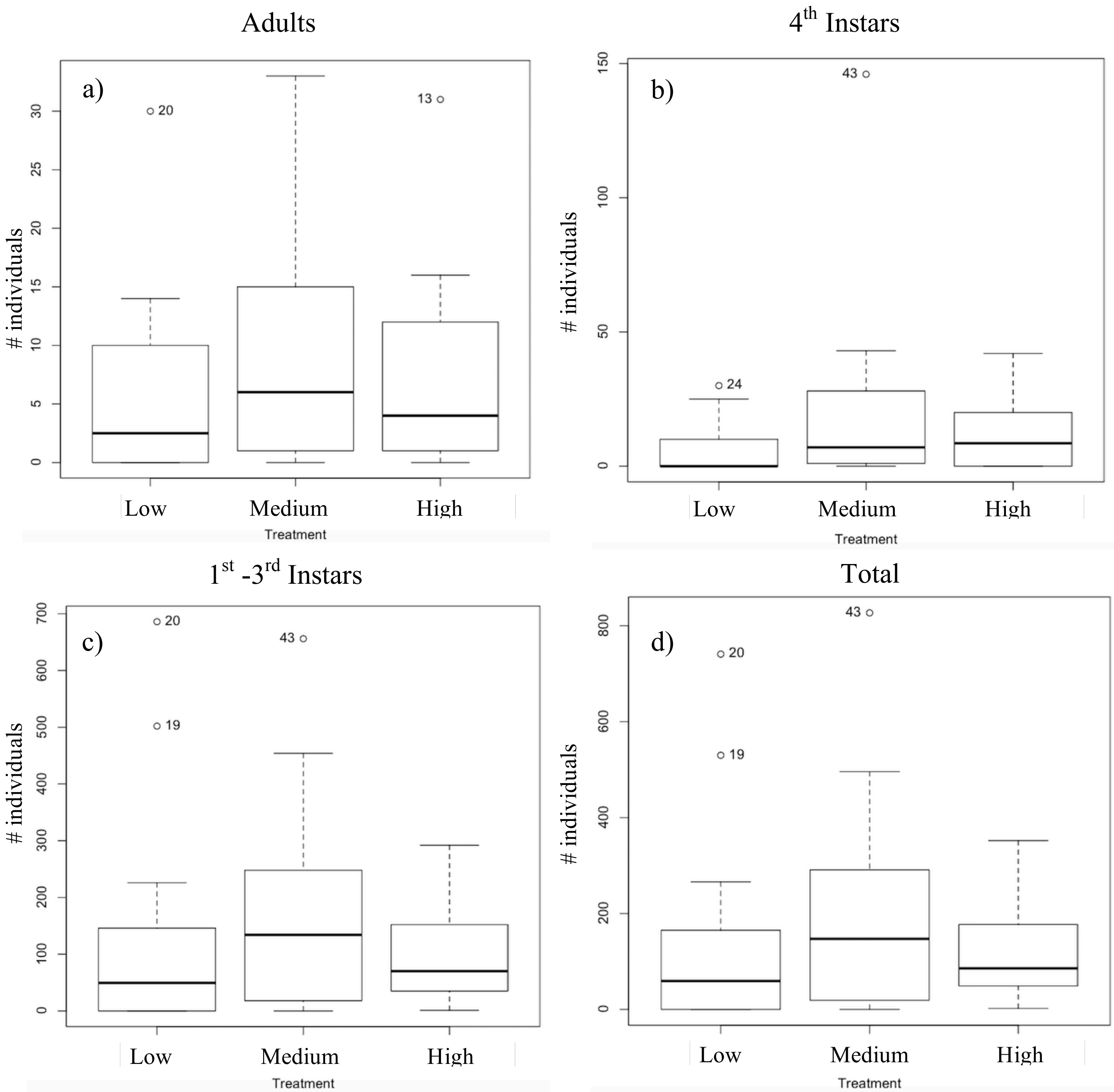

Figure 1. Distribution of Aphids by Instar and Treatment. a) Adults b) 4th Instars c) 1st-3rd Instars d) Total

I did a power analysis test that showed low levels of power for all aphid size classifications, indicating that there was a low probability that the test would correctly reject the null hypothesis when the alternative was true. I used the program pwr in R to find the power level of the data when doing a one-way ANOVA test. The effect sizes were calculated using the equation:

$$f = \sqrt{\frac{\sum_{i=1}^{k} p_i * (\mu_i - \mu)^2}{\sigma^2}}$$

where $p_i = n_i / N$,
n_i = number of observations in group i
N = total number of observations
μ_i = mean of group i
μ = grand mean
σ^2 = error variance within groups

The effect sizes for Adults, 4th Instars, 1st-3rd instars, and the total number of aphids were 0.075, 0.165, 0.155, and 0.149, respectively. These values indicate that more samples would be needed to have the possibility of showing a significant result **(Table 2)**.

Table 2. Power levels for different numbers of trials

Number of Trials	**Average per plant**	**1-3rd instar**	**1-3rd instar**	**adults**
18	0.14	0.15	0.17	0.07
60	0.41	0.44	0.49	0.13
90	0.58	0.61	0.67	0.18
120	0.71	0.75	0.80	0.23

DISCUSSION

Walnuts are an important California crop, and it is crucial for agricultural scientists to understand both the biotic and abiotic interactions that affect the production of walnuts. I researched the effect of soil water levels on walnut aphid (*Chromaphis juglandicola)* populations on walnut seedlings to determine effects of the California drought on this crop-pest system. There was no significant difference between treatments of High, Medium, and Low drought on aphid population composition. However, the data was non-normal and the power of the experimental design was low, reflecting that a larger sample size was needed. Despite these experimental constraints, previous studies have shown conflicting results of the effect of drought on sap sucking insects (White 1984, Huberty and Denno 2004, Banfield-Zanin and Leather 2015).

Nitrogen effects on aphid populations

The lack of significant difference between the three drought treatments may have been due to the lack of an effect of nitrogen levels on the plants. Increased nitrogen availability in phloem is hypothesized to be one main mechanism for an increase in herbivores on plants during drought conditions (Banfield-Zanin and Leather 2015). However, Mace and Mills (2015) found no effect of increased N on aphids feeding on walnut plants. These study results may mean that though nitrogen levels increase in droughted plants, they do not have any effect on the walnut aphids, resulting in no changes in the aphid populations due to drought. Under this assumption, there may be no effect of drought on the walnut aphids, which may explain the non-significant results in my study.

Conflicting results in other studies

Conflicting results in other drought studies of sap-sucking insects suggest that multiple factors are at play that could possibly counteract each other. In a meta-analysis of published studies that looked at insect responses to experimentally induced drought, there was a very negative effect on sap-feeding insects resulting from drought conditions regardless of intermittent or consistent drought (Huberty and Denno 2004). Other studies have found increased insect numbers with intermittent drought (Banfield-Zanin and Leather 2015). However, the Banfield-Zanin and Leather (2015) study had a low sample size of only 10 trees per drought treatment. This conflicts with my results, where I had a relatively low sample size but did not find significant results.

Some studies have found what they have termed “the plant stress paradox”, where they have found both evidence in support of plant stress helping insects and evidence against that theory. In a study by Mopper and Whitham (1992), they found that there was a sex ratio shift in females sawflies that negatively correlated with precipitation, meaning that there were more female sawflies after drought years. They also found that the density of pinyon shoot moths was negatively correlated with precipitation. Both of these results suggest that drought and plant stress help these insect populations. However, they also found that cocoon mass, proportion of females, and reproductive potential were all greater on watered and fertilized plants, and that sawfly reproductive potential was positively correlated with March precipitation levels. This demonstrates a commonly found paradox, where insect outbreaks correlate with plant stress in some studies, but in others there are negative responses to drought stress (Mopper and Whitham 1992).

Experimental design and effect size

Because the results of these studies have been so variable, it is difficult to interpret the ecological significance of my study on the walnut-aphid system. The majority of studies have either found positive or negative effects of stress, while my study did not find significant results. This could indicate that more trials were needed in order to produce normal data with a significant result, whether it be positive or negative, since non-significant results are uncommon in these types of experiments.

Limitations and future directions

The experiment had a small number of trials due to time constraints and feasibility, and high variability, which meant that the results had low power. This means that even if my results had been significant, there would have been a high probability that the results were due to chance. The data was also not normal, making it impossible to do an ANOVA test on it. The non-normal data was an indication that the sample size was not large enough, since with a large enough sample size the data should have had a normal distribution. The variability was most likely introduced because all other variables were not held consistent. The trials took place outside, where the humidity, temperature, sun, and wind could not be controlled. This also caused issues with establishment, which made it difficult to tell whether the insects did not establish due to the drought treatment, or due to the environmental conditions.

For future experiments, there are changes in the experimental set-up that I would recommend. More accurate soil moisture meters would keep the plants in more defined treatments. Introducing a more severe drought treatment would also increase the differences between treatments and make the effects of drought more visible. Having a more severe drought treatment would be possible if the plants were in a climate controlled room, with control over humidity and temperature. Being indoors would also allow for more consistent droughting of plants and would eliminate the variables introduced from being in an outdoor environment. Establishment may also improve if the initial distribution of aphids is replaced with 10 winged adult aphids. Finally, a larger number of trials would increase the power of the experiment, and increase confidence that any results are due to the experiment and not due to chance.

Conclusions

The data I collected yielded non-significant results, but it is impossible to determine if this was due the low power of the experiment or not. Repeating this experiment under more controlled conditions with more trials would help to confirm whether there is an effect of drought on walnut aphid populations. This could help to determine if drought actually does have an effect on this crop-pest system, or if there is no effect as may be indicated by my findings.

ACKNOWLEDGEMENTS

Thank you to Mills Lab and my mentor Kevi Mace. Thank you to the Undergraduate Research Apprentice Program (URAP) for providing a summer stipend for my project. Thank you to Tina Mendez and Kurt Spreyer for guiding us through the thesis writing process, and to my ESPM 175 workgroup for all of your help with editing.

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