Valuating the impact of specialist herbivore, *Aphis echinaceae,* on its host plant, *Echinacea augustifolia*

Alex Yeaney[1], Lydia English[2], Stewart Wagenius[2]

[1]Lake Forest College, 555 North Sheridan Rd., Lake Forest, Illinois, 60045

[2]Plant Conservation and Biology, 1000 Lake Cook Road, Chicago Botanic Garden, Glencoe, Illinois 60022

Research Questions:

1. Does aphid infestation affect the plant's fitness (measured by seed set)?
2. Does aphid infestation affect the weight of achenes?
3. Does aphid infestation influence the number of leaves senesced?
4. Does aphid infestation change the phenotypic expression of trichomes?
5. Does aphid infestation change the level of foliar herbivory on *E. augustifolia* in the two treatments negatively from the specialist infestation or positively due to protection from the mutualistic ants?

Methods and Procedures:

Lydia English continued the work of Katherine Muller transplanting aphids for the addition treatment and monitoring the exclusion treatment for infestation. Following the procedure outlined by K. Muller, aphids were added to the addition treatment twice a week and removed from the exclusion treatment once a week between July 2nd and August 29th. Senescence was observed by counting the number of yellow/brown leaves per plant on October 7th. Trichome counts were obtained through high-resolution photographs taken between August 23rd and the 27th. Those photos allowed a reliable count to be obtained from the edge of the leaf between 1cm and 2cm, measured along the center, originating from the distal tip. Herbivory for was recorded as the number of damaged leaves per plant on September 3rd. The total number of leaves for flowering plants recorded between August 2nd and the 14th was used to determine the percent damaged as well as percent senesced. At the end of the season, 27 heads from 15 plants were harvested and assigned a unique identifier. The unique identifier included the plant the head came from and the tag color of the head. The exclusion treatment contained 15 heads, and the addition treatment contained 12 heads. From the 15 plants harvested, 7 plants were in the addition treatment and 8 plants were in the exclusion treatment. The heads were dissected and organized into manila envelopes; I scanned and counted the achenes from all 27 heads in the Echinacea Lab at the Plant Conservation Science Center at the Chicago Botanical Gardens using the Echinaceae Project Website. From each of the 27 heads, I took a random sample of 30 achenes and weighed them using the automated Mettler Toledo scale. I constructed a line graph with weight on the y-axis and individual seeds arranged from lightest to heaviest on the x- axis. When determining which achenes contained an embryo (full) and those that did not (empty) a cut-off of 2mg had been previously established. For most of the heads, the 2mg cut-off used previously

to determine if the seed was full or empty was supported by the graphs. For those heads with unclear cut-offs, or no break was abundantly clear, an x-ray at 12 kV for 4 seconds was used to find the number of full and empty achenes. The 2mg cut-off used previously was supported by all but 2 heads. Heads ad-1004, au-1008, had some partially full achenes, confirmed by the x-ray that were then counted in the full treatment, making their cut-off 2.25 and 1.33mg respectively. An estimate of embryo weight was calculated by subtracting the average empty achene weight from the average full achene weight for each head.

Statistical Analysis:

For achene counts, seed set and weights among the flowering plants provided only a small sample (n=27). To test for significance between our addition and exclusion treatment a bootsrap analysis with random resampling of a null model with 10,000 iterations was performed.

Data from the field provided observations on the senescence, herbivory, and trichome counts for basal plants as well as the flowering plants creating a relatively larger sample. For trichome counts a linear model with categorical predictors and continuous response (n=92) was used. Senescence was also observed across the larger sample size an anova and a bootstrap with random resampling and 10,000 iterations were performed the bootstrap confirmed the findings from the anova (n=91). Field notes provided senescence observations on both September 3rd and October 7th. When performing the anova for the September 3rd dataset, an outlier was identified. In order to get a better sense of this outliers effect, it was removed and a second anova was performed. However, a bootstrap using the anova model without the outlier provided a very similar test statistic to the original. Herbivory observations were also taken across all plants, basal and flowering (n=93). The anova provided abnormal results for a parametric test and for this reason a bootstrap with random resampling of a null model with 10,000 iterations was also performed to confirm the results of the anova.

Results:

From the flowering plant, seed set averaged 15.8 ±1.5 full achenes in the addition and 13.5 ± 1.9 achenes in the exclusion. The lower number of full achenes in the exclusion treatment can be explained by normal variance (n=27, p=0.8678). Average weight of full achenes in the flowering plants was 4.15 mg ± 0.31 in the addition and 4.06mg ± 0.17 in the exclusion treatment. This difference can also be explained by normal variance (n=27, p=.7796). Empty achenes in the addition weighed slightly less (14.2 ± 1.6) than the exclusion treatment(16.5 ± 1.9). Again, there is no strong evidence that this difference is not accounted for by normal variation (n=27, p=.5866). Embryo weights were consistent between treatments: 3.09 ± 0.02 mg in the addition and 2.94 ± 0.15 mg in the exclusion treatment (n=27, p=.5644).

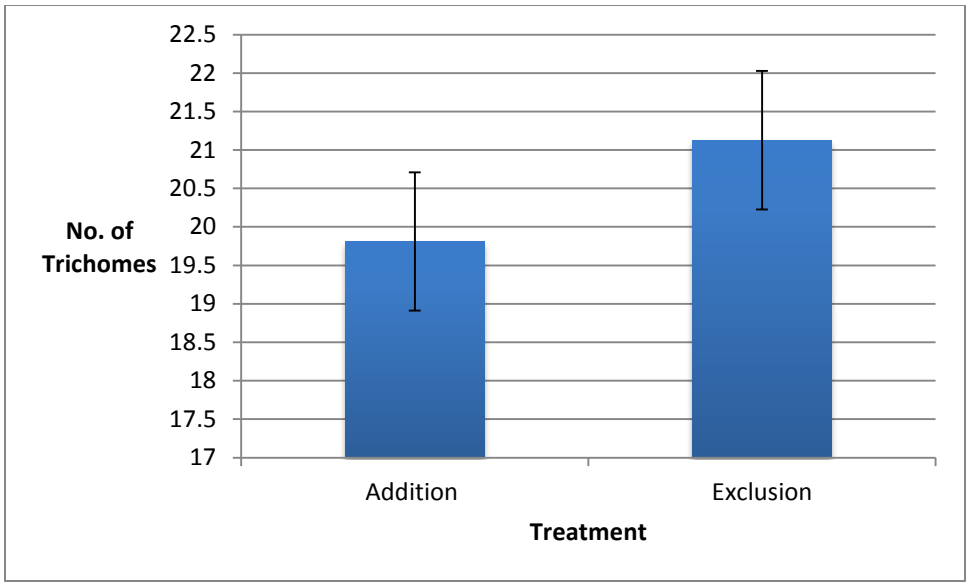

Figure 1. Trichome counts from the photographs taken in August 2013. (n=92, p=.2616)

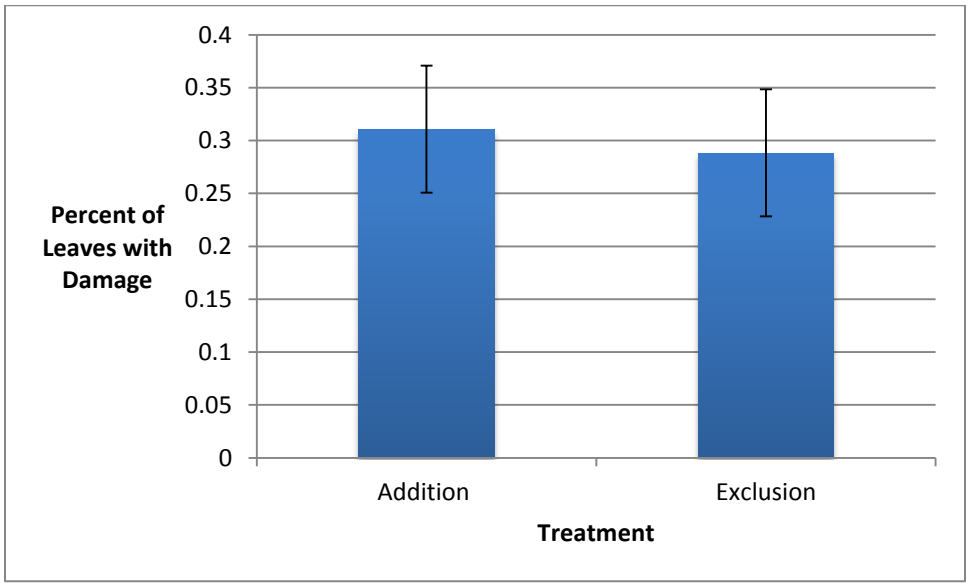

Figure 2. Damage from herbivory comparing the two treatments based on the recordings from September and August 2013 (n=93, p=.445).

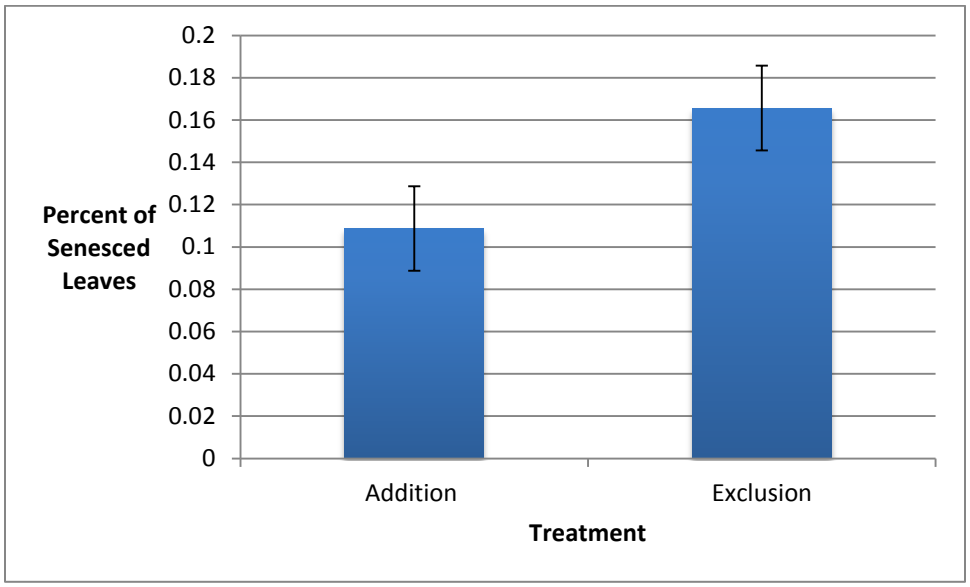

Figure 3. Senescence based on the recordings taken on September 3rd (n=91, p=.07).

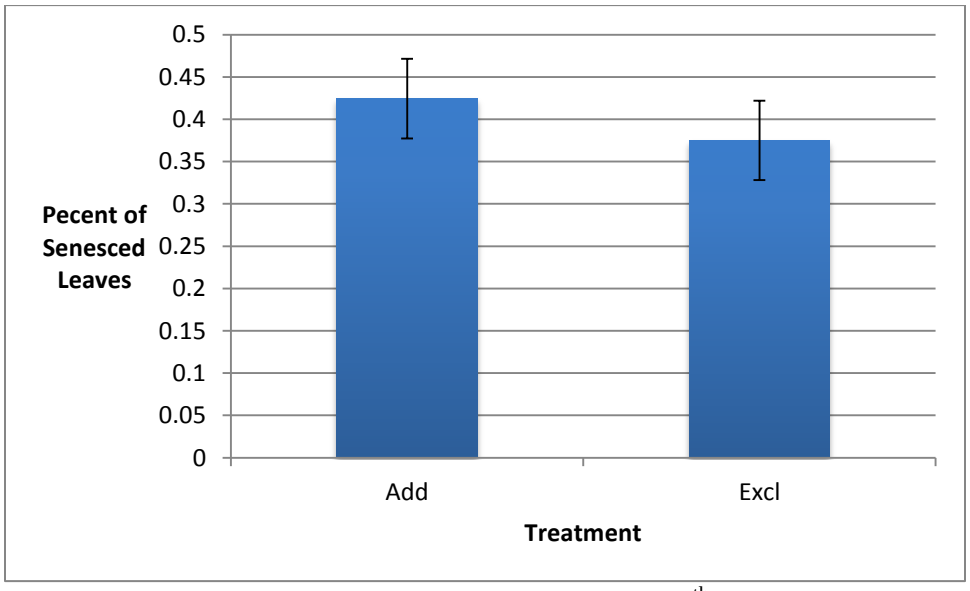

Figure 4. Senescence from the recordings taken on October 7th (n=91, p=.12).

Table 1. Averages for Addition and Exclusion Treatments Across the 27 Flowering Plant Heads:

Flowering Plants	Addition ± St. Error	Exclusion ± St. Error
Average Number Full Achenes	15.8 ±1.5	13.5 ± 1.9
Average Number Empty Achenes	14.2 ± 1.6	16.5 ± 1.9
Average Weight (mg) of Full Achenes	4.15 ± 0.31	4.06 ± 0.17
Average Weight (mg) of Empty Achenes	1.01 ± 0.01	1.14 ± 0.01
Embryo Weight	3.09 ± 0.02	2.94 ± 0.15

Discussion:

Though we found no concrete evidence while reviewing the data, a divergence in the treatments, number of full achenes, and weights of those full achenes could be seen between the addition and exclusion treatments. A larger sample size is necessary to gain further insight. The most statistically significant evidence of aphids affect on its host plant was that of senescence. The fact that the observations from September 3rd captured a more significant difference than those from October 7th suggests that the aphids have a greater affect on early senescence. It also should be noted that within the addition treatment, one head (ah-1026) was observed with aphids directly on top, unlike the other samples, where aphids occupied the leaves of the plant. This sample had a much smaller seed set and lower average full achene weight than the rest of the addition treatment. The greatest limitation for herbivory, again, is believed to be sample size; future research is necessary to conclude whether the specialized *A. echinaceae* benefit the plant by recruiting mutualistic ants to defend against other herbivores.

This is a review after the first year that flowering plants were analyzed in the aphid addition and exclusion treatment. It is very likely that changes in trichome expression and senescence will not be observed until even more generations have been exposed to treatment. Furthermore, artificially transplanting aphid colonies, though successful, is not a perfect substitute for well-established populations that do not require the relocation of *A. echinaceae*'s farming ants. Aphid abundance varies from year to year, and in the past, the challenge was maintaining the exclusion treatment. This year, however, it proved more difficult maintaining colonies in the addition treatment. It seemed when looking at individual heads rather than the average across heads, a greater difference could start to be seen. A greater understanding of this may lead to implications about metabolic rate and resource allocation. Perhaps the methods should be revisited to include adding aphids directly to the plant head for the greatest impacts. Continuing the research to increase the sample size is important to illuminate the shadows the current data cast. It is my hope that the research is continued. I know that personally after becoming involved in the project, and, as is the case with most scientific inquiries, we are now left with more questions than answers.