Affects of Transplanted *Aphis asclepiadis* and Ant Mutualism on *Asclepias syriaca*

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Introduction

Ants and aphids are known to share a mutualistic relationship. Aphids secrete a sugary waste (honeydew), which some ants harvest for carbohydrates, amino acids and other nutrients (Völkl, 1999). In return, ants provide aphids with protection against predators, parasites and lower rates of fungal attack (Buckley, 1987). When ants are not tending, aphids are believed to be more vulnerable to attacks by predators such as *Coccinellidae* (ladybugs) and *Chrysopidae* (lacewing larvae) (Deo, 1997).

Aphids must be able to access plant phloem in order to survive, and must avert plant defense responses in the process (Giordanengo, 2010). Certain ants may specialize on a specific aphid species. Specialization could be due to the ant's digestive enzymes for oligosaccharides (Lach, 2010). It has also been found that honeydew production of aphids is dependent on ant attendance (Völkl, 1999). In our experiment, we studied *Aphis asclepiadis*, which specialize on *Asclepias syriaca* (Common Milkweed). *A. asclepiadis* are almost always found to be tended by ants and in dense aggregations on apical leaves (Mooney, 2007).

We hypothesize that there will be a difference in plant chemistry to account for the presence or absence of aphids. We also hypothesize that the presence of aphids and ants will affect plant carbon to nitrogen chemistry.

Therefore, we predicted that if aphids are transferred to an uninhabited milkweed plant, then multiple species of ants should compete for aphids.

Methods

Our experiment took place in a field in Cheboygan County, Michigan, (T37N, R3W, Sec33, N45°56'09" and W84°67'86") during late July. We chose this field for its abundance of *Asclepias syriaca* (common milkweed) with *Aphis asclepiadis*.

<u>*Aphid Transfer*</u>

We transferred aphids by cutting an aphid-infested leaf (towards the top of the plant with a minimum of 20 aphids) from an "original" non-flowering milkweed plant and pinning it to a top leaf of a new "novel" non-flowering milkweed plant, which lacked aphids. Twenty replicates were spatially grouped into ten plants on the east side of the field (34x18.5m plot size) and the other ten plants approximately 15 meters to the southwest (22x26m plot size). Replicate plants were at least 1.5 meters apart.

In order to speed up leaf dessication and subsequently aphid transfer, we cut off excess parts of the leaf that did not have aphids. Any insects on the new plant were physically removed before transfer. This was done to help the aphids transfer without predation and also to make the chance of ant colonization more equal for all species present. We recorded the approximate number of aphids on the pinned leaf and then monitored the new plants every hour for the next 16 hours, including times after sunset. We recorded the number of aphids that moved to the new plant, the number and type of ants on the new plant as well as those tending the new aphids. In addition, if predatory organisms such as *Hippodamia convergens* (lady bugs) or *Chrysoperla carnea* (green lacewings) were present on a test plant, we removed them in order to protect the untended aphids while transferring. We waited a maximum of ten hours for aphids to transfer, after which point non-transferred aphids were gently brushed off the old leaf onto the new plant and the old leaf and pins were removed from the plant. We then placed sugar bait (sugar water in

50mL centrifuge tube with a cotton plug) at the base of the plant to attract ants. After approximately 24 hours the bait was removed. We monitored the test plants less frequently after aphid transfer (1-3 times per day).

Plant Chemistry

We tested the plant chemistry of each plant to determine carbon to nitrogen (C:N) levels. We cut leaf samples from the top of each milkweed plant used (original plants and novel plants). Leaves at the top of the plant were chosen because aphids were mainly observed on top leaves of the milkweed plants. We took these samples before the leaf transfer and then approximately four days after the leaf transfer. Leaf samples were desiccated for approximately 72 hours in a 60 ° C oven, finely ground and run through an elemental combustion analyzer. We compared the C:N of the original plants and novel plants to determine if there was a significant difference to potentially account for the presence/absence of aphids. In addition, we compared the C:N of each novel plant before aphid transfer to after aphid transfer and ant tending to determine whether the ant/aphid mutualism caused a measurable change in plant chemistry. We grouped plants into three categories,- non-tended aphids present, tended aphids present, and no aphids present. We compared the change in initial to final C:N of these groups using an ANOVA to see if they differed. We ran a regression of the change in C:N versus aphid density to see if they were correlated. Lastly, we compared the ant density and aphid density using regression.

Results

Aphid/Aphid Interactions

Four of the 20 novel plants had aphids that were actively tended by ants. Five other novel plants had aphids that were untended and 11 had no aphids present. All plants had ants present. We only observed interspecific competition of ants on 5% of the plants. However, several different ant species were on the milkweed plants. Ants present included *Lasius sp.*, *Formica sp.*, *Formica podzolica*, *Formica subsericae*, *Crematogaster sp.* and *Myrmica sp.*

Plant Chemistry

There was no significant difference in the carbon to nitrogen ratio of the original plants compared to the novel plants before aphid transfer (t=0.778, df=27, p=0.443, Fig.1). The change in carbon to nitrogen ratio of plants separated into three groups- plants with aphids present, plants with ants and aphids present and plants with no aphids present, showed no significant difference ($F_{2, 17}$ = 0.008, p = 0.992, Fig. 2). However, in all three groups the carbon to nitrogen ratio decreased, which indicates that nitrogen increased. The untended plants with aphids and tended plants had a smaller increase in nitrogen than the plants lacking aphids. There was no correlation between the novel plant's change in carbon to nitrogen ratio and aphid density (R^2 = 0.003, df=19, p=0.442, Fig. 3). The number of ants and aphids present per plant also showed no correlation (R^2 = 0.000, df=19, p=0.948, Fig. 4).

Discussion

After watching the milkweed plants for four days we only saw a direct interaction between two ants of a different species over the novel aphids. This could mean that the ant species we observed are able to both harvest the aphids at different times or maybe they are not that aggressive or protective over aphids.

In a previous study comparing *Aphis asclepiadis* to another species, *A. asclepiadis* densities showed a negative density dependence at higher densities, which suggests that there may be a threshold around 70 aphids where intraspecific competition is minimal (Mooney, 2007). Also, another study found that colonies with high aphid density had decreased or nonexistent benefits from ant tending. The effects of ants on aphids were most noticeable in populations of less than 30 aphids (Breton and Addicott, 1992). This could be a reason why some of the novel plants did not support aphids- the densities were too high to start with. This could also help explain why only 11 of the 20 plants had ants tending aphids. Another reason why we had low tending could have been due to the sugar bait we used. Ants may choose to harvest an alternative food over aphids. When given sugar in the presence of aphids, ants chose the sugar alternative and then ate the aphids instead, presumably as a source of protein (Offenberg 2001).

We found no significant difference in C:N between the original and novel plants. Originally we suspected that plants with aphids may have a higher C:N ratio in comparison to plants without aphids. In addition, less than half of the novel plants had aphids present after four days. Therefore, it is possible that aphid presence is due to random chance. The difference could also be due to another factor that we did not test.

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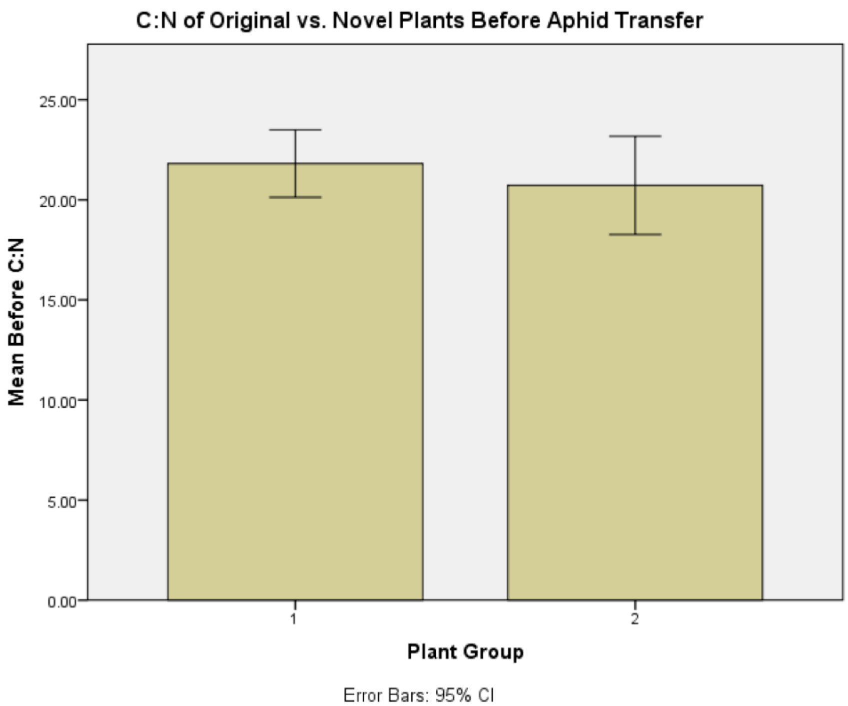
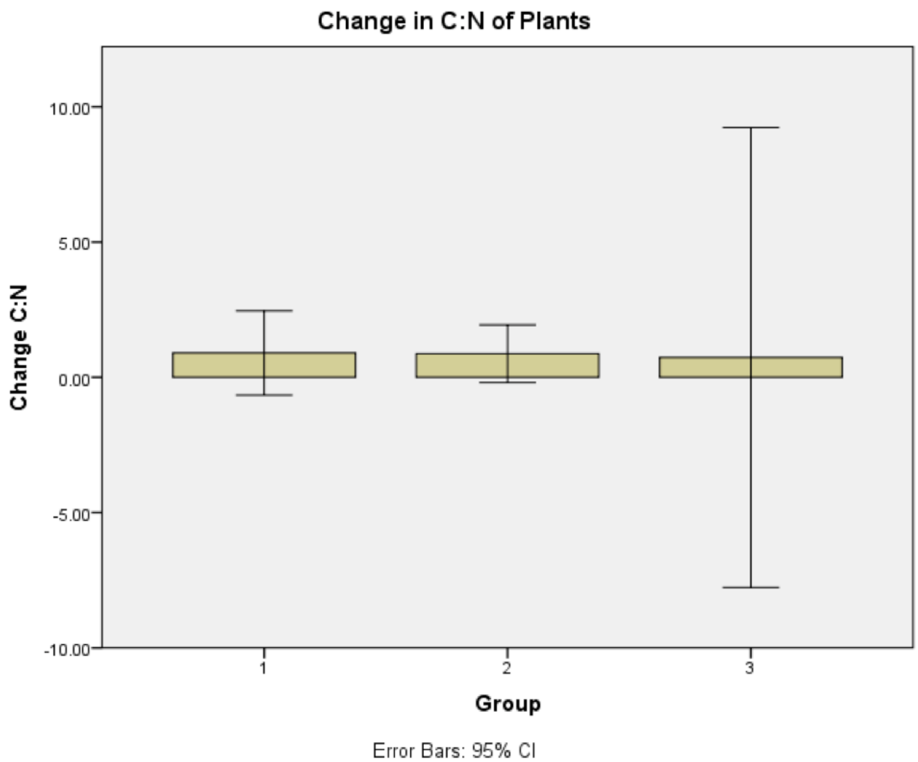
Figures



t = 0.778
df = 27
p = 0.443

1. **Novel Plant**
2. **Original Plant**

Figure 1. Carbon to Nitrogen Ratio of Original vs. Novel Plants Before Aphid Transfer.

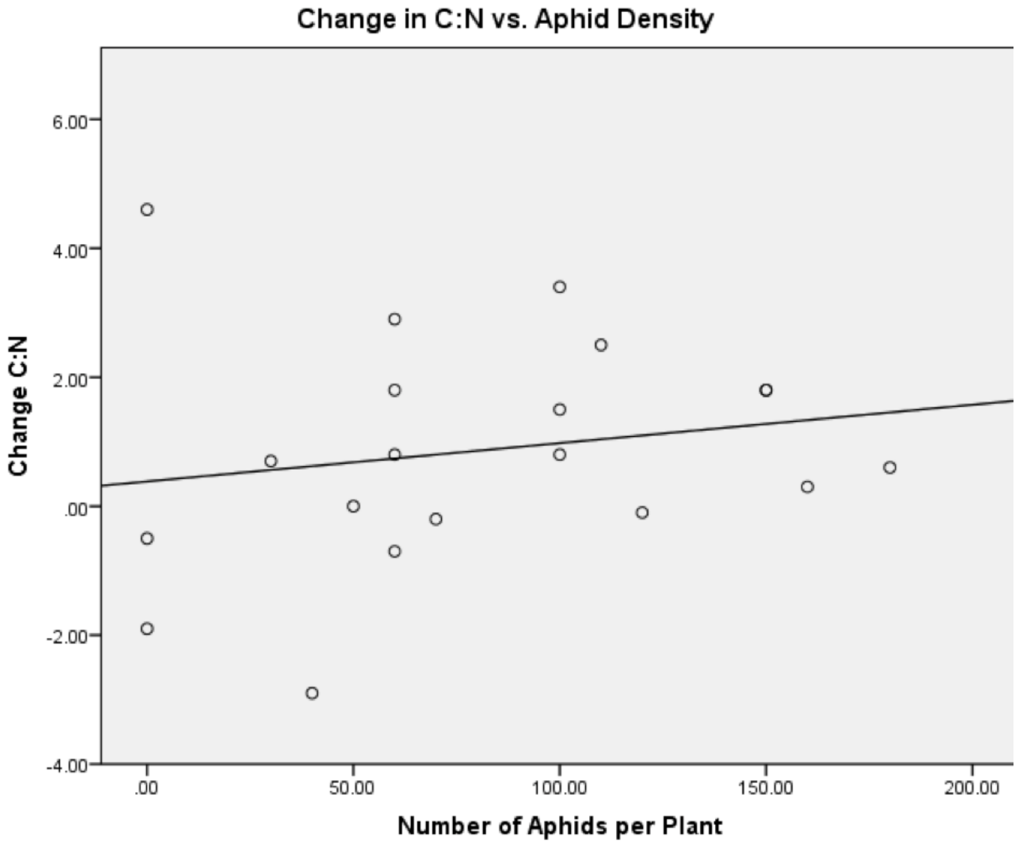


$F_{2, 17} = 0.008$

$p = 0.992$

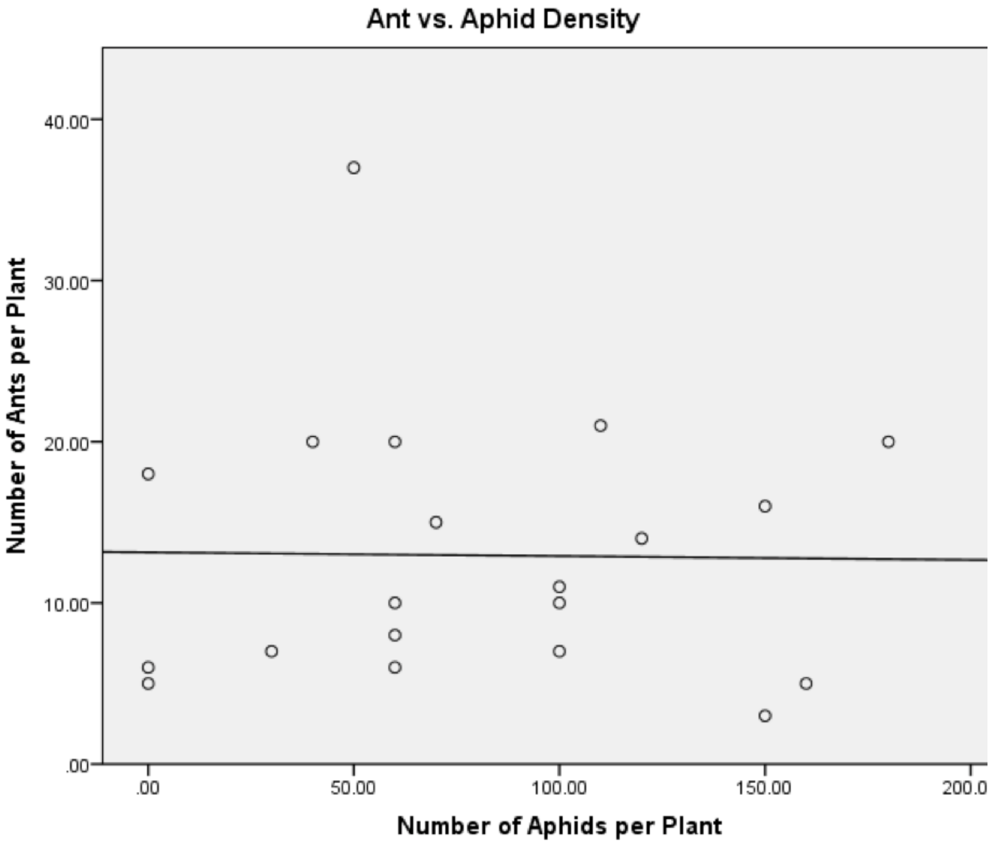
1. **Aphids**
2. **Mutualism**
3. **No Aphids**

Figure 2. Change in C:N of Plants with Aphids, Tended Aphids, and No Aphids.



R^2 = 0.003
df= 19
p = 0.442

Figure 3. Change in C:N vs. Aphid Density.



R^2 = 0.000
df= 19
p= 0.948

Figure 4. Ant vs. Aphid Density.

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