Abstract:

Mutualistic relationships are common throughout the natural world. Ants and aphids tend to have a mutualistic relationship. The purpose of this study was to observe interspecific competition between different ant species over a colony of aphids and what happened to the plants Carbon to Nitrogen levels as a result of varying amount of aphids and ants. We transplanted leaves from nine plants, to twenty novel or new plants in order to observe how the aphids would interact with or use the new plant as a source of food. Leaf samples from each plant were taken both before and after the introduction of aphid colonies, and these plants were then desiccated and analyzed for the C: N levels. We found no significant difference in the C: N of original vs. novel plants, and no correlation between the plants C: N ratios and aphid density. The number of aphids did not increase as the number of ants on each plant increased, this was unexpected, as ants tend to aphids and protect them from predation. We expected an increase in aphid density to lower the C: N levels on the plant but saw no strong correlation. This study has some practical applications such as looking at using aphids and ants as a source of biological control on plants that aphids colonize, and the long-term effects on the plant and plant chemistry from having an aphid colony feed from it.
**Introduction:**

Competition is defined as an interaction between multiple organisms or species, in which the fitness of one is lowered by the presence of another. Both ants and aphids benefit from each other. *Aphis Asclepiadis* feed on the plant for nutrients, and when an abundance of phloem is ingested they defecate a substance known as honeydew. This is a very rich resource that the ants then use as a food source (Deo, 1997). The ants protect the aphids from predators by running around the plant and removing predators. Ants demonstrate their aggressive behavior by pushing these predators off of the plants (Clover, 1991). Aphids release an alarm pheromone as an alarm, which is what the ants respond to (Beale et al. 2006). In many cases the ants farm the aphids by using their antennae to tap the aphids on their rear ends to facilitate the honeydew excrement. If the aphids did not have this protection, they experience increased mortality, which in turn decreases future reproduction compared to mortality with a lack of protection (Way, 1963). In addition to this mutualism between the ants and aphids, there are many other insects that come to feed on the milkweed and there is often competition for these scarce resources.

The objective of this study was to determine interspecific competition between ants over the introduced aphids, why the aphids weren’t initially present, and if the aphids affect the plant Carbon to Nitrogen chemistry. We believe that aphids influence plant chemistry by ingesting the plant they settle on. Carbon and Nitrogen interact to achieve C: N homeostasis, the plant allocates these nutrients for growth and development most efficiently (Sang, Y. 2012).
Ants regulate aphid populations at a level where intraspecific competition between aphids in a colony is low enough that the host plant is not harmed (Babb, 1993). Ants try to maintain the most productive rate of saturation of resources from aphids. Bryson (2000) found aphid density affected ant attendance, which in turn could affect the presence of aphid predators. This study investigated the interactions and abundance of ants and aphids when introducing an aphid colony on milkweed via a leaf from one milkweed plant, to another where there were no aphids previously. If competition between ants and other organisms affects aphid colonies on milkweed plants, then there will be a difference in the plants’ Carbon: Nitrogen.

**Materials and Methods:**

This study was conducted in the UV field at the University of Michigan Biological Station in Pellston, Michigan (Figure 5). This site was selected due to high density of milkweed plants in this area. We first clipped a milkweed leaf that had aphids and recorded the number of aphids. Then we introduced this leaf to another milkweed plant that contained no insects previously by pinning the leaf onto the new plant. We introduced the leaves with aphids from nine plants to twenty other milkweed plants that were scattered randomly throughout the UV field. We put sugar water in test tubes with a cotton ball at the entrance, at the base of every plant to lure ants to the aphids. Every hour, for the next sixteen hours, we checked and counted the number of aphids on the plant as well as the number of ants on the plant, the number of ants tending aphids on the plant, and the species of the ants.
We clipped a leaf of the original nine plants, and a leaf from the milkweed that it was introduced to both before, and after the experiment to examine the difference in plants Carbon to Nitrogen chemistry. The leaves were desiccated in an oven for 12 hours, and then ground into a fine powder. We then gave the local chemist this powder to see the Carbon to Nitrogen ratios and individuals percentages. The first test that we are performing was t-test to measure the change in C: N on the original plant vs. the change in C: N on the new plant, or the plant that received the leaf of aphids. This is being performed because we want to see if the aphids affect the Carbon to Nitrogen ratios on a new plant, and if it does, by how much. The second test we are performing is an ANOVA test to see the differences in C: N of plants that we put into three groups: plants that had aphids on them, plants that had both aphids and plants on them, and a control group of plants that had nothing on them. We are conducting this in order to determine how these groups compare to each other in regards to the change in Carbon to Nitrogen. We are performing a regression showing the change in C: N vs. the number of aphids per plant because we think the number of aphids on a plant can affect the change in C: N. The last test we are performing is a regression on the number of aphids per plant vs. the number of ants per plant to see if there is a correlation between the numbers of ants and aphids.

Results:

After conducting our first test, we found the Carbon to Nitrogen ratios of original vs. new plants before aphid transfer to be insignificant with a p-value being greater
than .05 (p = .443). From performing the second test, we found insignificant results pertaining to the change of C: N in relation to plants with aphids on them, plants with ants and aphids on them, and with neither aphids nor ants on the plants. The p-value found was greater than .05 (p = .992). We found p = .442 for our third test, giving us insignificant results for change in Carbon to Nitrogen levels with regards to aphid density. We did not find aphid density to have a significant effect on the milkweed's C: N ratio. After performing the fourth test, we found ant vs. aphid density to be insignificant as well, with p = .948

**Discussion:**

Although the tests that we ran proved to be insignificant it does not disprove whether aphid density affects plant chemistry. In another study, researchers found reproductive decisions in aphids tended to be based off of plant cues and that in the absence of suitable cues, the aphids would not colonize, feed or mate (Powell G, 2006). In another study conducted by Kindlmann et al, in 2007, they found that there was a strong selection pressure on aphids for finding high quality host plants to optimize dispersal. We may have tried to put the aphid colonies onto milkweed that were not nutritionally stable and or were not releasing cues, so the aphids may not have liked the site.

Aphids manipulate resource allocation within the plant, diverting nitrogen from atypical growth zones to its feeding site (Goggin, F. L. 2007). Goggin concluded that aphids increase the nutritional quality of the aphids feeding sites by increasing the import of resources from other sites in the plant. This could give the plant a higher
Nitrogen level where the aphids are located rather than equally distributed throughout the plant. We may not have seen this due to a short experiment time and not enough samples.

We did not find a significant relationship between the number of ants and aphids per plant. Ants and aphids have been shown to have a mutualistic relationship by other researchers, and in a study done by Hoffman, K.H. in 2007, this researcher found as ants helped to reduce predation for the aphids, the number of aphids rose. We may not have observed this due to a short experiment run time, however this study could be expanded over a longer time period to see if the ants significantly influence the number of aphids. We found no significant difference in C: N between the original and plants that were transplanted to, as well as the C: N of the different plant groups. Carbon and Nitrogen nutrients help regulate plant growth and development through metabolism and signaling pathways (Sang, Y, 2012). If the aphid density was large enough, the plant could be allocating more C and N to where the aphid colony is located. This can be measured by a plant response called compensation, which is when a plant limits the potential loss of fitness owing to herbivory by increasing growth (Zekveld, C. 2008). The milkweed, aphids, and ants could be in a three-way mutualistic relationship. Plants that suffer significantly from external herbivores, and lack an effective defensive, may indirectly gain from the colonization of the aphid if they attract ants that defend the aphids from predators and the plant from external herbivores (Buckley, R. 1987). Honeydew falling to the soil underneath the colonized plant may also facilitate plant growth if the soil is able to absorb it, and break down the nutrients for the plant to take up.
Despite not being able to quantify significant differences between ant and aphid interactions and the effects of plant chemistry on milkweed plants, we found some small increases in aphid density in relation to C: N chemistry of the plants, and if this study was expanded over a longer time period we may have found a larger difference. Ant and aphid interactions and the results on plants could be applicable to agriculture as a source of biological control. This study could also be expanded to pursue the interactions of various species of plants, ants, and aphids, and how they affect the C: N chemistry on various species of plants.
Figure 1

C:N of Original vs. Novel Plants Before Aphid Transfer

- t = 0.778
- df = 27
- p = 0.443

1. Novel Plant
2. Original Plant
Figure 2

- $F_{2, 17} = 0.008$
- $p = 0.992$

1. Aphids
2. Mutualism
3. No Aphids
Figure 3

Change in C:N vs. Aphid Density

R² = 0.003
df = 19
p = 0.442
Figure 4

$R^2 = 0.000$
$df = 19$
$p = 0.948$
Bibliography:


Bryson, T. C. 2000. Ant Behavior in the Presence of Aphids and Aphid Predators (Chiysoperla carneia or Coleomegilla maculata): An Indicator of Ant-Aphid Mutualism?


