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EEB 381 – Pelini
Ants and Aphids Project

Interspecific Competition in Ant Species and Change in C:N of Plants Following the Transfer of Aphids to Common Milkweed

Abstract

Ant-aphid mutualism is seen among many species of plants and throughout the world however only two species of aphids specialize on the common milkweed (*Asclepias syriaca*). For the purpose of this study we focused on the relationship between green aphids (*Aphis asclepiadis*), several ant species, and milkweed at The University of Michigan Biological Station. We observed the ants and aphids on 20 different milkweeds for four days and recorded any interspecific competition between ants over novel aphids. Additionally we analyzed plant chemistry before the transfer of aphids on the original and novel plants, also, after the transfer and tending of aphids on the novel plant. In order to find the effect of ant-aphid mutualism on the plant's C:N and to find an underlying difference for the presence or absence of aphids we ran plant chemistry. This experiment found no significant differences in interspecific competition or plant chemistry. Although we found no significant differences in plant chemistry we did find that plants with aphids and plants with the mutualism had a smaller increase in Nitrogen than the plants with no aphids present. Our experiment could be applied to an agricultural setting to measure the effects of pests, such as aphids, on the change in C:N of plants; in other words monitor the effect of aphids on plant growth.

Introduction

Many ants and aphids have a mutualistic relationship (Stadler and Dixon, 2008). The aphids feed on plant stems, then defecate sweet, sugary "honeydew" that is a result of the sugary sap (Müller-Schwarze, 2009). Ants then milk the aphids by tapping their abdomen to indicate to the aphids that it is feeding time. In return, the ants provide the aphids with protection from oncoming predators, such as the ladybird (*Coccinellidae*), and clean the smaller aphids (Lesko, 2012). At times ants also shelter aphids by herding them into their nests for protection from poor weather. Additionally, the aphids generally reside on younger leaves near the top of the plant. The leaves provide aphids with an abundance of nutrients including Nitrogen due to their increased metabolism (Zekveld and Markham, 2011). However, poor weather conditions, such as rain or hot temperature, can encourage the aphids to move down the shoot or simply drop off the plant (Lach, 2010).

In our experiment, we observed green aphids (*Aphis asclepiadis*) on the nonflowering common milkweed (*Asclepias syriaca*) and several ant species in the UV field at The University of Michigan Biological Station (UMBS). Carbon (C) and Nitrogen (N) are essential major nutrients that interact to modulate plant growth and development (Sun *et al.*, 2012). Signaling in the roots metabolize N upwards towards the shoots and leaves of the uppermost part of the plant (Sang *et al.*, 2012). In plants where aphids are present plant growth is stimulated as a result of nitrogen fixation (Zekveld and Markham, 2011). However, plant growth is hindered when aphids are feeding (Kindlmann *et al.*, 2006).

Green aphids, much like monarch butterflies (*Danainae* sp.), are a type of milkweed-specializing insect that have coevolved with its host in order to endure the cardenolide-barrier that the milkweed produces (Cohen, 1983). This specialization allows herbivory by aphids unlike most insects (Mooney *et al.*, 2007).

Interspecific competition between ants over novel aphids was expected; therefore we designed an experiment in which the aphids were transferred to an uninhabited milkweed (novel plant) from an original milkweed with aphids. If aphids were transferred to an uninhabited milkweed, then multiple species of ants were expected to compete over aphids because of the mutualism. Additionally, we performed chemical analysis on the milkweed in order to determine the C:N ratio. If ant-aphid mutualism played a role in plant chemistry, then we expected to see the C:N ratio to differ between plants with aphids, mutualism occurring, and no aphids present.

Methods

The plots in which we performed the experiment were roughly 34m x 18.5m (plot 1) and 22m x 26m (plot 2) (fig. 1). Orange flags marked 9 nonflowering milkweeds that were heavily infested with tended aphids (>60); these served as the original plants. Additionally, we marked 20 nonflowering uninhabited milkweeds (fig. 1) with numbered yellow flags; these served as the novel plant.

From the original plants we clipped a leaf at the top of the plant that was infested with aphids and recorded the number of non-reproductive aphids. Additionally, we clipped a leaf sample from the original plant and from the novel plant, prior to adding the aphids, for chemical analysis. We pinned the original infested leaf to one of the 20 novel milkweeds. The novel plants were at least 10 feet away from the original plant that the infested leaf originated. Every hour, for 16 hours we observed the novel plants for aphid migration activity to the novel plant from the original plant leaf. Eight hours after we pinned the leaves to the transfer plant we brushed the remaining aphids on to the novel plant using a soft paint brush. In addition, we recorded the number of aphids present, ants we observed on the plant or tending the aphids, and predators present. If a predator was present, we removed it from the plant to remove competition from the experiment since we were only interested in interspecific competition between ant species and plant chemistry.

Once all of the aphids were transferred to the novel plant we introduced sugar water at the base of the plant to bait ants. The sugar baits remained at the base of the plant for 24 hours post transfer. The bait consisted of table sugar and water (3:1). After the initial 16 hour observation period we recorded ant-aphid activity one to three times per day.

Four days post colonization of the new plant we clipped another leaf from the new plant for chemical analysis. We desiccated all of the sampled leaves (original plant, new plant prior to aphid transfer, and new plant post aphid transfer) in an oven at 60 degrees C for approximately 72 hours, or until completely dry. We ground the leaf samples into a fine powder and analyzed the Carbon to Nitrogen ratio in the milkweed using an elemental combustion analyzer (Costech Analytical). To compare ant and aphid density we performed a Regression of the highest number of ants and aphids recorded per plant from the four day experiment. An additional Regression model was performed to compare the change in C:N versus aphid density per plant. We measured the change in C:N of the original plant versus the novel plant before aphid transfer by using a t-test. We tested the change in C:N of plants among three groups – aphids, mutualism, and no aphids present on plant – by ANOVA.

Results

Over the four day period there was one observation of two different ant species tending aphids at the same time. Therefore, only 5% of the 20 plants had two species tending simultaneously.

There is not a significant difference in the number of ants or aphids present per plant (R^2=0.000; df=19; p=0.948) given the Regression analysis (fig. 5). Similarly, the change in C:N versus the number of aphids per plant is not statistically significant (R^2=0.003; df=19; p=0.442) given the Regression analysis (fig. 4).

According to a t-test (fig. 2) the change in C:N of the original plant to the novel plant before the aphids were transferred was not statistically significant (t=0.778; df=27; p=0.443).

Moreover, there was not a statistical significance ($F_{(2, 17)}$=0.008, p=0.992) in the change in the C:N of plants among three groups – aphids present (N=6), mutualism present (N=11), and no aphids present (N=3) – given the ANOVA graph (fig. 3).

Discussion

Although the number of each ant species was not recorded for this experiment we found that there were six different species of ants – *Crematogaster* sp., *Formica* sp., *Formica podzolica*, *Formica subsericae*, *Lasius* sp., and *Myrmica* sp., – either tending aphids or on the milkweed. With the multiple number of species observed we expected to see interspecific competition between the species over introduced aphids, however, our results were insignificant. Conversely, we observed 5% of the plants to have two different species of ants tending at the same time. Above all, ants such as *Formica podzolica* that are tending milkweed aphids protect aphid colonies against a lethal fungal disease caused by *Pandora neoaphidis* (Nielsen *et al.*, 2010). The ants are capable of detecting the deadly pathogen and either remove the aphid from the colony or sanitize the aphid, reducing the disease transmission in aphid colonies (Nielson *et al.*, 2010).

There was no significant relationship between the number of ants and aphids present on the plants (fig. 5). We predicted that if there were more aphids, there would be more ants to tend them. Thus, an increased number of ants assume an increased number of aphids on the plant because there would be fewer aphids consumed by predators (Hoffmann, 2007). However, this was not the case because there was no correlation between ant and aphid density.

There was no significant difference in C:N in plants with different aphid densities (fig. 4), opposite to the findings of some scientific research. Higher aphid density does not correlate to a higher or lower C:N in the plant. This may be because milkweed has coevolved with the mutualism between ants and aphids (Cohen, 1983). This species of aphids is a milkweed-specialist (Mooney *et al.*, 2007). Also, the plants may have developed a resistance to the aphids in an effort to protect itself from damage due to resource partitioning. Alternatively, aphids indirectly increase virulence and transmission of a monarch butterfly parasite due to weakened defensive chemistry of milkweed (De Roode *et al.*, 2011). The interaction of aphids with milkweed creates a series of indirect effects that greatly impact host defenses and thus influencing disease outbreaks. (De Roode *et al.*, 2011). This is why aphids are often seen as crop pests because of their damaging effects on plants (Seagraves, 2009).

There was no significant difference in C:N between the original and novel plants (Fig. 2). We tested this to try to explain why aphids were on some plants and not others – did the aphids choose plants with a high or low C:N? However, our results were insignificant. Thus, we conclude that the aphids might have been on some plants and not others due to random chance.

Finally, there was no significant difference in the change of C:N of the three plant groups (aphids, mutualism, and no aphids present on plant) (fig. 3). Every group had a decreased C:N, but aphids present made the decrease smaller. When Nitrogen increases, the C:N decreases. Some factor caused an increase in Nitrogen level to plants of all groups, but aphid presence on the plant had smaller N increases than plants with no aphids. This might be because aphids are taking some N from the plants when they consume phloem from the milkweed (Powell *et al.*, 2006).

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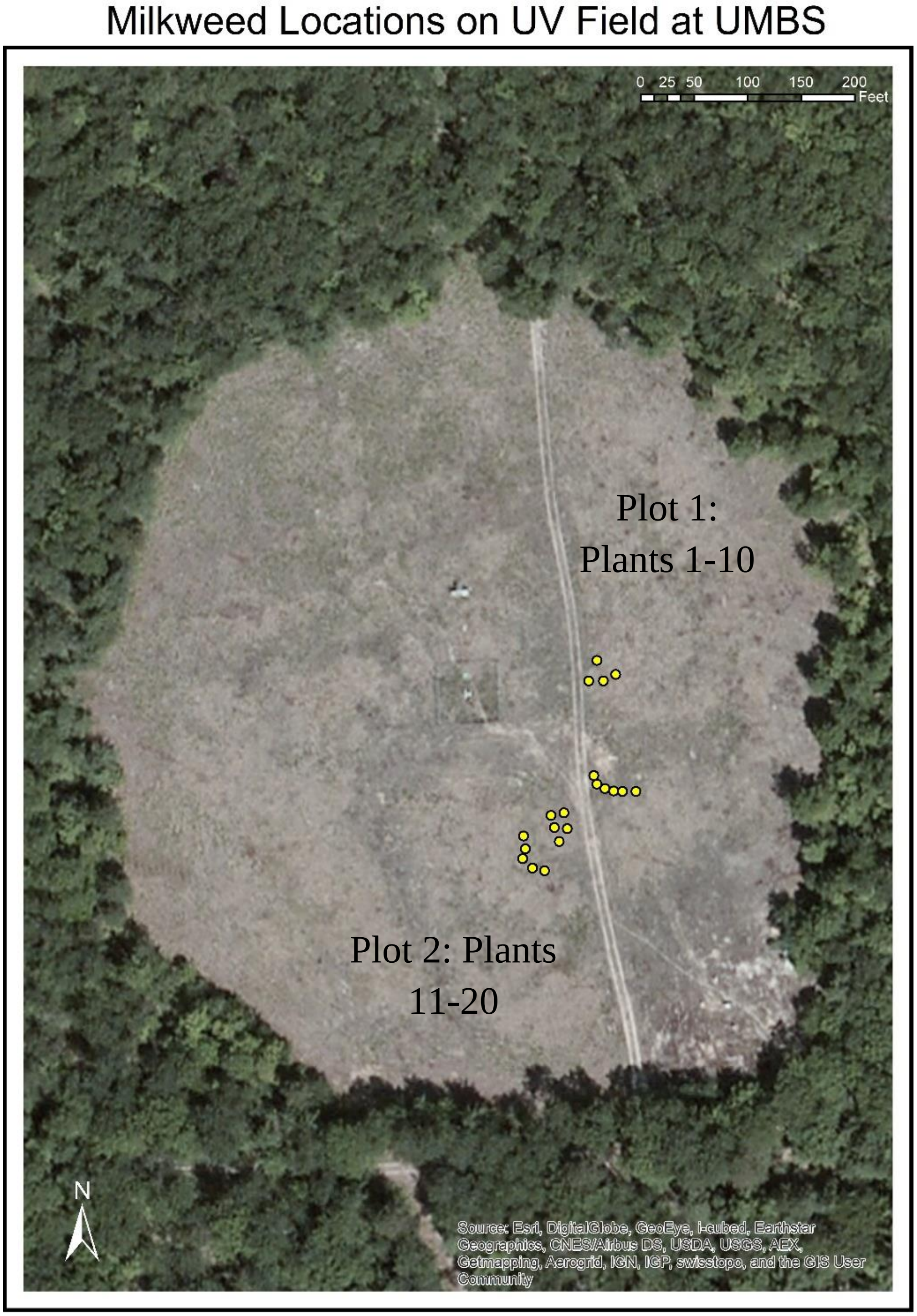
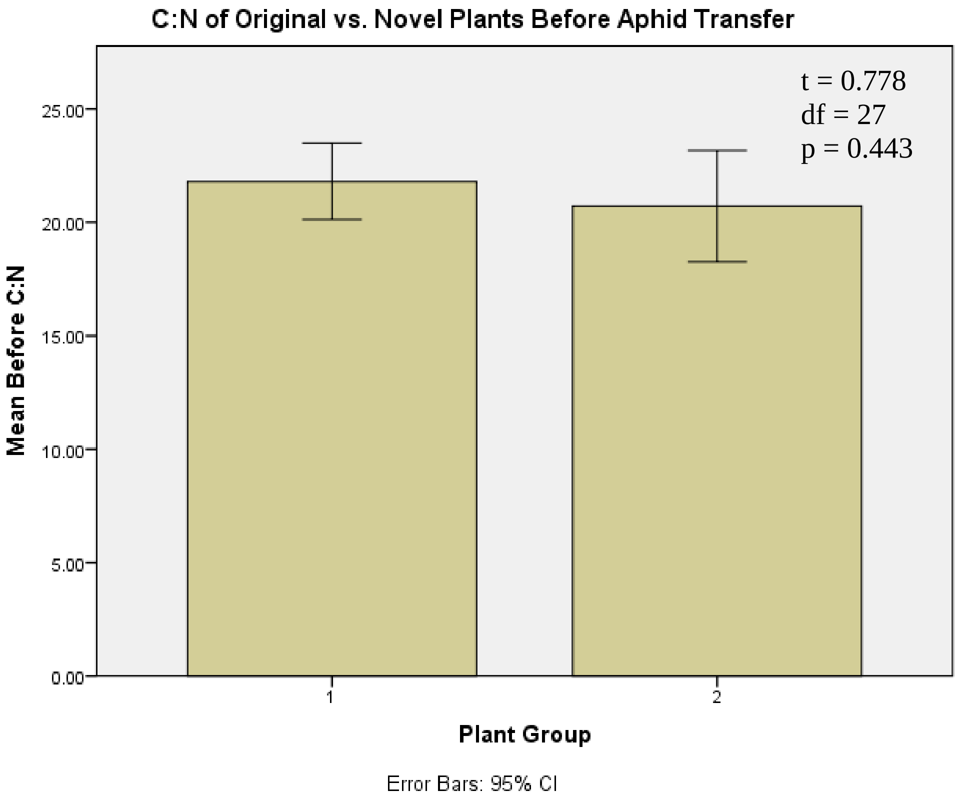
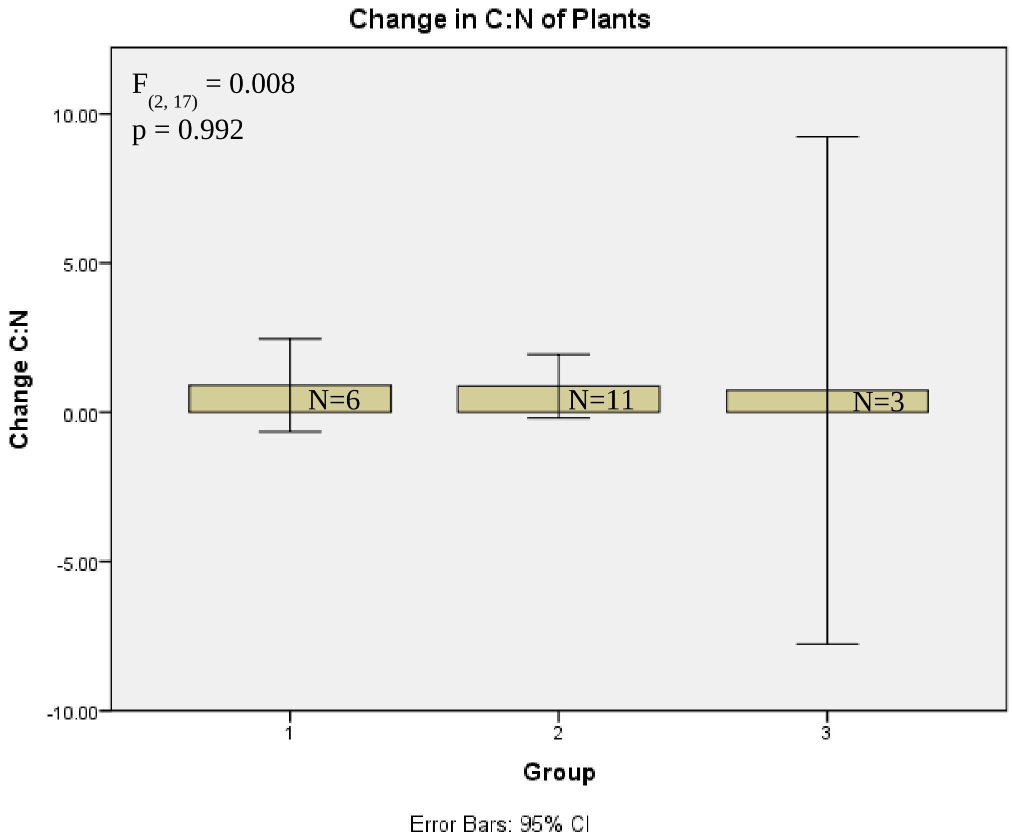


Figure 1. Milkweed Novel Plant Locations on UV Field at UMBS



1. Novel Plant
2. Original Plant

Figure 2. Change in C:N of Original vs. Change in C:N of Novel Plants



1. Aphids
2. Mutualism
3. No Aphids

Figure 3. Change in C:N Across Three Groups of Plants

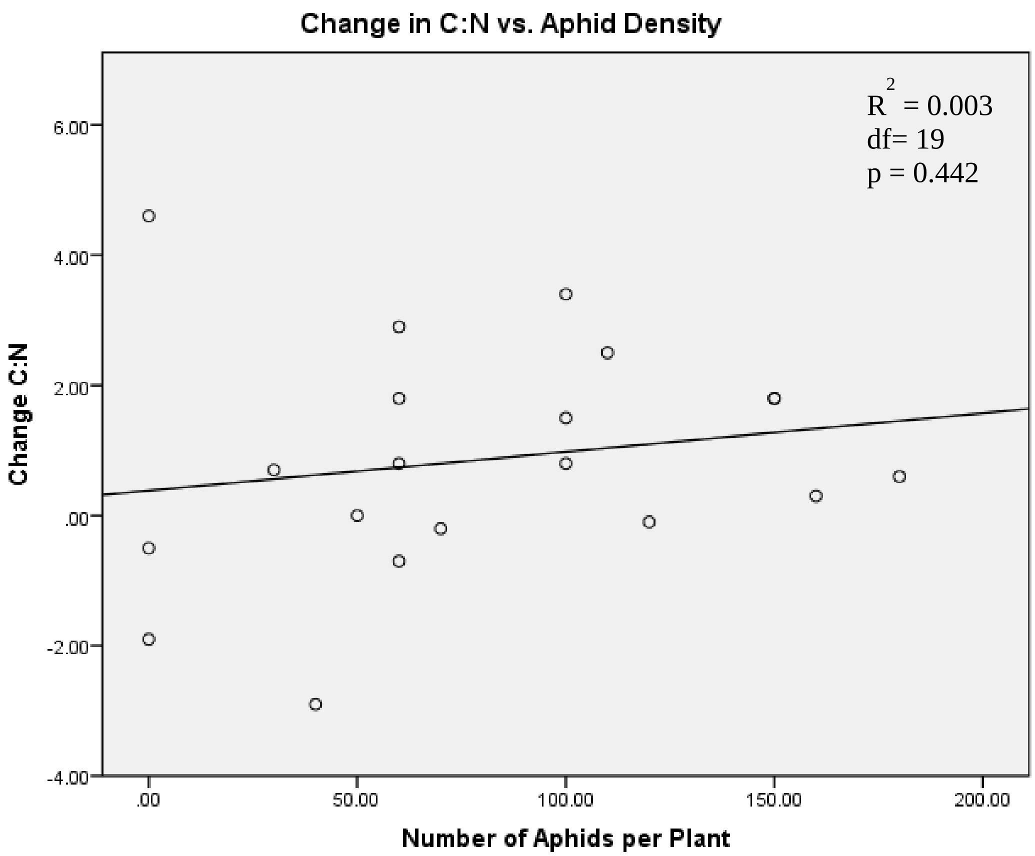


Figure 4. Change in C:N vs. the Number of Aphids

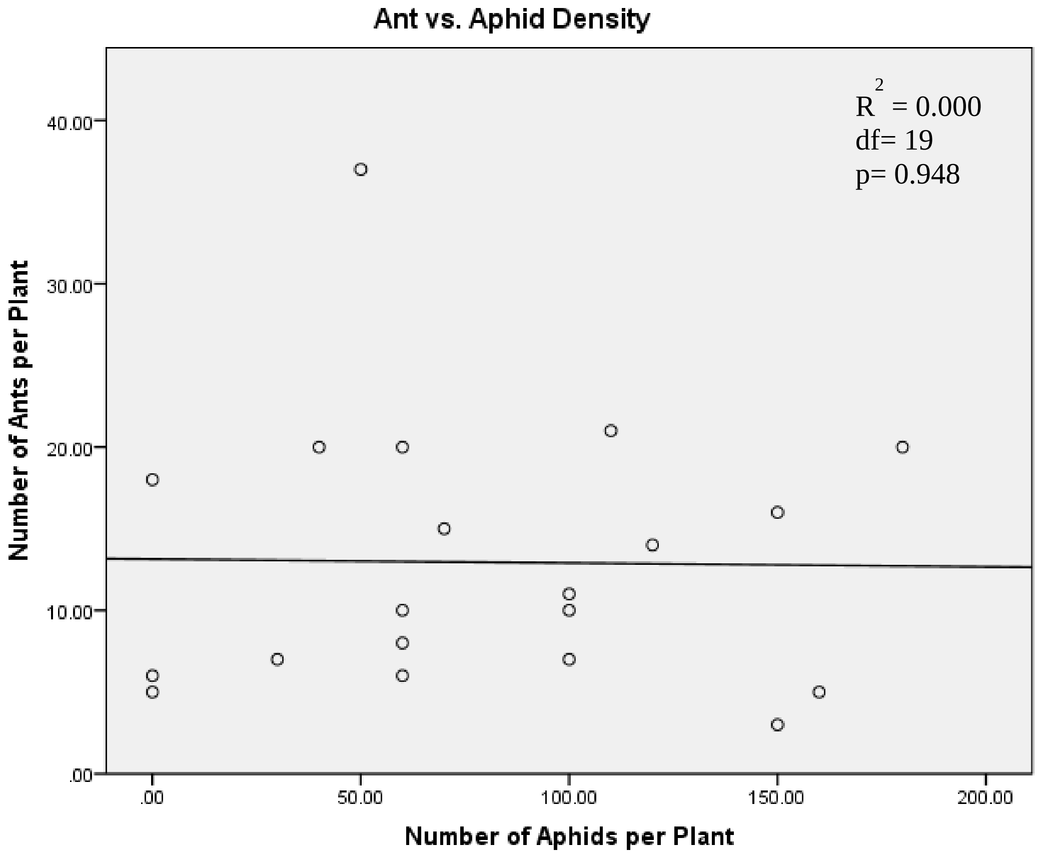


Figure 5. Number of Ants vs. the Number of Aphids Present per Plant

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