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Interspecific ant competition over novel aphid resources and changes in plant chemistry due to ant-aphid mutualisms on milkweed plants

Abstract

Ants and aphids have a mutualistic relationship in which ants tend the aphids for honeydew, a sugary substance that aphids excrete, and in turn, aphids receive protection from predators that the ants provide. We looked at this relationship on milkweed plants in the UVB field at the University of Michigan Biological Station in Cheboygan County, Michigan. We investigated how this mutualism affected carbon to nitrogen ratios of the plants, and how the ratio differed between the original and novel plants. We also looked at how the carbon to nitrogen ratio was affected by aphid density on the plant. Finally, we investigated interspecific ant competition over a novel aphid resource. To do this, we clipped leaves with aphids from an original plant, and moved them to a novel plant without aphids. We took leaf samples of the original plant and the novel plant that aphids were being moved to. We then recorded aphid colonization of the novel leaf and ant recruitment and tending on that plant. At the end of four days, we took a final sample of leaves from each novel plant. We used these to determine carbon to nitrogen ratios of the original plants, and before and after of the novel plants. There was no significant relationship between ant and aphid densities on the plants to effectively determine that a mutualism is present. There was no significant difference in the carbon to nitrogen ratios in the original plants compares to the novel plants before aphid transfer. There were no differences in the changes of plant chemistry of the plants with only aphids, plants with ants tending aphids, and plants with no aphids. There was no relationship between the carbon to nitrogen ratio and the

aphid density on the plant. We also saw only 5% of plants being tended by two ant species, so we could not conclude anything from this. The reason that none of our statistical analyses were significant might be because we did not give enough time for the aphids and ant-aphid mutualism to change plant chemistry.

Introduction

The relationship between ants (*Formicidae*) and aphids (*Aphididae*) has been referred to by many as a mutualistic relationship (Bristow 1985, Price 1984, Way 1963). A mutualism is an association between two species in which both species benefit from the other's presence (Cain et al. 2013). The individuals in a mutualistic association benefit by growing, surviving, or reproducing at a higher rate when in the presence of the individuals of the other species (Begon, Harper, and Townsend 1990). Aphids feed on the phloem, or sap, of a plant which provides them with critical nutrients. When an excess of sugar is ingested, the aphid excretes a liquid know as honeydew, which is a complex mixture of sugars, amino acids, proteins, minerals, and B-vitamins. Ants stroke the aphids with their antennae, and the honeydew is immediately released; it is then gathered by the ants as a food resource (Nixon 1951, Way 1963). In many cases, the presence of ants has been shown to benefit aphids because the ants stimulate growth, defend against predators by patrolling the area around the aphids for several hours at a time, and driving off intruders. Moreover, ants improve the sanitation of the aphid colonies, and occasionally carry the aphids to better feeding spots (Nault and Montgomery 1976, Way 1963). Aphids benefit greatly from the presence of ants other than protection from natural enemies. Flatt and Weisser (2000) found ant-tended individuals lived longer, matured earlier, had a higher rate of reproduction, and a higher expected number of offspring than aphids not tended by ants.

The host plant of the aphids plays an important role in the ant-aphid mutualism by directly and indirectly affecting the fitness of the aphids. Phloem fluids and chemical composition of leaf tissue directly affect the survival, growth, and reproduction of aphids (Auclair 1963, Dixon 1985). Other plant characteristics, such as surface type and architecture, can directly affect the ability of herbivores to acquire food resources (Juniper and Southwood 1986). Ant recruitment to aphid colonies has been found to be strongly influenced by the nutritional content and quantity of attractants produced by the aphids depending on what plant they were on (Taylor 1977). Feeding sites used by aphid colonies strongly influence their ability to attract ants. For our experiment, we wanted to see if plant chemistry made a difference in why aphids were on some plants but not others. We were also interested in how the ant-aphid mutualism affected the chemistry of the plant.

The interspecific competition of ants over the resources provided by aphid colonies was the main focus of our experiment. Ants will remove a predator from the aphid colonies, and we wanted to see if more than one ant species would tend the ants together, or if one ant species would out-compete the other for aphid resources. We investigated the interspecific interactions of ants tending aphid (*Aphis asclepiadis*) colonies on common milkweed (*Asclepias syriaca*). We also studied how milkweed nitrogen levels affected the presence of aphid colonies, and how the ant-aphid mutualism affected nitrogen levels in milkweed plants. We hypothesize that ant density relies on aphid density and vice versa, because there is a mutualistic relationship between ants and aphids. Multiple ant species will compete for limited aphid resources because no two species can coexist using the same resources. Original plants that the aphids were transferred from will have different carbon to nitrogen ratios than the novel plants without aphids, because aphids are on the original plants and not the novel plants because of a difference in plant chemistry. Finally, ant-aphid mutualistic relationships will increase the carbon to nitrogen ratio in those plants because plants raise carbon levels as defense.

Materials and Methods

This study was conducted from July 25 to July 29, 2014 in the UVB field of the University of Michigan Biological Station (Cheboygan County, T37N, R3W, Sec33, N45°56'09" and W84°67'86"). We used a total of nine non-flowering milkweed plants with aphid colonies present on the leaves. We took a leaf sample from the top of each of these plants for carbon to nitrogen chemical analysis. We then cut with scissors at least one leaf with an aphid colony off of each plant, and recorded the approximate number of aphids on each clipped leaf. If ants were present, they were brushed off with fingers. We then transferred each leaf with aphids to one of twenty non-flowering novel milkweed plants with no aphid colonies present. The original leaf was pinned to a top leaf on the novel plant. If ants or other insects were present on the novel plant, they were brushed off with fingers. We checked the plants every hour until most of the aphids had transferred to the novel leaf from the original clipped leaf. If not all aphids had transferred, a small paintbrush was used to brush the aphids onto the novel leaf. The original leaf was then unpinned from the plant. We then recorded the number of aphids that transferred to the novel leaf. After the aphids transferred to the novel plant, sugar bait (15ml of sugar water in a centrifuge tube with two cotton balls in the top) was placed under each of the twenty milkweed plants to recruit ants. The bait was left under each plant for twenty-four hours. We observed each plant every hour for seventeen hours after the aphids were moved from the original to the novel plant and recorded how many aphids were present, how many ants were present, the species of ants present, and if the ants were tending or not. After this first day, the plants were then observed for the next three days every six to eight hours. On the fourth day, July 29, we clipped with scissors a leaf from the top of each of the novel twenty milkweed plants to determine if the ant-aphid mutualism affected the carbon to nitrogen ratio in the plant.

We placed each leaf sample into a labeled paper bag, and desiccated them in an oven set to sixty degrees Celsius for three days. We then ground each leaf sample into a powder, and an elemental combustion analyzer to determine the carbon to nitrogen ratio present in each plant.

For the statistical analysis, we used an independent samples t-test to determine whether or not there was a difference in the carbon to nitrogen ratio of original plants with aphids and the novel plants before the aphids transferred. We ran an ANOVA test to determine whether there was a difference in the change (before and after aphids were transferred to the novel leaf) of the carbon to nitrogen ratio in plants with just aphids, those that had an aphids with ants tending them, and those that had no aphids. We ran a regression to determine if there was a relationship between ant density and aphid density on the plants to see if there was a mutualistic relationship between the two. We then ran another regression to see if there was a correlation between aphid density on the plant and the carbon to nitrogen ratio of that plant. We defined a statistically significant difference for this study as having a p-value of less than 0.05.

Results

There was no correlation between the ant and aphid densities on the plants ($R^2 = 0.000$; df = 19; p = 0.948, Fig. 1). There was no significant difference between carbon to nitrogen ratios of original and novel plants before aphid transfer (t = 0.778; df = 27; p = 0.443, Fig. 2). There was no significant difference between the changes (before and after aphids were transferred to the novel leaf) in carbon to nitrogen ratios of novel plants with aphids, aphids with ants tending, and those that had no aphids at any time point that we checked ($F_{2, 17} = 0.008$; p = 0.992, Fig. 3).

There was no relationship between the aphid density on a plant and the change (before and after aphids were transferred to the novel leaf) in carbon to nitrogen ratios ($R^2 = 0.003$; df = 19; p = 0.442, Fig. 4). We only observed 5% of plants at one time period with an interspecific ant interaction (defined as ants of two different species tending the aphids at the same time). Since this was only a small subset of the data, we were not able to run statistical analysis on this information.

Discussion

Overall, we did not see any interspecific ant competition over novel aphid resources in our experiment. Only 5% of milkweed plants (one plant out of twenty) at one time period had two species of ants tending aphids at the same time. Since this was an outlier in the experiment, we were not able to run any statistical analysis to see if this was related to plant carbon to nitrogen ratios or aphid density. Our hypothesis on interspecific ant competition was therefore incorrect. We observed ants on milkweed plants both with and without aphids, and were able to identify 4 genera (*Lasius, Formica, Crematogaster,* and *Myrmica*) and two species (*Forimca subsericae* and *Formica podzolica*) of ants found on some of the twenty plants on our experiment. *F. podzolica* was found by Nielsen et al. (2009) tending milkweed aphids and protecting aphid colonies from lethal fungal infections caused by an obligate aphid pathogen.

Unfortunately, we did not find a significant relationship between ant and aphid densities on the plants, so our hypothesis was incorrect. If there was a mutualism, we would be able to see high numbers of ants on the plants with high numbers of aphids, and vice versa. This is because if there is a high aphid density, there will be more ants to tend the aphids for honeydew. If there is a low ant density, the number of aphids will decrease due to lack of protection from predators that the ants provide (Begon, Harper, and Townsend 1990).

We wanted to know why aphids were on some milkweed plants and not others, but there was no statistical difference in the carbon to nitrogen ratios of the original plants and the novel plants before aphids were transferred. This means that our hypothesis was incorrect, and that the aphids did not choose one plant over another because of the carbon or nitrogen levels. There was either another factor influencing the aphid's choice of milkweed plant, or it was due to random chance that aphids were on some plants and not others.

Our next question was if aphids or ant-aphid mutualism changes the milkweed plant's chemistry. We found no differences in the changes of plant chemistry between only aphids, ants tending aphids, and no aphids on the plant, so our hypothesis was not correct. The standard error was very large especially for the no aphids group because the sample size was extremely small. In all three groups the carbon to nitrogen ratio decreased, meaning that nitrogen levels in plants of all three groups increased. The aphids only and the mutualism groups had a smaller increase in nitrogen than the group with no aphids. This means that something was increasing nitrogen levels in all plants that we tested, but the aphids either prevented the plants from increasing nitrogen levels as much as plants without aphids, or the aphids used the increased nitrogen in the plants to lower the levels.

There was no significant relationship between aphid density and the change in carbon to nitrogen ratios of the plants. Overall, greater aphid density does not mean higher or lower carbon to nitrogen ratios. This could be due to small sample size, but could also be due to milkweed resistance. Chemical defenses can serve as a repellent or deterrent to herbivores based on the post-ingestive effects of some secondary metabolites to enable insects to learn to reject a plant (Bernays 1998). Specialists, herbivores that feed on one particular species, often show less deterrence to the toxins due to the fact that the specialist's sensitivity has evolved with the plant (Bernays 1998). The species of aphid we saw on our plants, *A. asclepiadis,* is a specialist on milkweed, so it is well adapted to using milkweed as a host plant. In turn, milkweed could be well adapted to withstand the aphids and subsequently the ants that form a mutualism with the aphids. The milkweed could be compensating for changes in carbon and nitrogen levels, so we would not see a significant change depending on the aphid density on the plant. A raise in the carbon to nitrogen ratio in a plant is a way for the plant to defend itself.

One reason that none of the statistical analyses were significant might have been because the four days that the aphids were on the plant might not have allowed enough time for there to be a significant change in plant chemistry. It would be valuable to do a long term experiment in a controlled environment in which carbon to nitrogen levels in milkweed plants are compared before and after an ant-aphid mutualism is established.

Figures

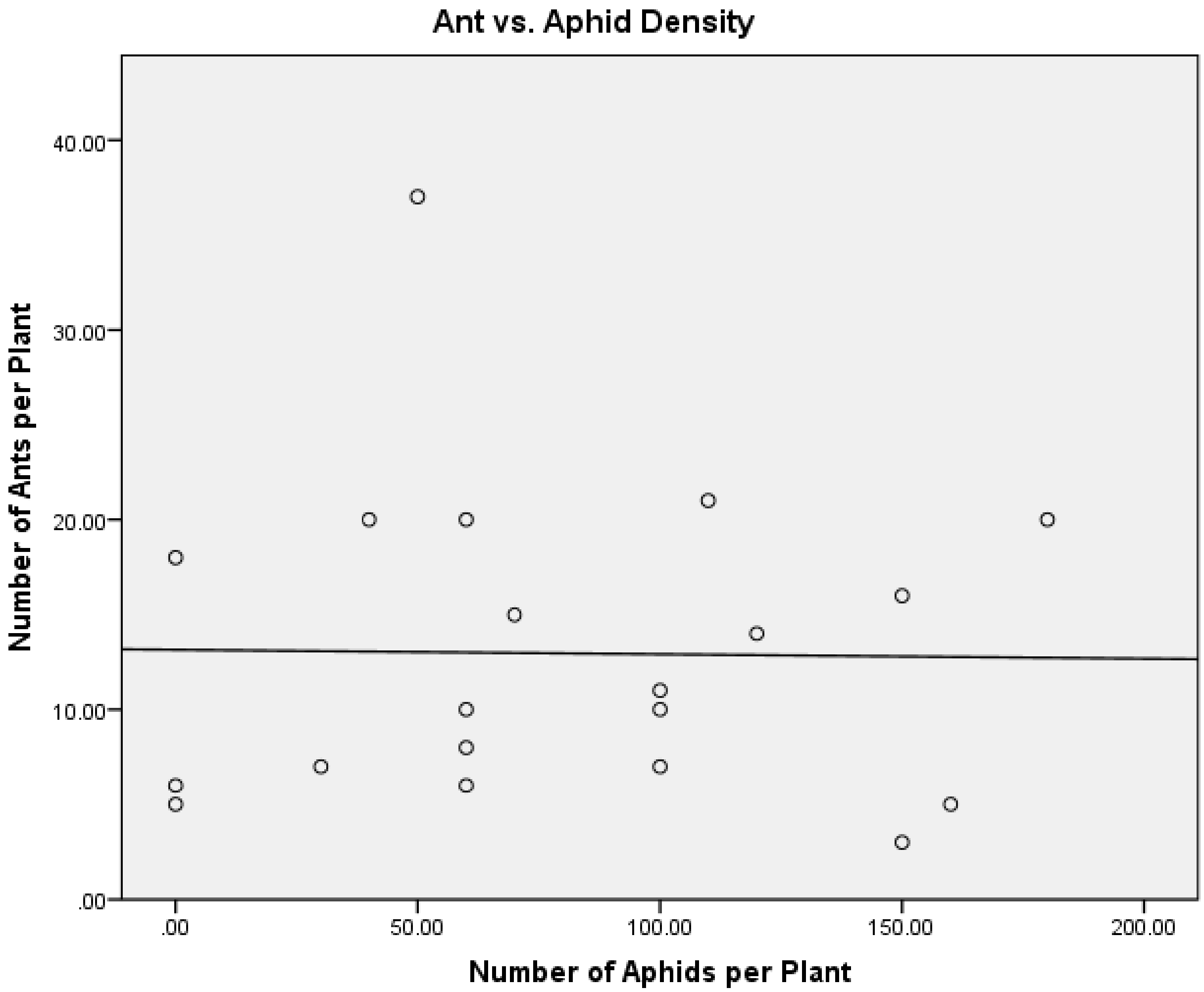

Fig. 1. Regression of number of aphids per plant vs. number of ants per plant.

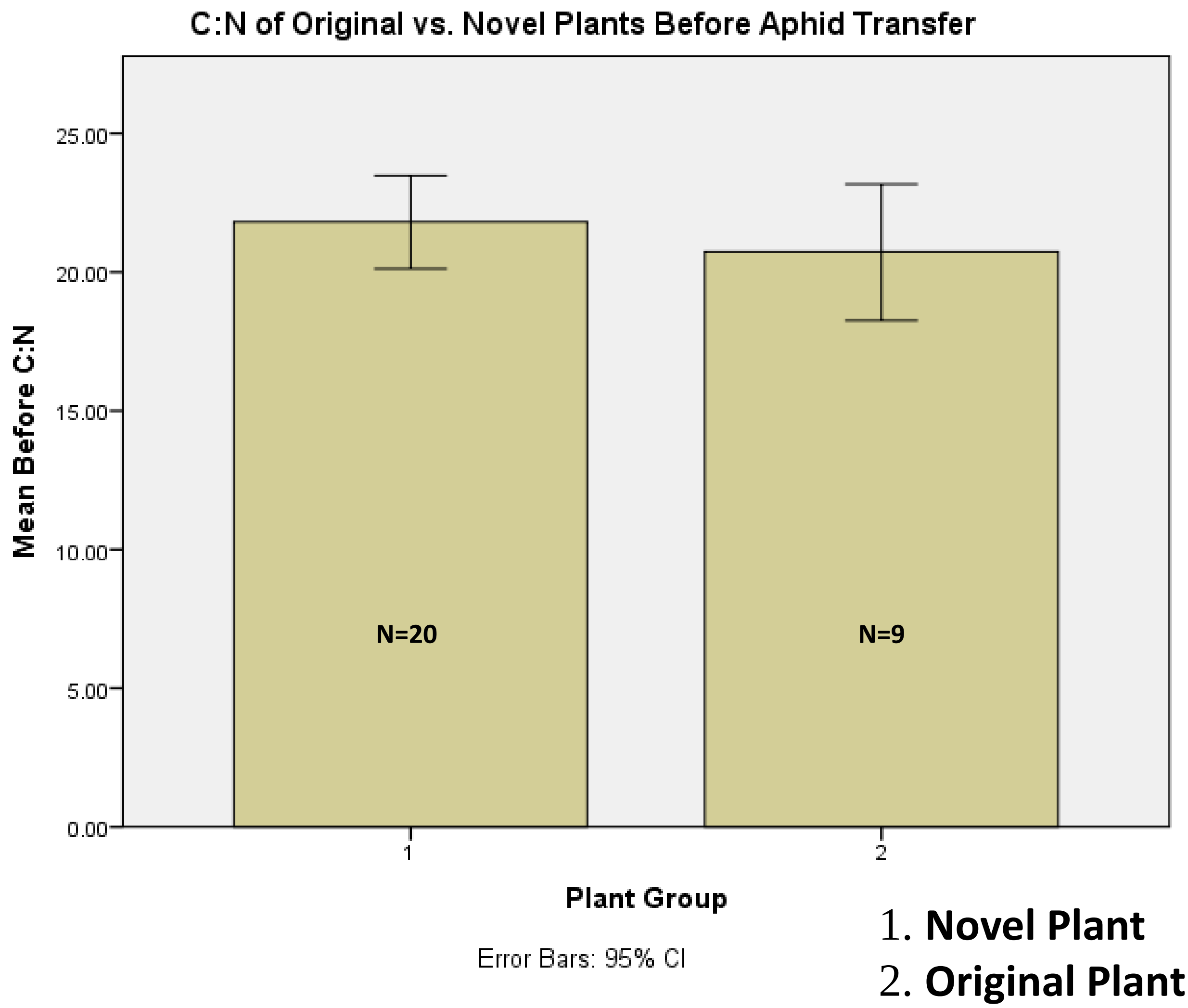

Fig. 2. Independent T-test in carbon to nitrogen in Original plants vs. carbon to nitrogen in Novel plants before aphid transfer.

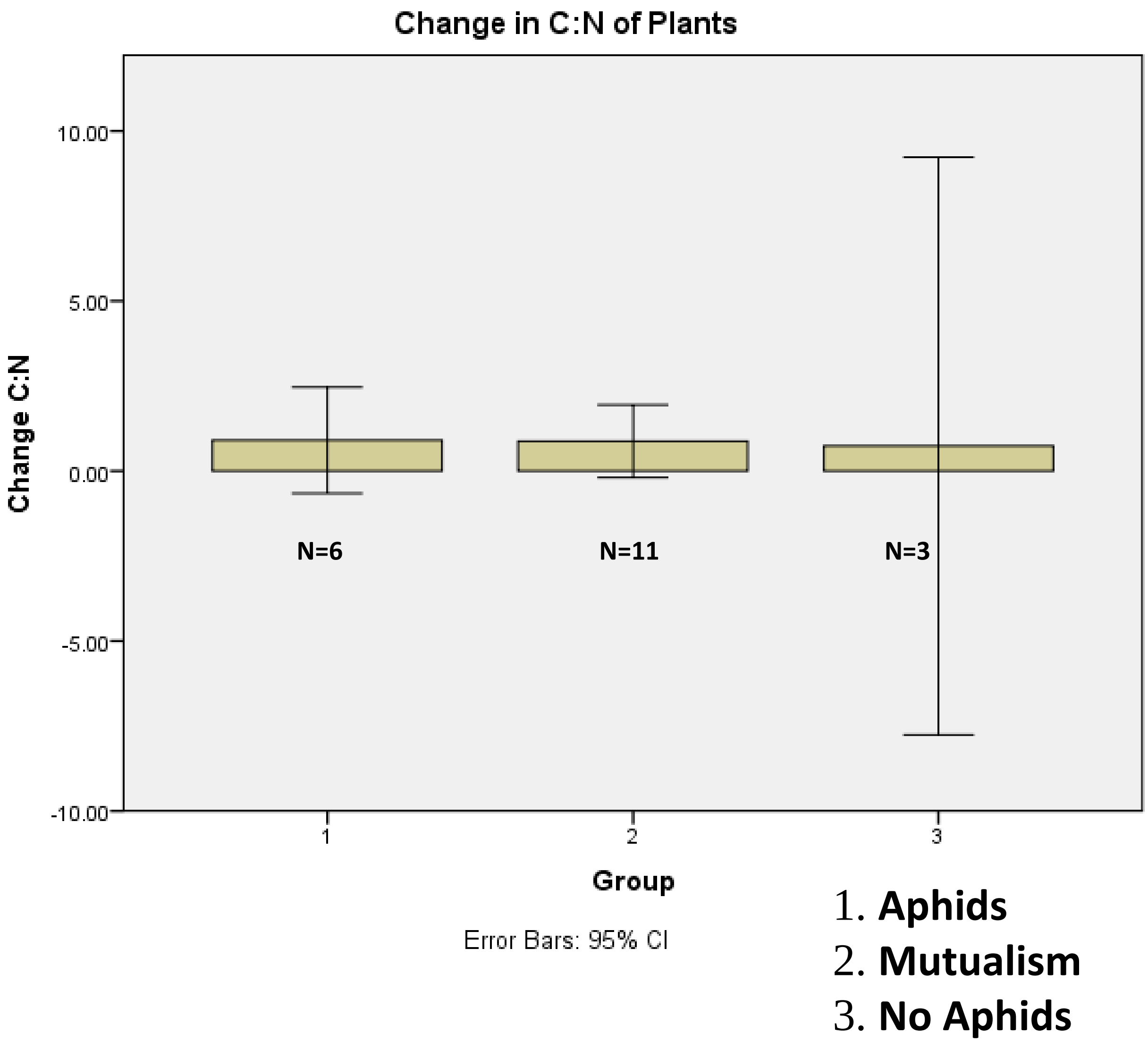

Fig. 3. ANOVA of the change in carbon to nitrogen ratios in groups of 3 plants: only aphids, mutualism (aphids with ants tending), and no aphids.

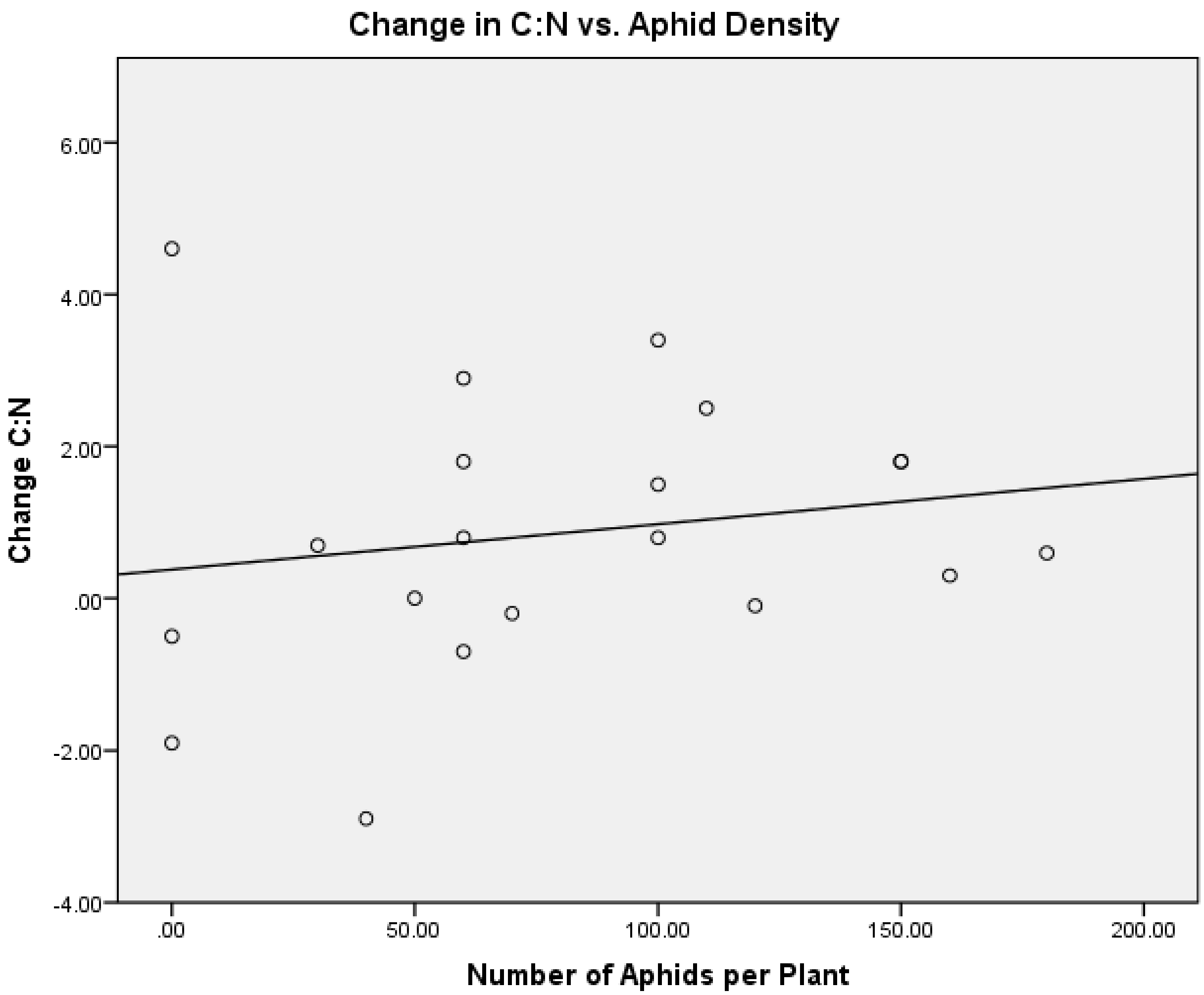

Fig. 4. Regression of the change in carbon to nitrogen ratio vs. number of aphids per plant.

Sources

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