

**Effects of *Helianthella quinquenervis* Extrafloral Nectaries
on Ant Abundance and Community Structure**

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Abstract

The beneficial effects of ants to myrmecophilous species is well established, but there is little empirical evidence demonstrating the benefits of such interactions for ants. The intent of this experiment was to determine how ants are affected by their interaction with the Aspen sunflower (*Helianthella quinquenervis*). Ants forage on Aspen sunflower extra floral nectaries (EFN) as a carbohydrate source. Twenty 7 m diameter plots were established in a field site (Maxfield Meadow) located at the Rocky Mountain Biological Laboratory in Gothic Colorado. Half of the plots had EFN removed, the others did not. Through pitfall traps it was found that four groups of ants occupy the meadow, *Tapinoma sessile*, *Myrmicinae*, *Formica fusca*, and *Formica rufa*. By doing visual surveys of plant stems, it was found that *Formica fusca* and *Tapinoma sessile* are the predominant taxa foraging on EFN. Forager and colony abundance was not affected by EFN removal based upon pitfall trapping and examination of artificial nesting structures (bricks), respectively. However ants within the EFN removal plots recruited to (marginally significant) and consumed significantly more 20% sugar solution baits than do the ants within the control plots. These findings gave evidence that ants do not necessarily depend on EFN from Aspen sunflowers as a carbohydrate source, however, they utilize the carbohydrate source as much as possible, and most likely receive less carbohydrates when they are deprived of EFN.

Introduction

A mutualism occurs when both of the organisms within the relationship are receiving positive benefits. Mutualisms can lead to structuring ecosystems all throughout the world. This has been exemplified multiple times, for example, coral reefs are formed by positive interactions (mutualisms) between corals and microalgae (zooxanthellae) and offer shelter from predators as well as from waves and currents for a vast diversity of invertebrates and fishes. Some of these animals are herbivores that benefit corals by mediating competition with macroalgae (Stachowicz, 2001). This mutualism helps to structure the ecosystem in which it occurs by allowing other organisms to take shelter, therefore allowing a population of different organisms to survive. Another mutualism is one between plants with birds, ants, and other organisms involving seed dispersal. While feeding on the fruit pulp or flower nectar, birds act as seed

dispersers or pollinators (Armesto et al. 1996). These relationships contribute significantly to species diversity in many tropical forests.

Ants are eusocial insects that have developed mutualisms with a variety of different organisms like plants, trees, aphids, and caterpillars. The benefits tend to be protection in exchange for a food source. Ants tend to forage on plants, this interaction has evolved into four ant-plant mutualisms in which ants: 1) protect plants from herbivores and other enemies, 2) feed plants essential nutrients, 3) disperse seeds and fruits, and 4) pollinate (Beattie et al. 1985). The mutualisms that ants engage in often have large effects on the communities and ecosystems in which they occur, which is most terrestrial ecosystems. This has also been exemplified multiple times where recent work suggests that opportunistic ant-plant mutualisms play a key role in structuring food webs in tropical forest canopies (Martin et al, 2003).

While there is much known about the effects of ants on mutualists and other species, there is little known about how the mutualists affect the ants. One study has shown that certain carbohydrates increase ant density by 35%, while certain proteins have no effect on ant density (Kaspari et al. 2012). Another study was done on the effects that plant-based nutritional resources had on colony productivity of an invasive ant species *Solenopsis invicta*. The study found that the ants that were exposed to an unlimited amount of insect prey, and nothing else, grew substantially. However, the ants that were exposed to unlimited insect prey and plants that were colonized by honeydew producing Hemipterans, grew about 50% larger than the ants that were only given access to insect prey (Helms et al. 2008). While these studies suggest myrmecophilous species such as aphids provide a benefit to ants, evidence is somewhat limited, especially from field-based studies.

Another mutualism that occurs with plants and ants is one in which plants can secrete extra floral nectar (EFN) which contains carbohydrates necessary for the ants and its' colony. *Helianthella quinquenervis*, commonly known as the Aspen Sunflower, secretes EFN, and has been observed with as many as 25 ants foraging on the plant (Inouye et al. 1979). This mutualism occurs in order to benefit both species, the plant gets protection from the ant, while the ant gets a carbohydrate food source in return. The EFN food source from involucral bracts of *Helianthella quinquenervis* has shown to have more nonprotein and total amino acids than have been reported for any other extra floral nectar. Seven to twelve additional unknown components of EFN, probably nonprotein amino acids, have also been found. The concentrations of amino acids (8-10 on the histidine scale) were found to be the highest ever reported for extrafloral nectar (Inouye et al. 1979). While the effects on the plants of the ant and *H. quinquenervis* mutualism is known, the effects of the EFN on ant abundance and the ants' community structure has not been studied. It is important to understand how mutualisms also affects ant abundance and community structure because ants play important roles in most terrestrial landscapes. Understanding how ants are affected by mutualisms can help to understand the workings of ant behavior and their necessities in an ecosystem. Ant and plant mutualisms are expected to alter ant abundance because the plant supplies the ants with a food source, therefore, creating a change in abundance for ants to go to the plant for foraging.

In this study, the question as to whether removal of EFN increases or decreases ant abundance was studied. Also, within this experiment, the question as of how ants are affected as a community when EFN's are taken away were tested. The experiment tested for if the effects differ between ant species. The study also tested for whether the ants' dietary needs would be affected by the removal of EFN. The colony density was tested for as well so as to see if the removal of EFN affected colony density among species of ants. It was hypothesized that ant abundance decreased in plots with EFN's taken away, that ants within the removal plots would visit baits more often than ants in the control plots, and that the ants' community structure was negatively affected via having decreased fitness, or size. This study is important to perform in order to fully understand the importance and effects of the mutualisms between ants and plants as well as how the mutualism directly affects the ants, which could have a large role in determining ecosystem structure.

Methods

Study system

Research was conducted outside of Gothic Colorado in Maxfield meadows. There are five different types of ants that typically occur within the vicinity of Rocky Mountain Biological Laboratory, *Myrmicinae* (subfamily), sub family *Dolichoderinae* (species, *Tapinoma sessile*), *Formica rufa* (species), *Formica fusca* (species), and *Camponotus* (genus). *Helianthella quinquenervis* has extra floral nectaries where the leaves meet the stem, usually wherever an inflorescence is about to form.



Figure 1. A photograph of a *Formica rufa* ant visiting an extra floral nectary.
<http://blog.wildaboutants.com/2012/02/11/gardening-for-ants-yes-you-can/>

Experimental design

I worked within a previously setup experiment. Within this previous setup, 20, seven meter in diameter plots were established with a two meter by two meter square center. Outside of the square, there were ten bricks in a circle around the square, in order to provide some sort of shelter for the ants to colonize. When I arrived at the site, each of the plots were numbered and paired to another plot via either having clipped, or unclipped *H. quinquenervis* to allow or inhibit growth and production of EFN. The plants were clipped by using scissors to cut on the stem beneath the inflorescence on July 9th, 2016 where the extra floral nectary would form.

Assessing ant community and composition and effects of EFN

Pitfall traps were used to observe what ants were occurring within the plots. Pitfall traps were made by filling 50 mL vials halfway with soapy water. In order to deploy the pitfall trap, it was placed in a hole, level to the ground so that the ants may fall into it while walking on the ground. The pitfall traps were collected after 48 hours had passed since deployment, and all ants were counted and identified to their taxonomic group.

Baits were used in this experiment in order to determine the abundance of ants foraging within the plot, and to observe their nutritional needs throughout the experiment. The baits are meant to somewhat imitate the carbohydrates that the ants would need to forage for their colony and for themselves. Eighty 10 mL 20% sugar baits were made, labeled, and weighed initially and then weighed at end of the experiment in order to calculate how much the ants consumed and/or foraged. These baits were made by filling a 15 mL vial with the sugar water, and placing half of a cotton ball into the vial to soak the cotton in the sugar water for the ants to feed on. 20 control water baits were made and weighed the same way as the sugar baits in order to account for the evaporation factor while calculating how much the ants consumed or foraged. The baits were placed on the ground slanting downwards so as to keep the cotton ball soaked with sugar water. A water control bait was placed one meter away from either of the 4 sugar baits in the same manner as the sugar baits. All of the assays were performed in the center of the seven meter diameter area of the plot because it is assumed that the ants that live within that plot, would not go outside of the seven meter diameter in order to forage. It is possible that some ant colonies live on the perimeter of the plot, and those ants may forage outside, however, the idea is that the ants will stay within the manipulation. The sugar baits were deployed in the morning at least three times throughout the summer and checked on three to four times throughout the day. When the baits were checked, the species of ant was noted, along with the amount of ants. The time that the baits were checked was noted as well. The 20% sugar baits were set out on June 29th, 2016, July 14th, 2016, and July 28th, 2016.

The sugar baits were considered biased during analysis due to the fact that ant nutritional needs may differ due to the manipulation in the experiment. Therefore, the ants are being manipulated and the baits are favoring the ants that are more efficient at defending and allocating carbohydrate resources.

We compared the pitfall observations with the bait observations, to see if the ants that were present in the plot were actually foraging within the plot as well. Pitfall traps were weighed before and after deployment to get a sense of how much the ants were consuming, this way we could

assess their dietary needs. Eighty pitfall traps were made and labeled to assign four to each plot similarly to the baits.

Pitfall traps were placed near each corner stake of each plot, each about one meter away from the bait placed at the same corner. The pitfall traps were deployed in the morning three times throughout the summer similarly to the sugar baits. When the pitfall traps were checked, they were checked in the same manner as the sugar baits. The water control baits were deployed in the morning in the same manner as the sugar baits, however, they were not checked regularly, as they were used primarily for calculating the evaporation factor. The dates that the baits and pitfalls were set out were the same as the 20% sugar baits, June 29th, 2016, July 14th, 2016, and July 28th, 2016. Each was done throughout the whole day starting in the morning and ending the baiting in the late afternoon.

Visual surveys were done six times total noting the species and amount of ants present on a stem of a singled out *H. quinquenervis*. To do this, three corners of each plot were chosen to be surveyed, giving 60 units of data each time a round of visual surveys was done. The visual surveys were done in order to document which ants visit the EFN's in the control plots. Visual surveys were performed on July 11, 12, 13, 22, 26th of 2016. Each was done during the late morning into the afternoon.

After the last check of baits and pitfall traps of each day, the baits and pitfall traps were collected and stored in a refrigerator. During analysis, the pitfall traps and visual surveys were considered unbiased since they do not manipulate the ants in their normal lives in any way other than capturing them and leading them to their impending demise. The visual surveys and pitfall traps give estimates of ant abundance and community composition without manipulation of the ants.

The bricks mentioned in the experimental design were checked on August 4th, 2016, and photographs of the undersides of each of the bricks were taken so that the colony size could be observed and written down as either large or small.

Statistical analyses

All statistical analyses were conducted with R statistical package (v. 3.2.4) using the "car" package. The residuals from all analyses were normally distributed based upon analysis of untransformed variables.

Ant community composition was described in control plots (only) based upon both pitfall data and stem observations. In each case, all replicate observations (multiple stems and pitfall traps, multiple time points) were averaged to produce a mean for each ant species in each plot. The effect of ant taxa was tested for in each case to determine whether there was variation in ant abundance among the four taxonomic groups.

The effects of EFN removal on ant abundance was based upon data from pitfall traps and ant occupancy of bricks. Because preliminary analyses suggested all ant species responded similarly to experimental manipulations, the reported analyses were conducted on total ant

abundance across all ant species. For pitfall data, all replicate observations (multiple pitfall traps, multiple time points) were averaged to produce a mean for total ant abundance across all ant species in each plot. For data on brick occupancy, the number of occupied bricks was recorded (regardless of ant species).

The effects of EFN ant recruitment to baits was based upon data on ant occurrence at baits and bait consumption (mass loss over time). Because preliminary analyses suggested all ant species responded similarly to experimental manipulations, subsequent analyses were conducted on total ant abundance across all ant species. For data on ant counts and bait consumption, all replicate observations (multiple baits, multiple time points) were averaged to produce a mean for total ant abundance and bait consumption across all ant species in each plot.

Results

Four groups of ants were found when pitfall trapping. *Tapinoma sessile*, *Myrmicinae*, *Formica fusca*, and *Formica rufa*. There was no presence of *Camponotus* found while pitfall trapping. There was no significant difference in the abundance of the four ant taxa based upon pitfall traps (Figure 3; $F_{3, 36}=1.2356$; $P=0.31$). In contrast to pitfall trapping, the four ant taxa differed significantly in abundance on *H. quinquenervis* stems (Figure 4; $F_{3, 27}=13.612$; $P=1.341 \times 10^{-5}$), with *Formica fusca* and *Tapinoma sessile* being the dominant taxa.

There was no significant effect of EFN removal on ant abundance in the pitfall trap (Figure 5; $F_{1,18}=1.456$; $P=0.24$), and the numbers of ant colonies that nesting under bricks (Figure 6; $F_{1,9}=0.0533$, $P=0.82265$)

There was a marginally significant effect of EFN removal on ants abundance at baits (Figure 7; $F_{1,18}=3.3903$, $P=0.08212$) and a significant effect on bait consumption (Figure 8; $F_{1,19}=8.3709$, $P=0.009316$) with more ants being present and greater bait consumption with EFN removal.

Discussion

The ants that were commonly found in Maxfield Meadows research site were *Tapinoma sessile*, *Myrmicinae*, *Formica fusca*, and *Formica rufa* (Figure 3). During visual surveys on the control plots to find out which ants were foraging EFN, it was found that there were significantly more *Tapinoma sessile* and *Formica fusca* than *Formica rufa* and *Myrmicinae* foraging on EFN's (Figure 4). It was expected that *Tapinoma sessile* would be present in large numbers at the EFN's due to their presence being commonly found while walking through the meadow. However, though it was not unexpected, *Formica fusca* was not as expected to be significantly present on the Aspen sunflowers due to it not being found as commonly throughout the meadow when walking about. When ant abundance was tested for in the EFN removal plots versus the control plots, no significant results were found. The ants were not more present in the control plots more so than the EFN removal plots or vice versa. Also, when ant colonies were checked for under the bricks and the results were compared with control plots versus EFN removal plots,

no significance was found between the presences of colonies at the two different types of plots. Ants present in the EFN removal plots were found to consume significantly more 20% sugar baits than did the ants in the control plots. With all of this, ants seem to be tolerating the removal of EFN, either this is the case, or the ants are able to compensate for the reduction of carbohydrate resources provided by EFN's. EFN removal affected ant foraging preferences, however, did not affect forager or colony abundance.

Against what was expected, EFN removal did not have significant effects on ant forager or colony abundance. It was hypothesized that there would be more ant foragers (assessed by pitfall trapping) and more abundant ant colonies (assessed by inspecting bricks) in the EFN removal plots than the control plots, but neither occurred (Figure 4 and Figure 5, respectively). The found result may have occurred due to the fact that it was raining on the day that the bricks were assayed. Nevertheless, pitfall traps also failed to detect a difference in ant forager abundance, and these assays were conducted across multiple, sunny days. Perhaps there was not a change in ant colony/forager abundance when EFN's were removed because the ants may have physiologically compensated for the resource by making due with fewer carbohydrate resources. The ants may have also foraged on other carbohydrate resources other than EFN allowing them to make up for their lost resource.

Consistent with my predictions, ants recruited more heavily to the sugar baits in plots with EFN removal treatment. The fact that more ants visited the baits in the removal plots and also consumed more of the bait tells us that ants are in fact affected by this removal of EFN. While the results tell us that the ants' dietary needs are affected, they also tell us that the ants do not necessarily need the EFN. The ants may not need to forage for carbohydrate resources, however, whenever it is present, they do so for the extra carbohydrate source. Also, due to the result of ants being abundant in both the removal plots and the control plots, ants may be substituting another carbohydrate source instead of EFN. For example, they may change to tending aphids in order to collect their produced honeydew as a carbohydrate source, they may also visit other floral nectaries. Due to these possibilities, there may be community wide consequences of EFN removal that occur due to behavioral changes.

Further studies to discover more about how this ant-plant mutualism affects ants should be conducted. An experiment could be done to determine how far species or taxonomic groups of ants travel to forage on EFN. This would determine whether ants within the removal plots were able to travel outside of the plot to forage elsewhere. If certain species that are located within Gothic Colorado and in Maxfield Meadows are able to travel more than seven meters to forage, then our experiment would need to be modified in that we would need to make the plots larger to prevent the ants from foraging outside of their plot. Also, it may take more than two years for an effect on ant abundance through the removal of EFN to be observed. In order to test for this, the study must be prolonged in order to see if an effect actually occurs after longer periods. Another experiment that could be done is to offer ants other carbohydrate resources to determine if in fact the ants are actually compensating for their loss of EFN, and to see what the broader community wide consequences of EFN removal are.

Climate change has been causing the time of snowmelt to come earlier and earlier throughout the years and as a result, Aspen sunflowers inflorescence abundance has been decreasing due to frost damage (Inouye 2008). Since mutualisms have a strong influence on the structure of ecosystems, if Aspen sunflowers die out due to massive frost damage, large ecosystem consequences may occur. The ants would suffer a massive loss to their primary carbohydrate resource, the death of the Aspen sunflowers would also cause for the ending of the ants mutualism with the flower. This in turn could have effects on the populations of other predators that the ants may have kept in check through the defending of Aspen sunflowers.

Bibliography

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Figures

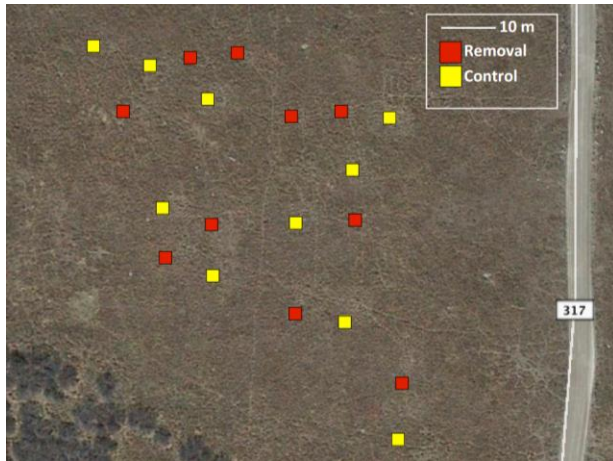


Figure 2. Map of field site in Gothic Colorado, located specifically in Maxfield meadows. The yellow squares indicate control plots, and the red indicate removal of *Helianthella quinquenervis* plots.

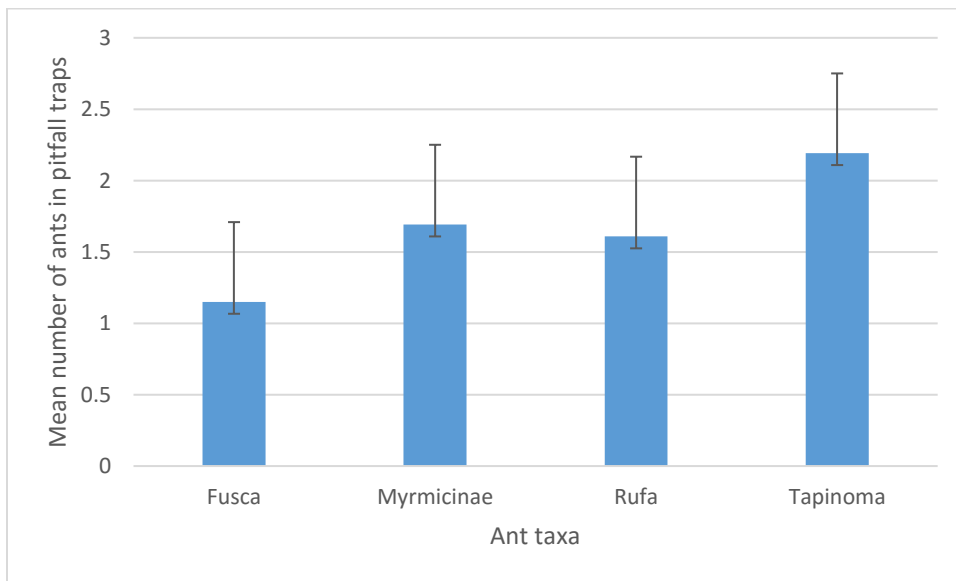


Figure 3. Shows the pitfall ant community located in Maxfield Meadows. The mean of all ants found throughout all of the pitfall dates at all plots are shown. The error bars are SEM and no significance was found between any of the data shown.

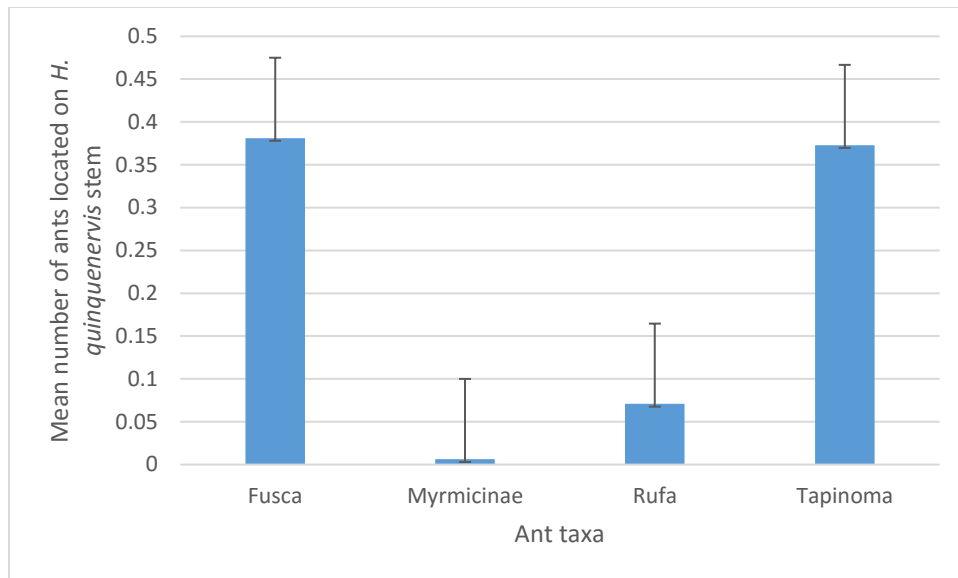


Figure 4. Shows the ant community within the control plots by showing the mean number of species of ants present on the stems, inflorescences and leaves of *H. quinquenervis* throughout all visual surveys. The error bars were SEM and strong significance was found for the difference of abundances of ants.

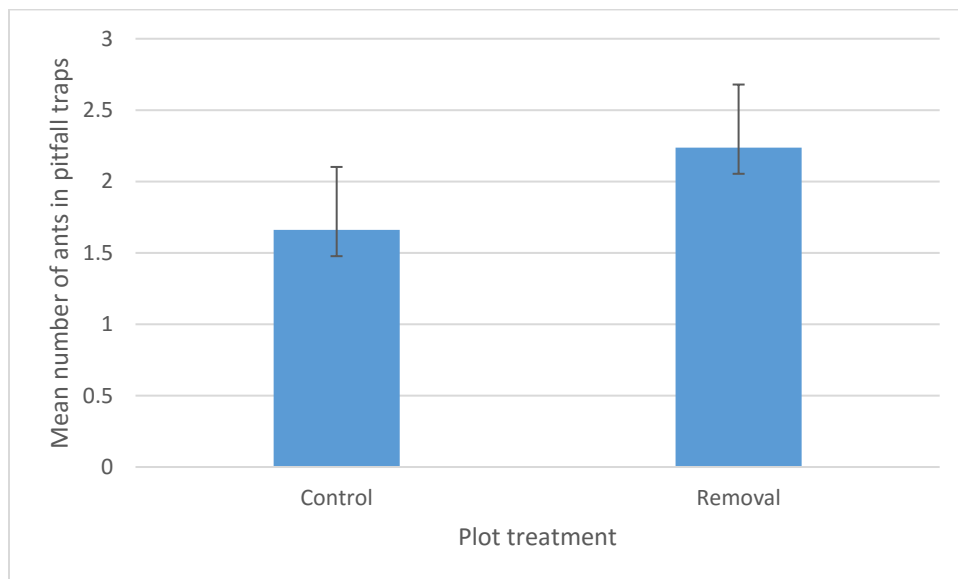


Figure 5. Shows the means number of ants found in pitfall traps in the control plots versus the EFN removal plots throughout all data collection dates. The error bars were SEM and no significance was found between control and EFN removal plots.

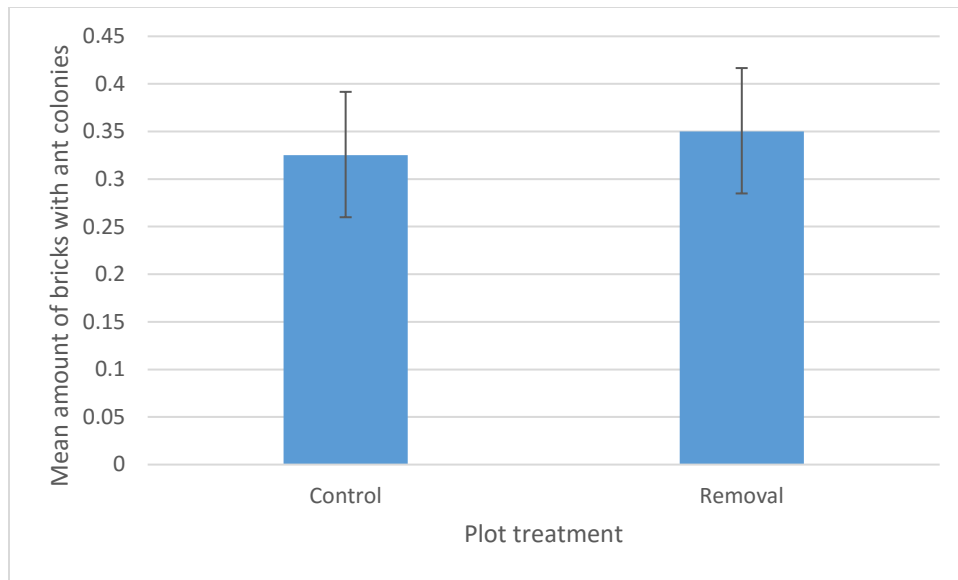


Figure 6. Shows brick occupancy by displaying the mean number of colonies that were under the control plot bricks, versus the EFN removal plot bricks. The error bars were SEM and no significance was found between the control and EFN removal plots.

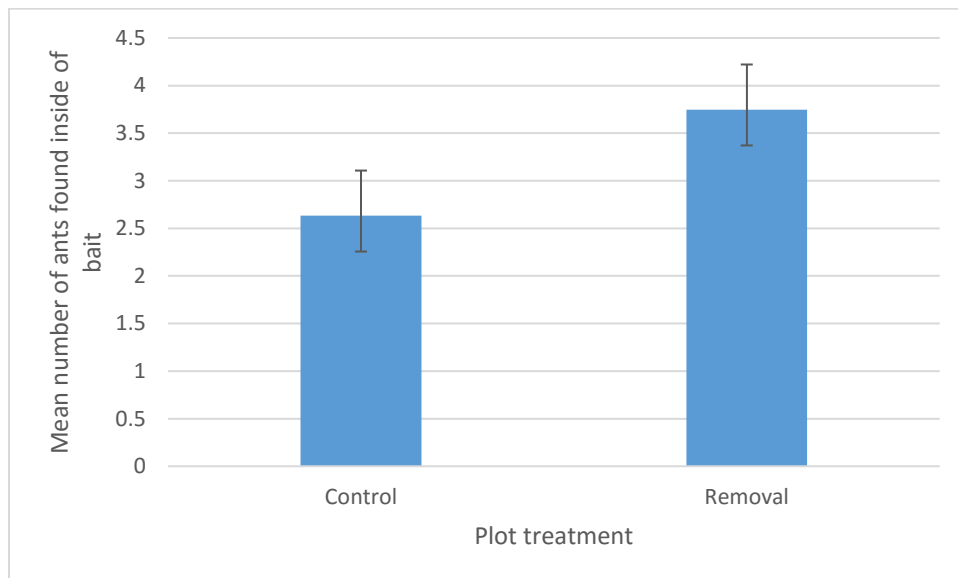


Figure 7. Shows the bait counts by displaying the mean number of ants visiting baits at control plots versus EFN removal plots. The error bars were SEM and no significance was found statistically between the data.

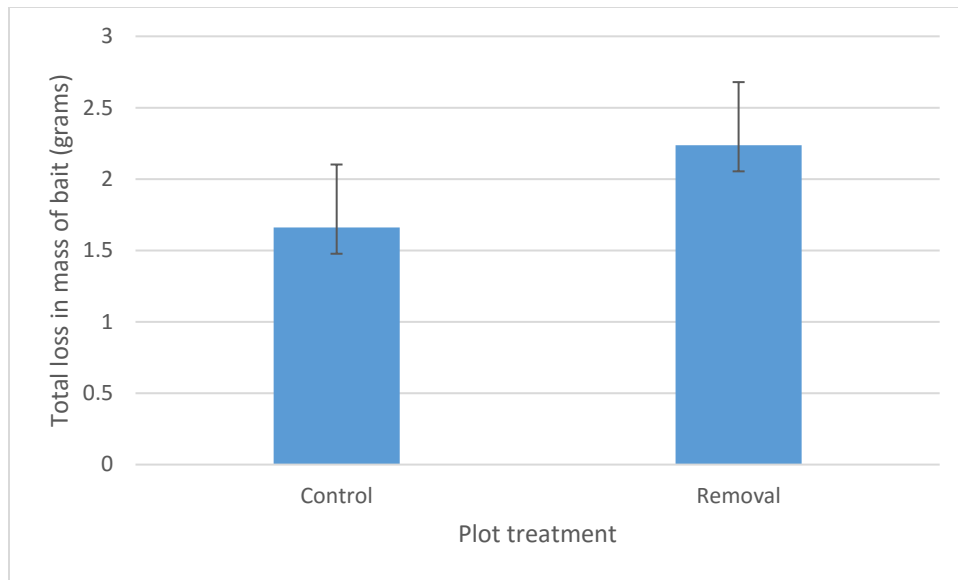


Figure 8. Shows baits consumption by the ants in the study by showing the means of how much of the sugar baits ants consumed at the control plots versus the removal plots. The error bars are SEM and strong significance was found between the control plot data and EFN removal plots data.