

Reciprocal Effects Among the ant *Formica obscuripes* and the Asters *Chrysothamnus*
viscidiflorus and *Artemisia tridentata*

Mitchell Lopez

Josh Grinath, Ian Billick

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Rocky Mountain Biological Laboratory, SUR

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Mentor: Josh Grinath, Ian Billick,

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Abstract: Ants play a large role within the community in which they live in and can affect many different organisms that they come into contact with. Through behavioral traits, ants have a large impact on the local vegetation, as well as there is a reciprocal effect in that many ants are dependent on the vegetation as a resource for survival. In order to discover the effects of ants on the local vegetation as well as contributions to soil nutrient, seeds of *Chrysothamnus viscidiflorus* and *Artemisia tridentata* were grown in soils near and far distances from ant nests. The condition of the plant would be determined by weight, biomass and size. To determine if there was a lack of nutrients that is significant, fertilizer was added to one set of soils and not to the other. Unfortunately the seeds did not germinate so no data was able to be gathered. Additionally an observational study was conducted to look at the trend of vegetation density at 3 distances away from ant nests of *Chrysothamnus viscidiflorus*, *Artemisia tridentata* and *Symphoricarpos oreophilus*. These densities were taken from nests that were at most 5 years old. This was determined from a survey that was taken in 2004 and compared to a survey taken in 2009. This study suggests that ant nests are located in areas that have *Chrysothamnus viscidiflorus* and *Artemisia tridentata* nearby. It was found that there is a significant higher density of *Chrysothamnus viscidiflorus* and *Artemisia tridentata* near nests than in areas that were chosen at random that were further than 5m away from nests. Further studies could determine the mechanism behind this observation. Through a better understanding of how ants affect a community we can begin to understand what factors shape that community.

Introduction: The arthropods and vegetations of an area represent large factors that define an area as a distinct ecological community. These species influence the evolution of each other, and the community composition will ultimately impact the success of the individuals of each species. For example, ants have a strong impact on plants through herbivory, seed dispersal, foraging, and soil nutrient content. These factors have a large impact on the vegetation of an

area. Equally so, the local vegetation can determine the success of an ant colony by providing resources and protection. By studying these interactions, one can begin to understand the niche that each species plays within the ecological context of the system. Through a better understanding of the relations that ants and plants have on each other we can learn how a community has become what it is today.

Ecologists have demonstrated that ants affect plant populations through herbivory, myrmecochory, and changes in soil properties (Beattie *et al.* 1977). For instance, the chemical composition of the soil within and directly surrounding an ant colony can be much different than soil further away from the colony (Culver and Beattie 1983). Culver and Beattie (1983) found significantly higher levels of potassium and phosphorus in soils close in proximity to ant nests, but micronutrients like Fe, Zn, and Cu had higher concentrations further away from the colonies. The authors reasoned that the high levels of potassium and phosphorus can be explained by the foraging behaviors of the ant species, whereby *Formica* species collect fecal and vegetative debris to build their nests, causing these materials to accumulate near nests (Culver and Beattie, 1983). How this behavior impacts the vegetation in the surrounding area is poorly understood and needs further investigation.

Ants have an impact on many of the arthropods within their local community. For instance, a mutualism exists between hemipteran species and ants, where the hemipterans produce sweet excrement called “honeydew” that the ants feed on in exchange for protection. Grinath *et al.* (In prep) have shown that the ant, *Formica obscuripes*, has both positive and negative indirect effects on the host plants of hemipteran honeydew producers. For example, the survival of *Publilia modesta*, an abundant honeydew-producer, is increased by the presence of *Formica obscuripes*, and this has a negative effect on plant reproduction (Grinath *et al.* In prep).

Conversely, ants can deter other herbivores that cause leaf damage, such as the beetle *Monoxia schyzonycha*, which causes less plant damage in the presence of ants (Grinath *et al.* In prep). In the Boreal Forest, ants involved in this mutualism impact the organic matter levels on the forest floor (Jurgensen, 2008). These influences are due to soil turning and litter removal behaviors, which will cause higher levels of nutrients available to plants in the presence of ants in this ecosystem (Jurgensen, 2008). The indirect effects of ants on plants via herbivores has been well documented, but the direct effect of ants on the plants that host honeydew-producing hemipterans through soil nutrient alterations is still unclear.

Studies have shown that ants do have a significant effect on trees that host ant/membracid mutualism through the availability of water and nutrients, in that there is a higher fine root biomass near the nests than in other mineral soil (Jurgensen *et al.*, 2008). As well it was shown that conifer trees had a reduction in above ground growth when *Formica rufa* was present (Jurgensen *et al.*, 2008) due to the ant/hemipteran mutualism. This higher biomass in fine root material is hypothesized to be because there is a higher concentration of nutrients and water available for trees. The vegetation studies relating to ants found that there was more homogeneity in the number of species for mound areas, which was hypothesized to be a result of fewer plants that were able to withstand the effects of the mound (Culver and Beattie, 1983).

There are also many reciprocal effects that plants have on ant. In general, ants have been known to nest in cavities in bark, roots of epiphytes, and constructed or natural cavities in stems and branches (Hölldobler and Wilson, 1990). The plant species in the mating ground of ant might have an effect on the location of new ant nests. *Formica obscuripes* has been observed to perch on top of shrubs and wait for a mate. This plant that the queen perches upon might be the location of a new ant nest. If there is a relation between new colony location and proximity to

shrubs, then vegetation could potentially predict the location of new colonies. Ants use plants and vegetation for shade, thermoregulation and protection, but is this a consideration to the new queen when choosing a site for her nest? By looking at the location of new sites and the sites that have survived and relating this to the plant density I can interpret if this is a factor that can help predict new ant nest location.

This study investigated the effects of ants on the nutrients available for *Chrysothamnus viscidiflorus* and *Artemisia tridentata* growth, and the reciprocal effect of the vegetation on the location of new ant nests. Do ants have a considerable effect on soil nutrients that has an impact on the surrounding vegetation? As well, is there a significant relationship between the density of *Chrysothamnus viscidiflorus* and *Artemisia tridentata* and new ant nests. The soil nutrient content will be addressed by germinating *Chrysothamnus viscidiflorus* and *Artemisia tridentata* seeds in soil that is both near and far from ant nest. If there is a deficiency in nitrogen, potassium, or phosphorus, the seedlings will show positive growth responses to fertilizers that were added, in comparison to control groups that have no added nutrients. Additionally a survey was conducted for vegetation densities as a means to show how vegetation affects new ant colony location.

Methods and Materials:

Experimental Site and Species

The experiment took place in Gothic, CO, which has an elevation of 2896m and is approximately 35km north of Almont, where the soil and seeds were gathered. The location of

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where the plants were grown was seen as irrelevant because all plants had the same environmental factors and conditions. This experiment aims to show the ability of different soils to support seedlings, not the environmental factors of Almont. Almont is a sagebrush community at 2700m elevation that is subject to snow and has an annual precipitation level of 23 in/yr (Terra Fly Point Data).

Chrysothamnus viscidiflorus and *Artemisia tridentata* are dominant plant species that are present in dry areas across the Western United States. These plants support ant/membracid mutualisms in most environments that both ants and membracids are present in. These plants were chosen because they represent important and wide spread species of plant, and information on these species could possibly be applied to many regions in the Western United States. As well *Formica* species can be found in most every state in the US, and in countries in Europe, which makes this study applicable to many other systems.

Both *Chrysothamnus viscidiflorus* and *Artemisia tridentata* were grown in small wax paper cups. Both species of plants were grown from seeds collected in 2008 from the Almont Triangle Wildlife Management Area, CO, ensuring that the seasonal and environmental factors that affected seed production are the same for both species. All soil samples were collected in the Almont site,

Ant Effect on Plants

Both plant species were grown in soils collected at three distances from ant colonies. The seeds were gathered from Almont, from similar area that the soil was obtained. Soil was collected at 0m, 2m, and 5m away from ant colonies and the soil samples was divided between a nutrient addition group (n) in one container and a control group (c) that had no added nutrients in

another container. I expected that 0-2m will represent the area that the ants have the greatest impact and at 5m the ants will have a minimal affect on plant growth and soil chemistry. This is because the effect of ant foraging behavior will be less further from an ant nest. If this behavior of foraging and discarding material near the nests positively contributes to the soil nutrient levels, then seedlings further from ant colonies would grow less than the seedlings of a closer proximity.

Both *Chrysothamnus viscidiflorus* and *Artemisia tridentata* were grown side by side in a small container. This container was randomly assigned to one of the 3 distances, as well as having nutrients or not. All seedlings were grown in the same greenhouse and there were 20 replicates of each treatment. The location of each container was randomized and containers were redistributed systematically to reduce experimental bias. Each replicate soil group came from 20 different ant mounds from Almont and was kept separate according to each mound. The containers were split in two by a cardboard divider, which had *Artemisia tridentata* on one side and *Chrysothamnus viscidiflorus* on the other side. The soil was sifted to remove all organic plant matter, seeds, and rocks. Both species of plants were grown from seeds collected in 2008 from the Almont Triangle Wildlife Management Area, CO, ensuring that the seasonal and environmental factors that affected seed production are the same for both species.

Through this study I attempted show if *Formica obscuripes* has multiple effects on plant species, specifically those that host ant/membracid mutualism. In all, I intended to grow a total of 120 seedlings for each plant species. Along with the soil sample set, I attempted to germinate *Chrysothamnus viscidiflorus* seeds in moist filter paper to check seed viability, there was not enough seeds for *Artemisia* to do this experiment. I intended to measure the biomass of the plant, and leaf, stem and root size as an indication of plant performance.

Plant Density Effect on New Ant Nest Location

To observe the affects of vegetation on ant nests, GPS points were taken of the ant nest within a 250x60 m area and compared to an existing map that was created in 2004 in the same area. The new nests were compared to the old nest and the vegetation density within a 1x1 m area was estimated at 0, 2, and 5 m away from the new nest. Each transect was always taken due north of the nest or random point. 20 new nests and 20 random points that are at least 5 m from an ant nest were measured for density of *Chrysothamnus viscidiflorus*, *Artemisia tridentata*, *Symphoricarpos oreophilus*. The random points were generated by Excel, were an (X,Y) coordinate was created for the 20 random points. Another survey was performed on ant nests were the vegetation density was measured in an area north of the Upper Meadow, where the random points and new nest data was taken, called the Lower Meadow. The same measuring techniques used in the Upper Meadow as were used to determine the percent cover of *Artemisia tridentata* and *Chrysothamnus viscidiflorus*.

I used an ANOVA, Wilcoxon, and Tukey test to find significant differences and relationship between data sets. The Tukey test was to determine which distance was significantly different that the other distances. The ANOVA was used to determine the significant relationship between distances. Wilcoxon test was used to determine the relationship between new nest and random sites.

Results

Ant Effects on Soil: The soil was watered for two weeks and there was no germination during this time. The seeds that I attempted to germinate in the filter paper did not germinate either.

Another researcher was also growing *Chrysothamnus viscidiflorus*, and she was able to germinate seeds in potting soil, though the success rate was low. Within these two weeks there was no incidence or accident that happened that I can determine that would affect the germination of the seeds.

Plant Density Effect on New Ant Nest Location: The correlations between plant cover and sites, Nests or Random, were different between plant species. This is supported by a Wilcoxon test, which showed that the density of *Chrysothamnus viscidiflorus* in the Nest sites, at 0m, are significantly greater than it is in the random sites at 0m ($Z=-3.77$, $P=0.002$, Figure 1). There was also a significant difference between the Random sites and the Nest sites for *Artemisia tridentata* at 5m, with *Artemisia tridentata* having a greater density at new nest sites ($Z= -1.72$, $P= .004$, Wilcoxon), and a marginally significant difference for the 2m ($Z= -1.72$, $P=0.0842$, Wilcoxon). There was no other significant difference between the Nested / Random sites for *Symphoricarpos oreophilus* at any distance using a Wilcoxon test.

There was a considerable relationship between *Chrysothamnus viscidiflorus* and distance from new ant colonies using an ANOVA ($F=3.96$, $P=0.0247$, Figure 2), and it was determined that 0m from ant nests is significantly different than 5m distance from ant nests, in that there was a higher amount of *Chrysothamnus viscidiflorus* near the nest sites. There was no correlation between *Chrysothamnus viscidiflorus* and distance for any of the random sites, though there was a significance between *Artemisia tridentata* and distance for the random site at 2m ($Z=3.23$, $P=.0485$, Figure 2).

For the Lower Meadow there was significantly greater coverage was found near the ant mounds for *Chrysothamnus viscidiflorus* ($t= -2.175$, $P= 0.0338$, Figure 3), whereas no such

relationship was observed for *Artemisia tridentata* ($t = 0.121$, $P = 0.904$). A Wilcoxon non-parametric test shows that there is a significant difference between the vegetation densities of *Artemisia tridentata* at the 2 different sites. Because of this difference the sites cannot be compiled together and compared as a group.

Figure 1. Comparisons of the mean percent cover of Nest sites to Random of *Chrysothamnus viscidiflorus*, *Artemisia tridentata*, *Symphoricarpos oreophilus* at three distances. Standard Error bars included. * Denotes the significant difference between the percent cover and Sites were $P < .01$.

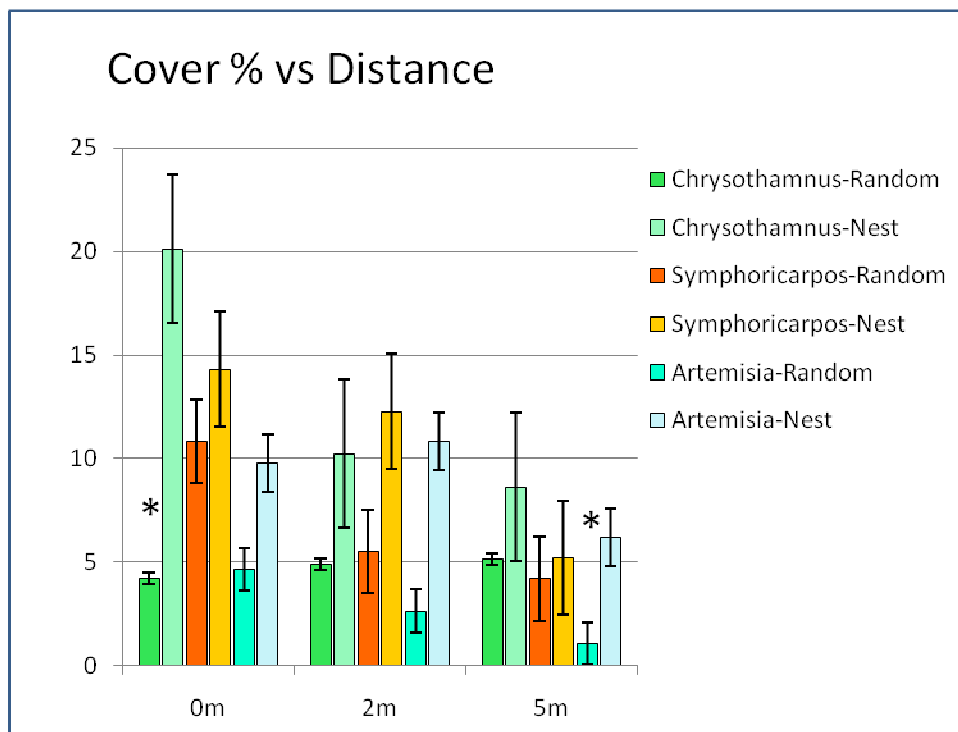
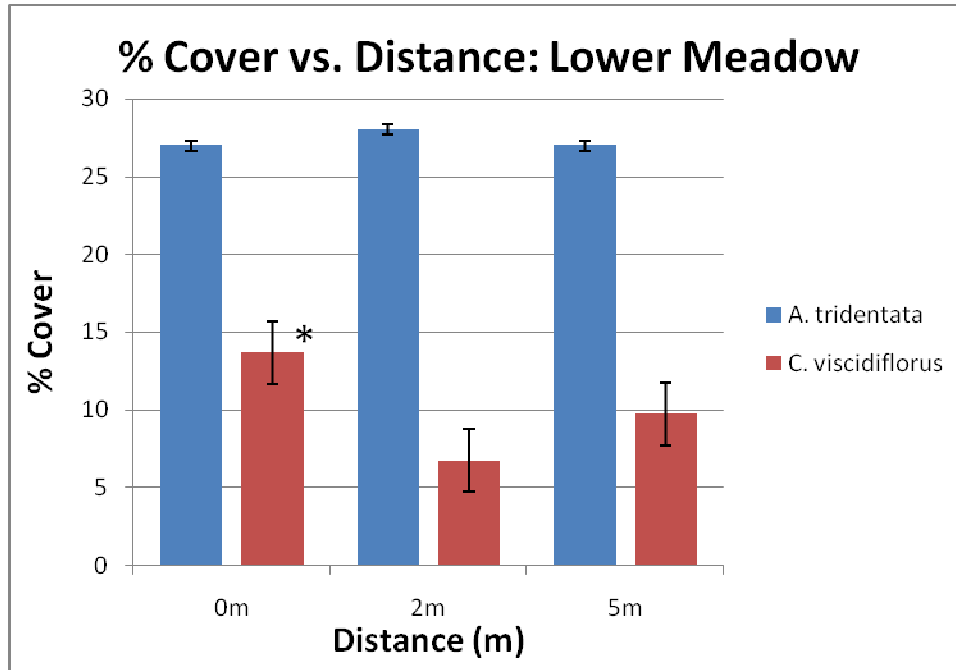


Figure 2. Comparison of Mean Vegetation Density of *Chrysothamnus viscidiflorus* and *Artemisia tridentata* for the Lower Meadow. * Denotes the significant relationship between Distance and Percent Cover were $P < 0.05$.



Discussion

Ant Effects on Soil : The affect of ants and the soil nutrient content of this area are still undetermined because there was no germination of either of the experimental plant species. Possible reasons that the seeds did not germinate was that I was unable to mimic the environmental conditions needed for the seeds to activate and undergo respiration. Additionally the seed set may have not been viable for germination. Further study of the nutrient content of the soil is needed. Soil core sampling of the nests as well as under the studied plant species would be helpful in determining the nutrient levels. In addition to determining the nutrient composition, a microbial and fungal analysis would be useful to see if there is a difference in the presence and density of nutrient fixing bacteria and mycorrhiza.

Plant Density Effect on New Ant Nest Location: The observational data of this project suggests that there is a significant difference in vegetation between random sites and sites that are near ant nests. As shown in Figure 1, we can see that there is a significant difference between the vegetation densities of *Chrysothamnus viscidiflorus*, and *Artemisia tridentata* in the nested areas than in areas that are farther away from nests (at least 5m). Previous studies have found that there is more homogeneity of species within a close proximity of ant nests. The vegetation of an area and its relation to ant nests is contextual, some species in a given area are better adapted to the conditions that occur around ant nests, and some are not. Due to disturbances and soil heaping activities near ant nests, the fitness and diversity of plants can be negatively affected.

Through this study we have seen that there is a significant relationship between distance from ant nests and *Chrysothamnus viscidiflorus*. Within 1m of an ant nest, there is a higher probability that there will be a higher density of *Chrysothamnus viscidiflorus* than 5m away in the lower meadow, in addition to there being a higher density near new nests than in random areas further than 5m away in the upper meadow. This suggests a trend within Almont, though the densities of *Chrysothamnus viscidiflorus* are slightly different between the Upper and Lower Meadows and the sites are too few to draw strong inferences.

There is a considerable difference in the density of *Artemisia tridentata* between the random areas and the nest sites at 5m, in that there is a higher density in the areas that are near nests than areas that are randomly dispersed. Although there was no significance difference between vegetation density and distances for the ant nest areas. Perhaps *Artemisia tridentata* is not a crucial resource for new ant colonies. Furthermore, *Artemisia tridentata* could possibly be well adapted to the disturbances of ant nest and encroach upon the ant nest.

The original alternate hypothesis was that there would be a significantly higher vegetation density of *Symphoricarpos oreophilus* near ant nests, but this study shows that there was no significant difference in vegetation density among site locations. Additionally there was no significant difference in vegetation density and distance from ant nests or random locations. From this we have determined that there is no relationship between the densities of *Symphoricarpos oreophilus* and new ant nest location.

The mechanism of how the presence of *Chrysothamnus viscidiflorus* affects the location of the ant nest also needs more investigation. Mechanisms to be considered would be if young queen ants chose areas that have a higher density of *Chrysothamnus viscidiflorus*. There might be a higher survivorship of young ant nest that are closer to the aphids and membracids that live on *Chrysothamnus viscidiflorus* and protection that the plant offers. It has been observed that queen ants will fly to the top of shrubs and forbs and signal males through pheromones. After mating, the queen ant might use this plant as the place of her new nest location. It is also possible that the disturbance of ant mounds regulates what plants are adapted to withstand these disturbances. *Chrysothamnus viscidiflorus* may be adapted to withstand these effects of ant nests and thus would have a better abundance and density than those species that are unable to withstand these disturbances.

The presence of ants has a large affect on the abundance and diversity of both arthropod and plant communities. There is an effect on arthropods through predation as well as mutualism. Ants impact the vegetation indirectly through ant/membracid mutualism, but also directly affect it through nutrients levels and, soil turning, and herbivory. The effects of ants can explain aspects of community structure like the population abundance of plant and arthropod species, which can affect the larger vertebrate species that rely on either plants or arthropods as a resource. By

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studying this super-organism we can gain an understanding of community dynamics and interrelations of different ecological systems across the earth.

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