The Effect of Predation on Ant-aphid Mutualism in *Ligusticum porteri*

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Abstract

Predation and mutualism take up a large part of interactions within communities. These multi-trophic level interactions affect the makeup of the community. We explored how predation impacts the strength of mutualism between ants and aphids on the plant *Ligusticum porteri*. It has been a base assumption that ants mediate the effect of predation on aphids. We manipulated half of the plants by adding lygus bugs and measured ant density of each site. Through this study, it was discovered the presence of predators broke down the mutualism and disrupted it from forming ($P < 0.0056$). Ant density had no correlation to the efficiency of the mutualism forming or the strength of it ($P = 0.360$). Larger aphid colonies did attract more ants, and the larger the number of ants resulted in greater services for the aphids ($P < 0.0056$). Higher ant abundance resulted in a greater growth rate for aphid colonies. The predation impacts the mutualism potentially affecting aphid, ant and lygus abundance, as well as revealing how ants mediate the effect of predation.

Introduction

Top-down processes affect herbivore survival and their performance (Wimp and Whithman, 2001). Top-down studies have shown the effect of predators can influence multi-trophic levels, such as herbivore abundance, plant growth, and plant reproduction (Moran et al., 1996). It is important to understand the top-down and bottom-up processes in trophic level interactions in order to grasp how the community is affected directly and indirectly (Wimp and Whitman, 2001). Predators have the potential to extirpate aphid population, therefore mutualism between ants and aphids can be crucial for survival. Ants perform a service by protection aphids from predators in return for honeydew (Ness et al., 2010). Mutualism allows both species in the interaction to have positive benefits and potentially expanding their distribution. In this case, mutualism is important for the survival, protection and reproduction of aphids. For ant's honeydew is a valuable nutrient resource that contributes to their survival (Ness et al., 2010).

Predators have the potential to alter this mutualism by changing ant and aphid behaviors (Mooney, 2006). An example of predation affecting the mutualism revealed birds reduced the abundance of ants as well as the quality of services they performed (Mooney, 2006). The birds had more of an effect on the aphids with a mutualism than the aphids without a mutualism. Other studies have also shown aphid populations can be affected positively and negatively by organisms of higher trophic levels (Wimp and Whitham, 2001). The performance of aphids would vary with the absence of predators and ants. Therefore, aphid's realized abundance is altered by their mutualism with ants as well as the intensity of predation. The strength of the mutualism is influenced by the abundance and behaviors of other species (Boucher et al. 1982; Stanton, 2003). These species can alter the mutualism until it is no longer a mutualism, but a different relationship such as commensalism or a complete breakdown (Bronstein and Barbosa, 2002).

In this system, there are a variety of trophic levels interacting and impacting abundance and behavior. These interactions take place on the host plant *Ligusticum porteri,* which has multiple flowering stocks that will go through a variety of phenological stages. As the flower progresses through these stages it will attract the aphids, as well as lygus bugs (Robinson et al., 2017). Aphids feed off the phloem of the host plant and produce a rich, sugary substance known as honeydew. This substance attracts ants, which depend on this resource and this results in the mutualism being formed. There are also lygus bugs, which are intraguild predators. Not only do they feed off of aphids, but they will compete with them to feed off of the host plant. The lygus bugs further complicate these trophic interactions by being a predator, as well as a competitor. They are directly impacting the mutualism in a variety of ways, the health of the plant, and potentially causing a trophic cascade. Intraguild predators' interactions are poorly understood and further complicate how they impact the makeup of the community (Moran et al., 1996).

The changes of aphid abundance could be a result of host plant phenological changes from different snowmelt periods as well as predator abundance (Robinson et al., 2017). Over the past nine years high snowpack data has correlated to high aphid abundance, while low snowpack data has correlated to high lygus bug abundance (Mooney et al., In Press). This pattern could be caused by a variety of factors. This study in particular will determine if lygus bugs are able to take advantage of this phenological mistiming between the host plant, aphids, and ants. It will focus on the mutualism between ants and aphids and how it may potentially be mediating the extent of the effect of lygus bugs predation.

The purpose of this study is to observe predation and its effect on ant-aphid mutualism on *Ligusticum porteri*. It is also attempting to understand this pattern of high lygus bug abundance and low aphid abundance during certain snowpack years. We will examine how the increased abundance of predators alters the abundance of ants and aphids. We will also look at behavioral changes of aphids and ants in the presence and absence of predators, such as predator avoidance. Observing the effects of predators on this mutualism can help explain the structure of the multi-trophic level community. It could also explain the direct and indirect effects aphids have on the surrounding biodiversity. By observing this ant-aphid mutualism at different densities of ant mounds, along with the added pressure of predation, the varying degrees of the strength of this mutualism can be observed.

Method

Observational Study:

For the observational study I used ten existing long-term monitoring sites located at the research meadow and near the Gothic town site. These sites are used to continue the long-term research of snowpack data, lygus bug, and aphid abundance. At each site along a 30 meter transect, ten host plants were identified. The host plants were picked randomly by using a random number generator and selecting the closest plants to those points. Data of aphid, ant, lygus bug, and other insect abundance on the host plants was collected every week for 6 weeks. I also observed six other locations surrounding the Gothic town site (Map 1.). Ten host plants were selected with the same method along a 20 meter transect. The overall density of ant mounds at each site was also recorded. A 6-meter radius was placed at the farthest end point plants. In this circle, the amount of active ant mounds were counted and recorded.

The weekly data collection at each site allowed for aphid and ant abundance to be tracked over time. Each host plant was nested within a site, with site as a random effect. The ant mound density was the independent variable (X). I tested how this affected observed aphid (Y1) and ant abundance (Y2) at each site. For the subset of host plants with aphid colonies, I tested how ant tending varied with ant density. The relationship between the number of aphids and ants' measures levels of ant tending (Mooney and Agrawal, 2008). I calculated how ant tending varied using a general linear model with number of ants as the dependent variable (Y) and aphid colony size (X1) and ant mound density (X2) as the independent variables. It was performed through a statistical analysis with an R program.

Experimental study:

For the experimental study, I examined how lygus bug predators affected the ant-aphid mutualism. The independent variables were the varying density of ant mounds and the addition of lygus bugs to each host plant. The dependent variables were (1) aphid abundance, ant abundance, and lygus bug abundance and (2) ant behavior.

I measured experimental data by looking at the plants at the six different locations, three low density and three high density. Plants were selected randomly, with the same method of the observational study. For each plant, an aphid colony was established. This was done by gently transferring approximately 10 aphids by using a paint brush to each host plant from other aphid colonies. Then a mesh bag was placed around the plant and tied off with a twist tie to protect it from flying predators. In addition, tanglefoot was also applied to the stem to stop predators from crawling up. Two days later the sites were revisited. A census of aphid, ant, and lygus bug abundance was taken. Ant bridges were also established to introduce the ants to the aphids. The tanglefoot was removed and an approximately 3cm long straw was taped to the location of where the twist tie was tied. The mesh bad was placed around the straw and stem and tied off. Three days later another census was conducted. Lygus bugs were also added to half of the host plants. The experimental plants were determined randomly through a coin flip. Lygus bugs were collected from the surrounding plants using a sweep net. Five lygus bugs were placed in mesh bags and added to each experimental plant. Experimental data of ants, aphids, and lygus bug abundance were collected approximately every three days.

I observed changes in ant behavior in the presence and absence of predators. I observed ant behavior by conducting focal observation (1 min each) of ants on control and lygus addition colonies. Behavioral observations were broken down into three categories: (1) aphid tending, (2) predator avoidance, (3) protection. Each category had two behaviors (1) contact with aphids: any physical contact between aphids and ants, and tending to aphids: ants stroking aphids with their antennae, (2) ant jumping: avoiding predators by jumping off the host plant, and fleeing: ants avoiding the predator by running down along the stem, (3) aggression: any attempt to locate the predator, and protecting the aphids: a physical attack of the ant on the predator.

The effect of ant tending on lygus bug predation of aphid colonies was analyzed using a general linear model. It was performed through a statistical analysis with an R program. I performed separate analyses for each dependent variable. For example, how a variable (ant behavior, Y1) is affected by density of ant mound (X1) which varies by how much lygus bugs added (X2). Significant interactions would indicate that the effect of lygus bug addition on a dependent variable would be mediated by the density of ants at a particular site.

Results

Ant density for each location did not vary significantly ($P = 0.360$). Ant foraging was low, with most sites below 20 ants, with the exception of one site ($N=73$) ($P = 0.0806$). For the observational study, the high snowpack year was consistent with a high aphid abundance (Figure.6). In the experimental study, the aphid colonies changed over the census period, either growing exponentially or went extinct ($P < 0.001$). It was also revealed lygus bug addition affected how these colonies changed over time ($P < 0.001$). The addition of lygus bugs had a negative effect on aphid abundance. The treatment of lygus bug also affected ant recruitment ($P < 0.0056$). The presence of lygus bugs deterred ants from forming a mutualism. The plants with no added lygus recruited more ants than the lygus treated plants. The behavioral observations displayed predation influenced the efficiency of aphid tending by ants. When there was an absence of predators all behaviors seen were tending to aphids. In the presence of predators, the actions were either predator avoidance or aggression towards predators.

Discussion

The purpose of this project was to determine the effect of predation on the ant-aphid mutualism and the strength of the mutualism. This was tested by using locations of varying ant density and adding lygus bugs to half of the total plants. Ant density within each site had little variation and did not vary significantly from site to site. Most locations were along open meadows and shaded areas. (Figure. 1). Ant density had no effect on ant recruitment, ant foraging, or colony size ($P = 0.791$). The combined effects of density, treatment, and colony size were not shown to have a significant effect ($P = 0.332$), however treatment and colony size did, suggesting a greater variation in density would show effects of ant recruitment. Ant foraging across each site was similar with the exception of site one with 73 ants foraging (Figure. 2). Colony size did not affect the number of ants foraging the surrounding areas. This suggests that ant activity is not affected by the number of ants or what they are foraging for, but rather than the environment itself. Abiotic factors, specifically light, impacts species distribution as well as influencing the strength of the mutualism (Mooney et al., 2016). Most site locations were in open meadows except for site 2 and 6 (Map. 1). These sites experienced the lowest ant recruitment and overall colony size. Although ant density did not vary significantly, the three lowest ant density locations did experience the most extinct colonies and overall smaller colony size.

Over the census period aphid colony size was impacted by time and treatment. The predation of lygus bugs decreased the abundance of aphid colonies over time (Figure. 3). Intraguild predators such as lygus bugs have demonstrated a variety of effects on the surrounding community unlike specialist predators that prey on few species (Moran et al., 1996). The control plants had a greater relative growth rate than the treated plants. The addition of lygus bugs also influenced total ant recruitment. The control plants attracted more ants than the treated plants, creating a positive top down effect by forming a mutualism (Wimp and Whitman

2001), (Figure. 5). The presence alone of predators was enough to impact the mutualism from forming. Ants may be aware that the cost of forming the mutualism with the aphids outweighs the benefit, meaning there are multiple factors in choosing an aphid colony. Not only is the presence of predators stopping ants, but as well as the health and size of the colony ($P < 0.001$). The greater the colony size, the more likely ants are to be recruited (Figure. 4)

From the behavioral observations, expected results from the ants were seen. There were two instances of ants transporting aphids to different umbels. In the absence of predators, the majority of behaviors observed were tending to aphids (Table. 1). In the presence of predators, all ant tending behaviors stopped immediately. The colonies with fewer ants fled, while the colonies with more ants protected the aphids. These ants displayed high aggression, turned their focus to the intruder, and physical fought off the predator (Table. 2). All tending behaviors stopped, which reveals the high stress of predators decreases the efficiency of aphid tending. Once the predator was removed, the ants returned to tending behaviors. The ants that immediately fled did not return during our observation period. This reveals the number of ants does play a role when fending off predators. The higher number of ants, the more resilient the mutualism. Repeated attacks of the predators could possibly break down the mutualism, especially those with fewer ants.

There are a variety of characteristics of aphid colonies that play a large role in whether or not ants will form a mutualism. Factors of aphid abundance, health, and the presence of predators were observed in this study alone. Mutualism is not a simple process, but rather an intricate one especially from the ant perspective. Predator presence and smaller colony size discouraged ants from forming the mutualism, most likely because the cost was too great. The behavioral observations revealed that once the mutualism is formed there is a threshold to the ant's capability. With fewer ants, the relationship becomes more of a commensalism. Especially in shaded areas the mutualism has been seen to shift to a commensalism (Kersh and Fonseca, 2005). The ants fled with no care for the well-being of the colony, but most likely returned when the predators left to benefit from the honeydew. It was more likely with a larger number of ants that the mutualism was stronger, but only in terms of physically protecting the ants. The mutualism varies in strength from forming the relationship to building enough commitment to stay and protect the colonies. Predation impacts this mutualism from forming and how efficient the ants can be at tending to the aphids.

This year particularly was a high snowpack year, which resulted in a higher aphid abundance and a lower lygus abundance. This study revealed lygus bug presence affected the mutualism from forming. In low snowpack years, lygus bugs are active before ants meaning they reach the host plant first. Their presence drives away the ants, allowing them to feed off of aphids and decrease the aphid's abundance, while increasing their own abundance. In this high snowpack year, more mutualisms were formed, mediating the negative effect of lygus bugs on aphid abundance. This study did not capture significantly different ant densities across the locations, which could be done in further studies. Capturing this would allow the effect of density on colony size and ant recruitment to be examined better. Although varying ant density was not captured, the three lowest ant density locations did experience the most extinct colonies and overall smaller colony size. Other studies have shown that over an 8-meter distance from ant mounds the abundance of aphids decreases (Wimp and Whitman, 2001). This suggest ant density could play a role in the strength of the mutualism in varying environments. Along with this, other abiotic factors should be measured, such as light. This study has found that a variety of factors contribute to ants forming a mutualism with aphid colonies. Predator presence deterred ants from aphid colonies. It was also found that there is a threshold to the ants' capability to protect the colonies, suggesting this relationship shifts to a commensalism.

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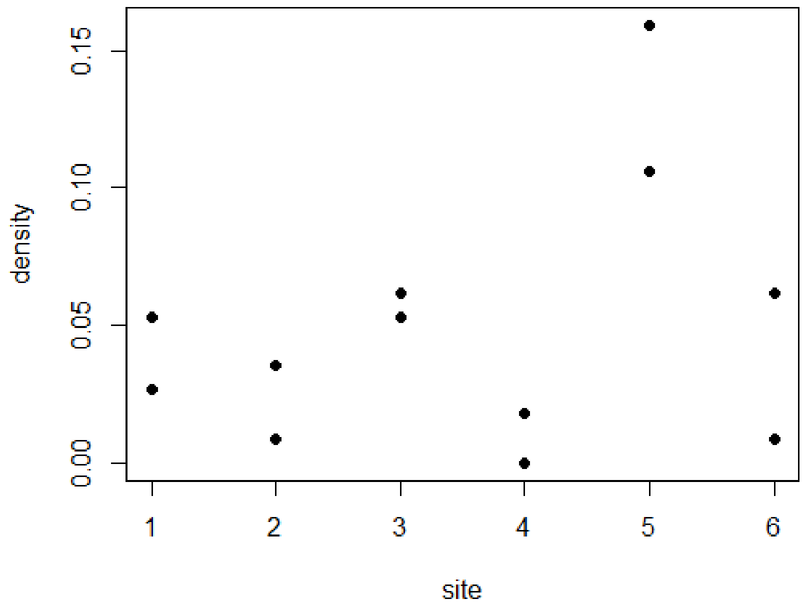


Figure 1. Ant density at end point plants for each site. At each site there is little differentiation between each plant for ant density. Low ant densities were typically at shaded areas and high at densities were found in open meadows.

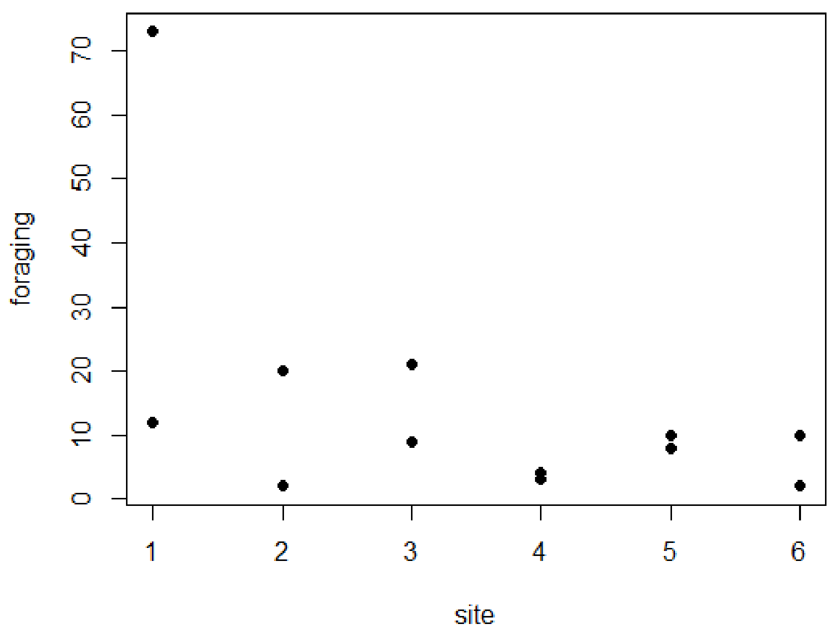


Figure 2. The foraging activity for each site location. Except for site one, with a high of 73 ants foraging, the foraging activity was relatively the same for each site.

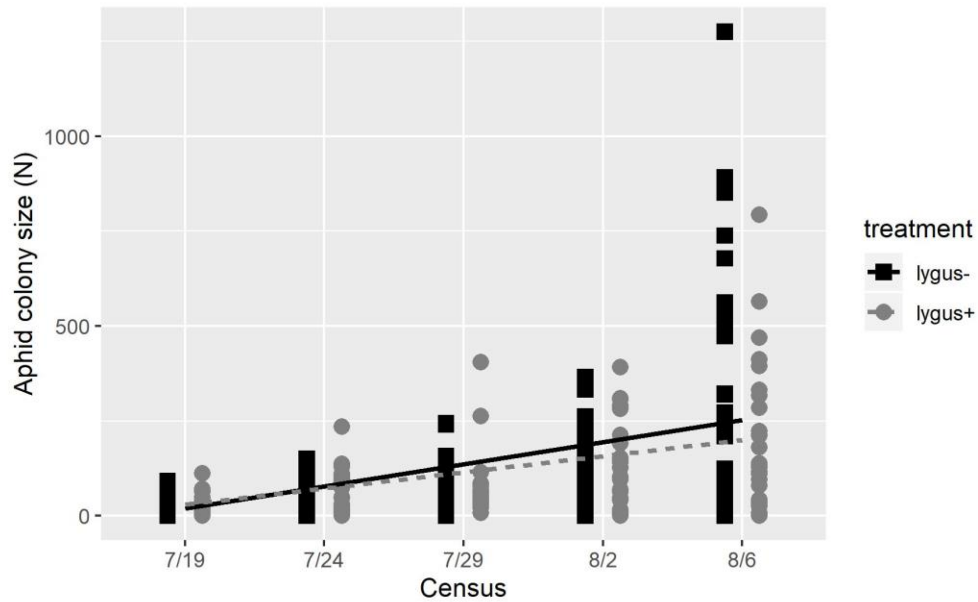


Figure 3. Aphid relative growth as a result of treatment over five census periods. The aphid colonies without added lygus bugs have larger growth rates than the plants with added lygus bugs.

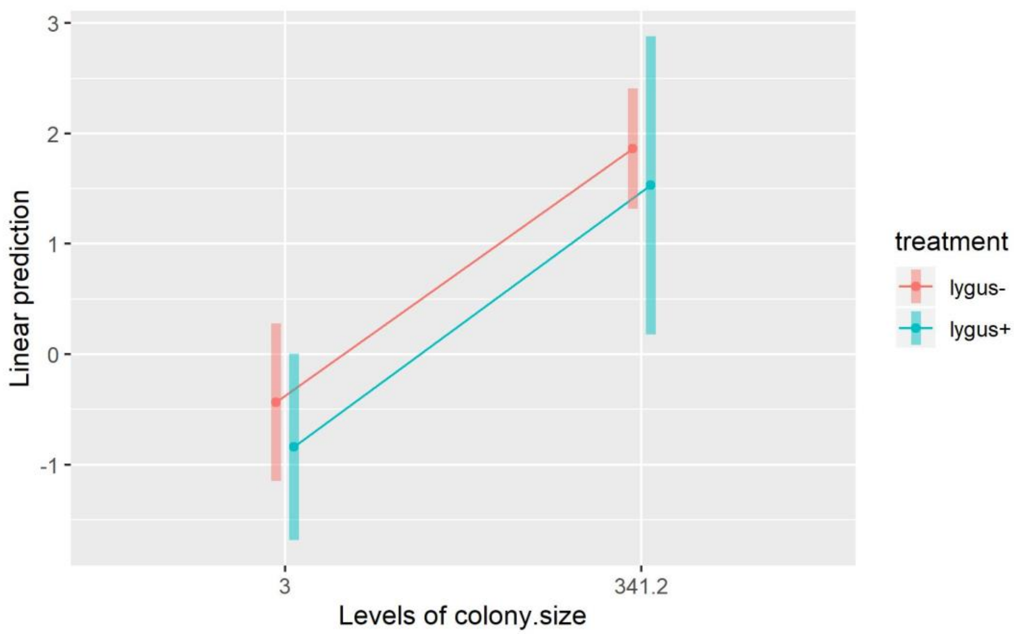


Figure 4. Linear prediction of total ant recruitment as a function of colony size. As the colony size increases, it is expected to recruitment more ants. The colonies without added lygus attract more ants than the colonies with added lygus.

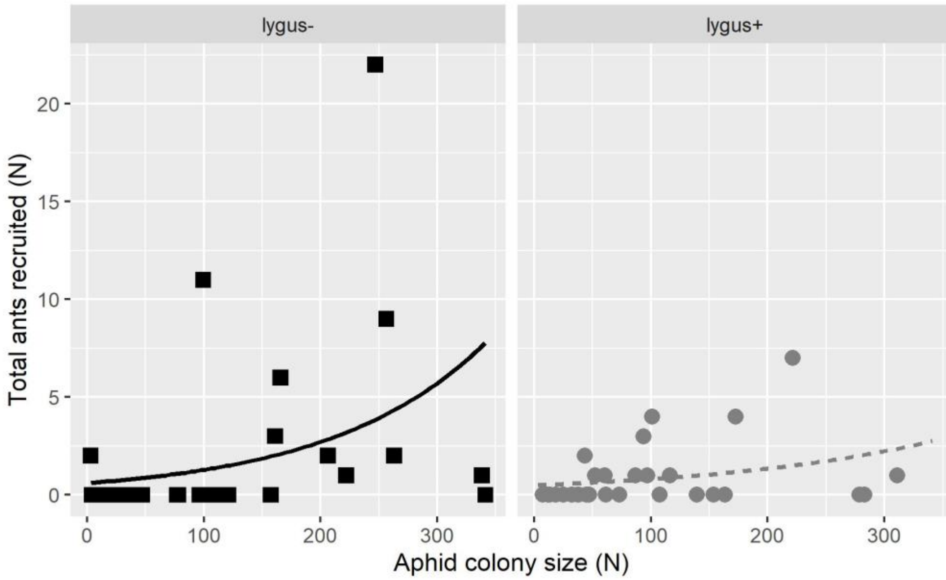


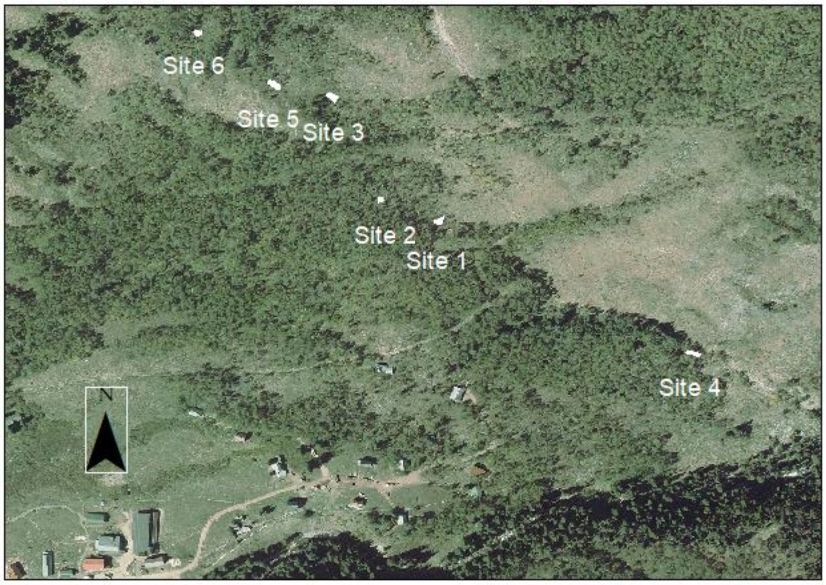
Figure 5. The number of ants recruited by control plants compared to the number of ants recruited by treated plants. Larger colonies without added predators attracted more plants.

Population	# of Ants	Ant Jumping	Fleeing	Contact with Aphids	Tending to Aphids	Aggression	Protecting Aphid
Plant 1	12	0	0	12	5	0	0
Plant 2	9	0	0	9	8	0	0
Plant 3	6	0	0	6	7	0	0
Plant 4	4	0	0	4	8	0	0
Plant 5	13	0	0	13	10	0	0
Plant 6	6	0	1	6	5	0	0

Table 1. Without Predators: When there were no predators the most abundant behaviors were tending to aphids and contact with them. There was no other behaviors observed.

Population	# of Ants	Ant Jumping	Fleeing	Contact with Aphids	Tending to Aphids	Aggression	Protecting Aphid
Plant 1	12	1	10	0	0	0	0
Plant 2	9	0	3	1	0	3	1
Plant 3	4	0	4	0	0	0	0
Plant 4	3	0	3	0	0	0	0
Plant 5	13	0	1	2	0	3	1
Plant 6	6	0	4	0	0	2	0

Table 2. With Predators: The amount of aphid tending actions decreased significantly. Only the larger groups of ants showed aggression and protected the aphids. The smaller number of ants fled and exhibited more predator avoidance behaviors.



Map 1. The Gothic town site is in the lower left-hand corner. The sites are along the Judd falls trail.

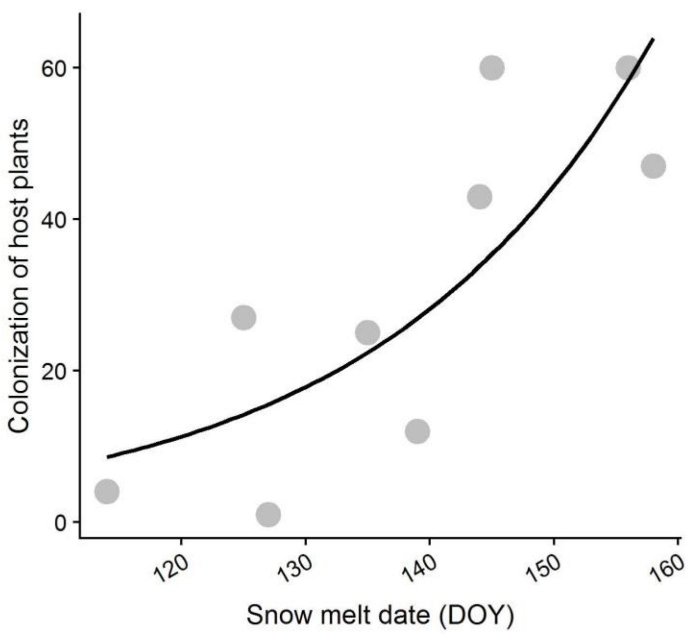


Figure 6. Aphid abundance as a factor of snow melt. As the snow melt data lasts further into the year there is a higher colonization of the host plant by aphids.