

# Does Ant Behavior in Aphid Mutualisms Vary by Distance from a Disturbed Area, Temperature and the Type of Species

## Prevalent?

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## Abstract

Interactions between organisms are essential for maintaining natural communities. Something as small as the mutualism between ants and aphids can influence a variety of plant species. Our experiment aimed to understand how ants change their behaviors in the presence of a predator in high and low traffic areas. Using a paintbrush as a simulated predator, we attacked aphid communities on milkweed plants in two sites in the University of Michigan Biological Station's UV fields. We found no significant difference for leaving, tending, assisting, and attacking when related to the presence or absence of a predator and the distance from the road. However, we did find a significant difference for ignoring behavior in ants closer to the road and those unprovoked by a predator. This may occur because if the aphid nest is not being attacked then the ant will be doing something else, often categorized as ignoring. As humans invade new habitats, we can negatively impact these relationships causing a linked cascade of extinction throughout the community. By understanding the smallest relationships and how their responses may change, we can prevent large-scale changes and hopefully help future researchers understand the role of aphid and ant interactions within the whole system.

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*Sondra Halperin*

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DISTURBED AREA, TEMPERATURE AND THE TYPE OF SPECIES PREVALENT?

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## **Abstract**

Interactions between organisms are essential for maintaining natural communities. Something as small as the mutualism between ants and aphids can influence a variety of plant species. Our experiment aimed to understand how ants change their behaviors in the presence of a predator in high and low traffic areas. Using a paintbrush as a simulated predator, we attacked aphid communities on milkweed plants in two sites in the University of Michigan Biological Station's UV fields. We found no significant difference for leaving, tending, assisting, and attacking when related to the presence or absence of a predator and the distance from the road. However, we did find a significant difference for ignoring behavior in ants closer to the road and those unprovoked by a predator. This may occur because if the aphid nest is not being attacked then the ant will be doing something else, often categorized as ignoring. As humans invade new habitats, we can negatively impact these relationships causing a linked cascade of extinction throughout the community. By understanding the smallest relationships and how their responses may change, we can prevent large-scale changes and hopefully help future researchers understand the role of aphid and ant interactions within the whole system.

## **Introduction**

The study of ecology or the way organisms interact with each other and their abiotic environment is split into multiple branches. Within behavioral ecology, one species interaction, aphid and ants, is of particular interest. In the unique mutualism, ants and aphids work together, each giving and receiving a different

service (Oeller, 2014). Aphids are a herbivorous organism, feeding on the phloem or sap in a milkweed plant (*Asclepias syriaca*) and excrete the excess sugar, amino acids, proteins, minerals, and B-vitamins in a substance called honeydew (Oeller, 2014). Ants feed off of this nutrient source by touching the back end of the aphids with their antennae, which causes the release of honeydew (Oeller, 2014). In exchange, ants consume the excess honeydew; provide shelter for aphids, and protection from natural predators (Bryson, 2000). Within this relationship, ants exhibit different behaviors in response to different types of predators. They often discourage other insects from pollinating milkweeds by protecting aphids on the plant (Levan, et al. 2015). The aggression and territoriality that ants display can also be influenced by the quality of honeydew they receive. If aphids produce more honeydew, ants will be more willing to protect them (Lescanso, et al, 2012). Another study found that when no predator is present, ants tend to approach aphids more instead of using aggressive behavior, as aggressive behavior is a costly resource of time and energy (Bryson, 2000).

Researchers are now looking at how other variables influence this mutualistic interaction. One study looked at the possible effects ant-aphid interactions have on their host plants (LeVan, et al., 2015). They found that an increase in aphid density and ant density reduced the number of visits honeybees made to each plant (LeVan, et al., 2015). This negatively affects the plant's fitness as it loses potential pollinators (LeVan, 2015). Many ants visit flowers but do not pollinate them, which reduces the fitness of the plants (LeVan, et al, 2015).

However, in some cases ants help the plant because they get rid of bad pollinators

and other herbivores which are more damaging to the plant than aphids (LeVan, et al. 2015).

As humans disperse further into new habitats, many researchers are concerned how this mutualism and other ecological interactions will be influenced. One study found that pruned plants produced more extra floral nectaries, disturbances cause new foliage to grow which have more nutrients and less chemical defenses (Piovia-Scott, 2010). The plant produces more phloem and aphids can therefore produce more honeydew (Piovia-Scott, 2010). For ants, this means more of a reward for protecting aphids but also more work to protect them as these new foliage's are vulnerable to the threat of herbivory (Piovia-Scott, 2010).

In our study, we aim to learn how disturbances such as a road, influence the protective or non-protective behaviors of ants in their interactions with aphids. We predicted that if there are aphid and ant communities on plants within 0-10 meters away from the road they would more likely show aggressive behaviors than those in the 10-20 meter section, as they are used to more predators. Furthermore, ants will attack and assist each other more in the presence of a predator in warmer areas of land, as aphids metabolisms will work faster and produce more honeydew making ants want to protect their source of food (Youngsteadt, et al., 2014).

## **Methods**

Using a meter tape we sectioned off an area of 20 x 15 meters from the road in UMBS's UV field. We then split the area into two sections 0-10 and 10-20 meters to test the effect of human disturbances on aphid-ant interactions. We collected data

on two separate days giving us four total replicates of each section. In each section, we identified 10 milkweed plants for a total of 40 plants tested.

On each plant, except for the 4 controls, we mimicked a predator by brushing a paintbrush near the aphid nest, to stimulate ant behaviors. We disturbed the aphid nest for three minutes and recorded the presence of five behaviors. We described the aggressive behaviors as attacking the predator, climbing onto the paintbrush, providing assistance, when ants from other leaves came onto the leaf we observed, and tending to the aphids instead of attacking the predator (Bryson, 2000). We also recorded non-protective behaviors such as ignoring the predator and leaving the leaf (Bryson, 2000).

At the end of the observation, using tweezers, we took two ants from each plant and stored them in small vials filled with ethanol to identify in the lab. We had ten vials containing 80 ants from both days of collecting.

We collected data on two separate days because we did not want to reuse plants that were recently disturbed by a predator. To make sure the variance in temperature did not influence our results we took ground temperatures before and after our observations. We later averaged the temperatures from both days.

We ran a two-way ANOVA to discern the relationship between the different behaviors, protective and non-protective, and the distance from the road. We ran an independent samples T-test to compare distance from the road and temperature. Lastly, we ran an independent samples T-test comparing species and distance from the road.

## **Results**

### *Presence or Absence of a Predator*

We detected no significant difference between the ant behaviors of tending, ignoring, and leaving when related to the presence or absence of a predator (Figure 1, overlap in IQRs and error bars). However, our two-way ANOVA with five conditions found a significant difference in both attacking and assisting behaviors in the presence of a predator in comparison to our controls ( $p$  value 0.000 and 0.0003 for attacking and assistance respectively; this can also be seen in Figure 2 and 3).

### *Distance From Road*

We detected no significant difference between ant behaviors of ignoring, leaving, assistance, and attacking and the distance from the road ( $p > 0.05$ ). However, we found a significant difference for tending ( $p$  value 0.029). This relationship can be further seen in Figure 4, which illustrates that more tending behavior was recorded in plants closer to the road.

### *Presence and Absence of Predator and Distance From Road*

No significant difference was found between tending, attacking, leaving, and assisting behaviors when looking at the presence and absence of a predator as well as distance from the road ( $p > 0.05$ ). There was a significant difference for ignoring behavior ( $p$  value of 0.016). Furthermore, Ants closer to the road and not threatened by a predator were more prone to exhibit an ignoring behavior (Figure 5, 6).

### *Confounding Variables*

No significant difference between species composition (Formica, Lasius, Camponotus) and site type was observed (Figure 7). Additionally, we found no significant difference between temperature and site type. Therefore it is unlikely that species composition and temperature are confounding variables.

### **Discussion**

In our lab, we hypothesized that if there were aphid ant interactions on plants within 0-10 meters from a high traffic area then the ants would demonstrate more aggressive behaviors than those 10-20 meters from the road, as they would be more accustomed to predators. We also hypothesized that aphids in warmer areas of land, areas near disturbances, would produce a higher quality of honeydew causing ants to be more protective of this resource.

However, our results do not support our hypothesis as they indicate that there was no significant difference between protective and non-protective behaviors compared to the distance from the road and the presence or absence of a predator. Nevertheless, we saw an unexpected significant difference for ignoring behavior (non-protective). We found that ants observed in Site 1, or closer to the road, exhibited the ignoring behavior more frequently. A possible explanation for the response of ignoring is that ants were acclimated to disturbances and therefore do not attack as often, in order to preserve energy (Bryson, 2000). We may have seen this more in ants that were not provoked because when they are not attacked

they are tending the aphids or just doing something else. It is hard to classify what this other action might be and we may have recorded multiple actions as ignoring.

We also found that the influence of a presence or absence of a predator on behaviors significantly affects the behaviors of attack and assistance. This result supported our hypothesis that ants would respond to a predator with protective behaviors. When plants were left alone and observed in controls, ants would not waste their energy on attacking. A similar study, used lady beetles and lacewing as predators, found that ants are more prone to perform protective behaviors when a predator is present ( $p$  value  $<0.005$ ) (Bryson, 2000).

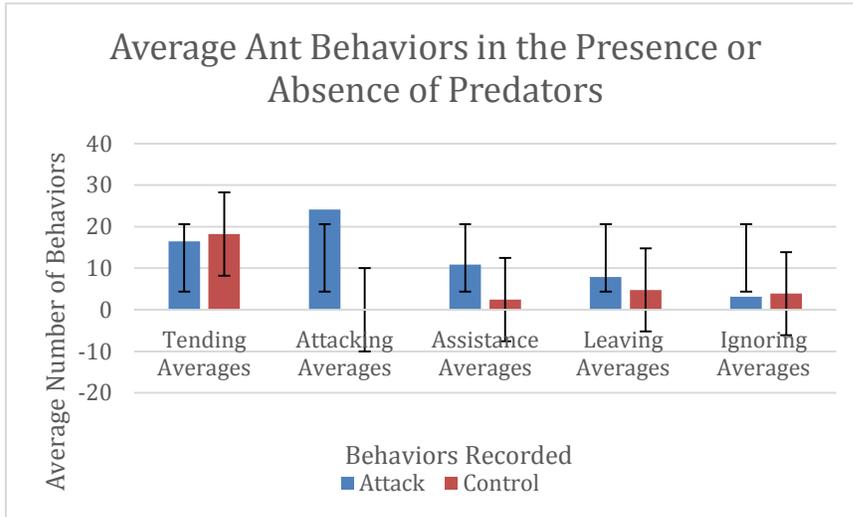
When we looked at behaviors and their relationship to the distance from the road, we saw more tending behavior closer to the road. We observed more of this neutral behavior in areas closer to the road, as disturbances can increase the amount of honeydew produced. In areas that are more frequently disturbed, plants may produce more new foliage and phloem, giving aphids excess nutrients to work with and produce better quality honeydew (Piovia-Scott, 2010).

In our study we found no significant difference between temperature and site. From this, we cannot conclude that temperature influenced ant behavior. However, other studies found that under warmer temperature aphids had higher population growth but their abundance greatly decreased in the presence of predators (Barton, et al., 2014). Further research indicates that this occurs because ants exhibited less protective behaviors and were less abundant in higher temperatures.

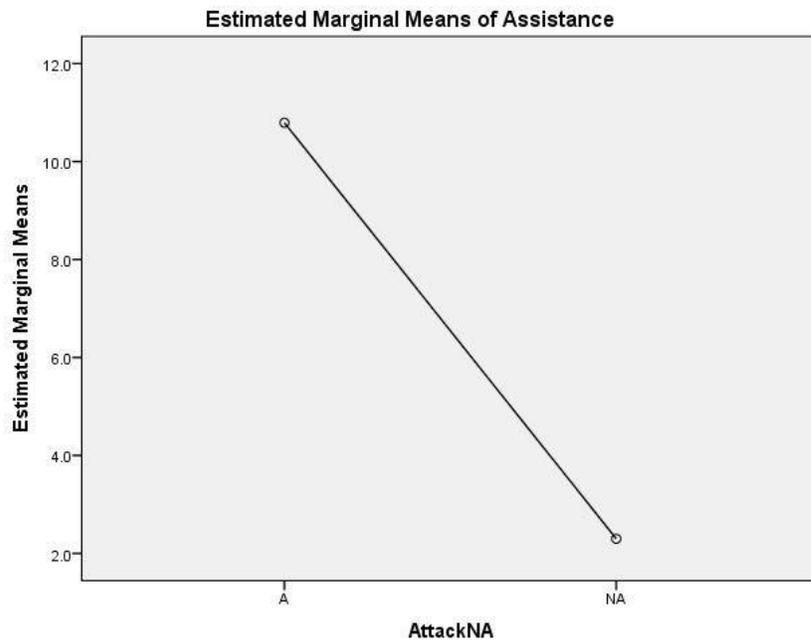
We also found no significant difference between species of ants and site. However, we only collected two ants from each plant, which may not have supplied us with enough data to account for error. Furthermore, we collected ants from each plant but did not keep them in separate vials preventing us from analyzing the relationship between species and exhibited behaviors.

Although our study was limited, we can see that ants do exhibit protective behaviors when a predator threatens aphid communities, and that some behaviors are influenced by disturbances. As humans expand into new territories, we influence the current ecosystem including many specializations such as the one between aphids and ants. These co-evolutions are sensitive to small climate changes as one species relies on the other (Barton, et al., 2014). The relationship between ants and aphids is important to understand as it affects other plant species and our own source of food (Breton, et al., 1989). The mutualism can be studied in the future with researchers using our experiment as a ground point and focusing on the influence of milkweed type and ant species on the mutualistic relationship.

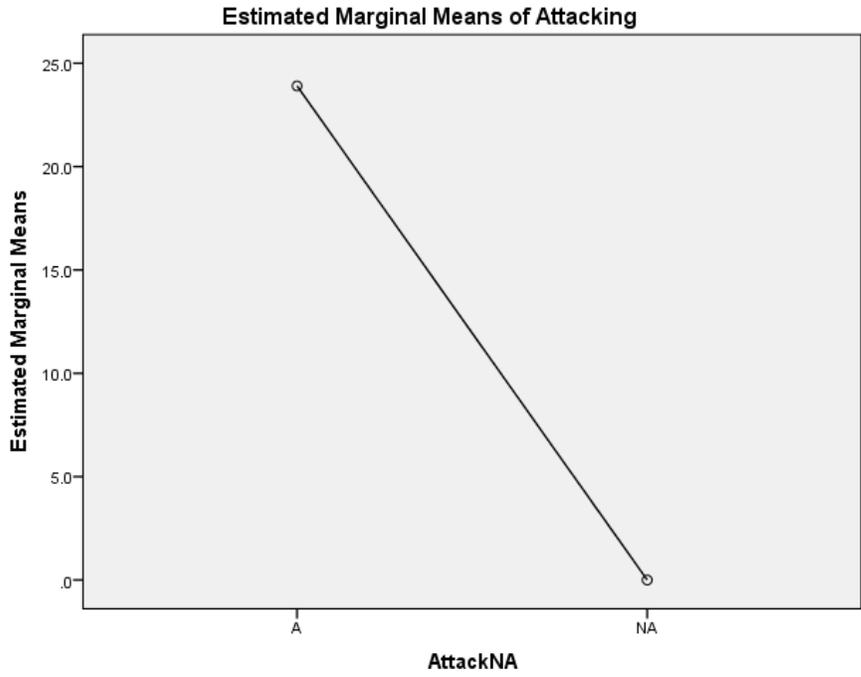
## Appendix



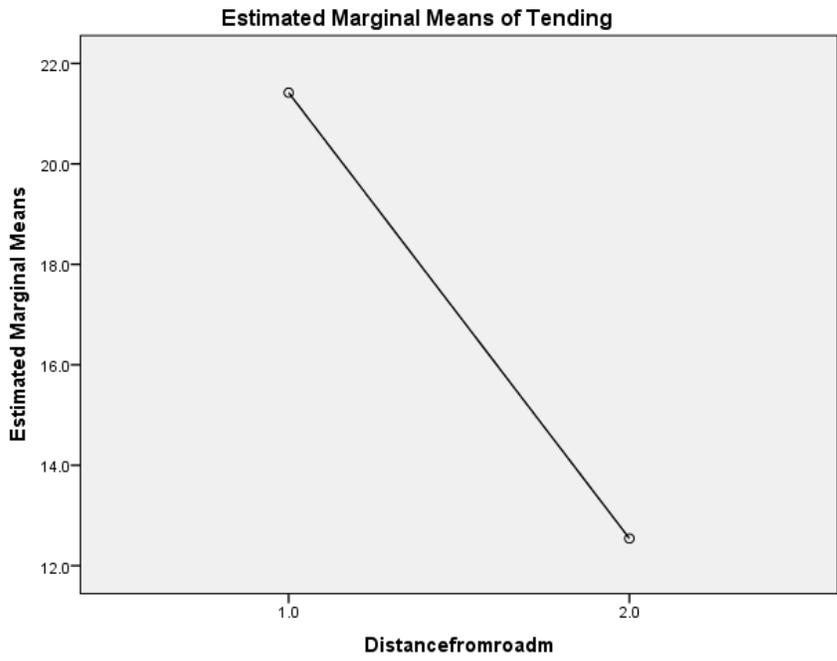
**Figure 1.** Shows the relationship between average ant behaviors and its variance with the presence or absence of predators. Error bars represent standard deviation.



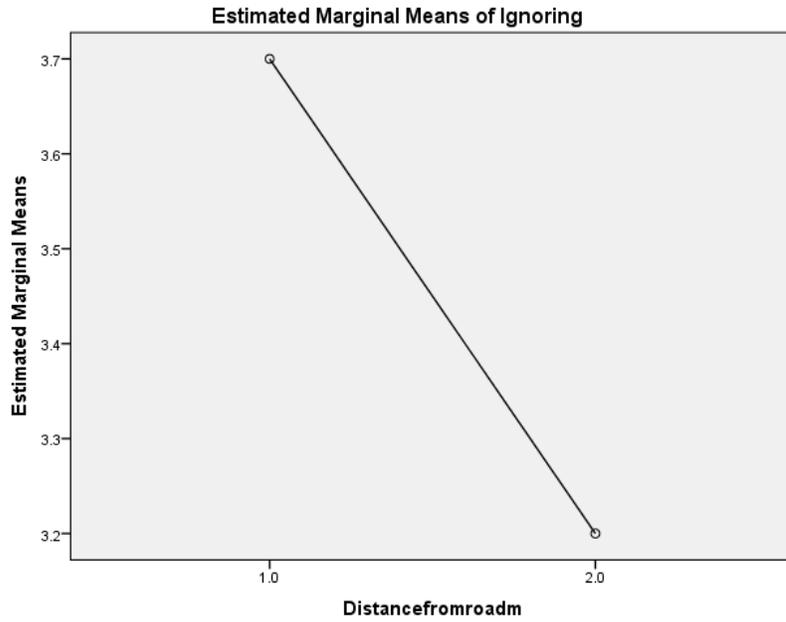
**Figure 2.** Shows the relationship between mean behavioral response of assistance and its variance between presence (A) and absence of a predator (NA).



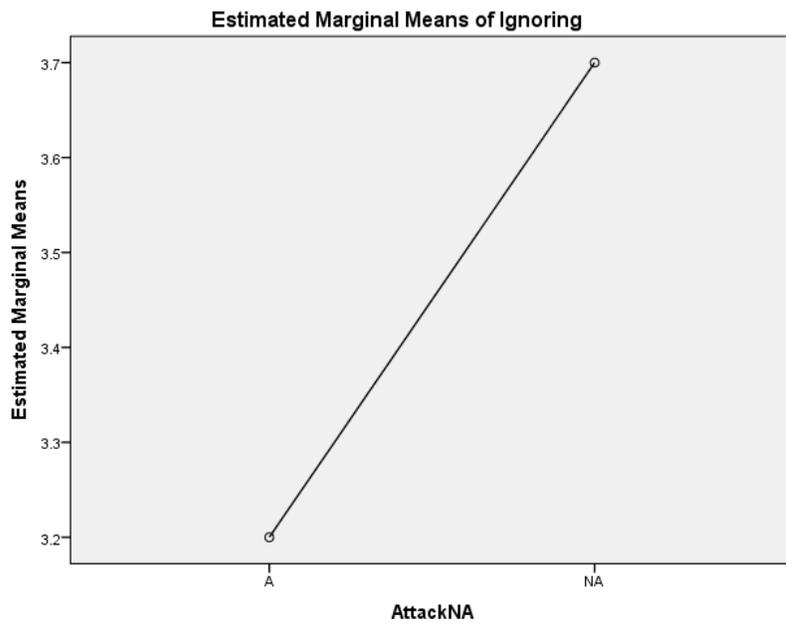
**Figure 3.** Shows the relationship between mean behavioral response of attacking and its variance between presence (A) and absence of a predator (NA).



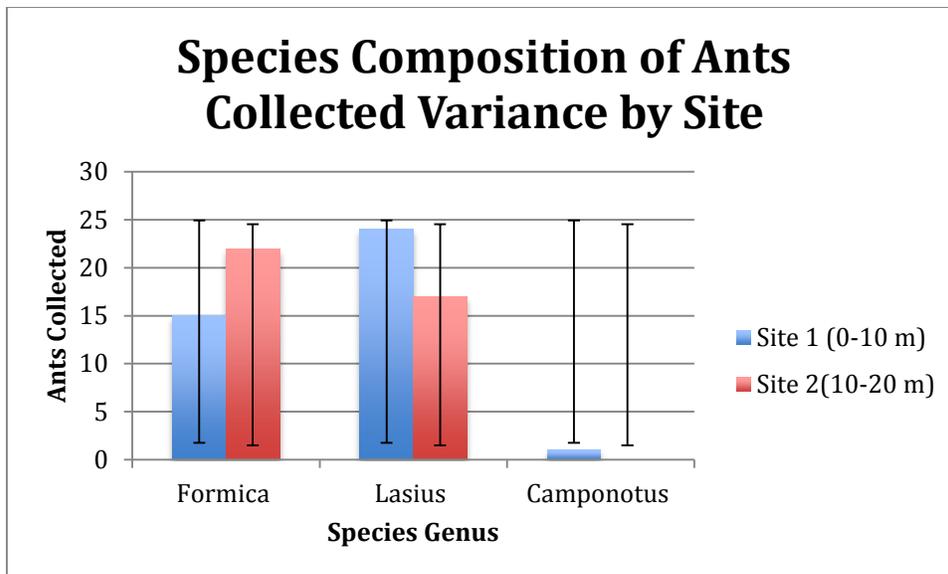
**Figure 4.** Shows the relationship between mean behavioral response of tending and its variance between site 1 (0-10 m from the road) and site 2 (10-20 m from the road).



**Figure 5.** Shows the relationship between mean behavioral response of ignoring and its variance between Site 1 (0-10 m from the road) and Site 2 (10-20 m from the road).



**Figure 6.** Shows the relationship between mean behavioral response and its variance between the presence (A) and absence of an attacking predator (NA).



**Figure 7.** Shows the relationship between species genus and site type (distance from the road). Error bars represent one standard deviation from the mean.

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