A study of the mutualism between Senna and ants via inaccessible extrafloral nectaries

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Abstract

Extrafloral nectaries play a very important role in attracting ants to Senna plants. Other insects, herbivorous ones, are also attracted to Senna, but these plants are often protected by the ants, who may eat these pests as prey. This extra mutualistic perk calls into question just how much the extrafloral nectaries matter in terms of attracting ants. To test this mutualism, I found 30 “wild” Senna plants, and divided them up into a control group and a treatment group that involved painting nail polish over the extrafloral nectaries to render them inaccessible. The specimens were monitored every few days for how many ants were on them and this data was compiled per group. The nail polish treatment group overall had statistically significantly fewer ant visits compared to the control group but experimental flaws make these results inconclusive. This paper advises several changes in the experimental setup.

Introduction

For the purpose of this experiment, the two species of plants monitored- *Senna herbecarpa* (Wild Senna) and *Senna marilandica* (Maryland Senna)- are referred to collectively as Senna plants. As opposed to Partridge Pea, which also bears extrafloral nectaries, Wild Senna is similar enough in hardiness, flower color, and height, to Maryland Senna (Prairie Moon Nursery, 2016) to warrant no significant distinction in this study.

Various species of ants are drawn to Senna plants, at least in part, because of its extrafloral nectaries (EFNs). These EFNs are located in between the flowers and the stem of each compound leaf and reward ants for visiting with their nectar. This is thought to be ideal placement for Senna's extrafloral nectaries, as the ants will ward off/attack other insects that could harm the plant (Borge, 2014). It has been speculated that the ants themselves may benefit from this protective interaction as well, with easy herbivorous prey (Borge, 2014). However, the main reason that ants are believed to be attracted to Senna is for the nectar itself. Therefore, limited information is available to discuss just how much this added benefit of easy prey plays a role in attracting ants to the Senna plant, beyond the extrafloral nectaries.

This experiment aims to test the attraction of ants to Senna plants themselves, by blocking the extrafloral nectaries. A treatment of nail polish was used on extrafloral nectaries to render them inaccessible and compared to a control group of Senna for how many ants continued to visit the plants. The prediction for this outcome was that there would be a statistically significantly greater amount of ants observed on the unaltered control group in comparison to the inaccessible EFN nail polish group. It was hypothesized that, if extrafloral nectaries were blocked or made inaccessible to them, then ants will stop visiting Senna plants in as high numbers but will still continue to visit for another benefit.

Methods

All 30 Senna plants chosen for this experiment came from three unique prairies and the woods found at Litzsinger Road Ecology Center. From this sample, treatments- a control and an applied light purple nail polish on EFNs- were randomly assigned. Each Senna plant was flagged with a number. Corresponding numbers were written on individual sheets of paper and then put into a bag, mixed up, and drawn at random to be alternately placed into the two treatment categories. This was a purely observational study so the Senna plants remained in their respective locations to be monitored.

A basic excel spreadsheet was created with the "Control" group in one column and the "nail polish" treatment group in another. The rows were labeled with what day of the experiment it was, starting with day 1 and progressing up to day 28, when the experiment was concluded. This spreadsheet was printed out, and in each cell, the total number of ants seen per treatment group were recorded each day of monitoring.

I counted every ant on each plant from top to bottom and recorded the number of ants seen, right away when I approached the plant, using a tally system corresponding to only the treatment group the plant belonged to.

Four days of monitoring were conducted, over the course of about a week prior to treatments being applied, to ensure that the number of ants seen per corresponding treatment group were similar overall. After day eight, the light purple nail polish was applied on every extrafloral nectary of each plant belonging to that treatment group. The same tally system mentioned before was utilized again every day of pot-treatment monitoring.

The Senna plants were not monitored every day. I monitored plants at similar times, 7-9 am typically, so similar conditions, could be attained. The order at which the plants were monitored changed daily.

At the conclusion of this experiment, an unpaired t-test was conducted to test for statistical significance.

Results

During the 28 days of this experiment, the control and the nail polish treatment groups experienced a visible difference in number of visiting ants, as exhibited in *Figure 1* and *Figure 2*. The initial four data points in both charts are pre-treatment. Data for the control group fluctuates, but generally seems to be increasing in number of ants seen total until Day 26, when the number of ants seen on its plants return to pre-treatment level (*Figure 1*).

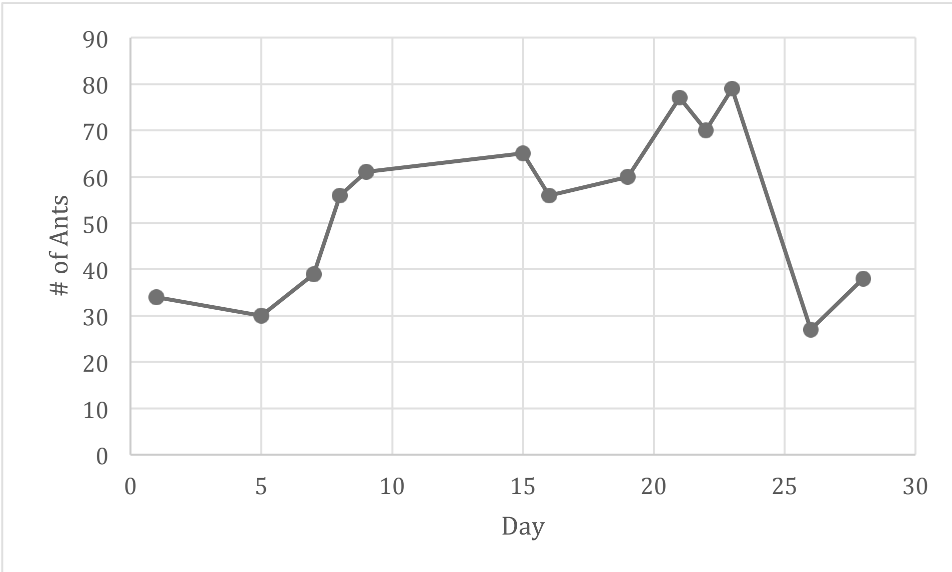

Figure 1. "Number of Ants Seen Daily on Senna Plants in the Control Treatment Group"

Data for the nail polish treatment group also fluctuates, but instead generally seems to be decreasing post-treatment, and never quite returns to pre-treatment level (*Figure 2*).

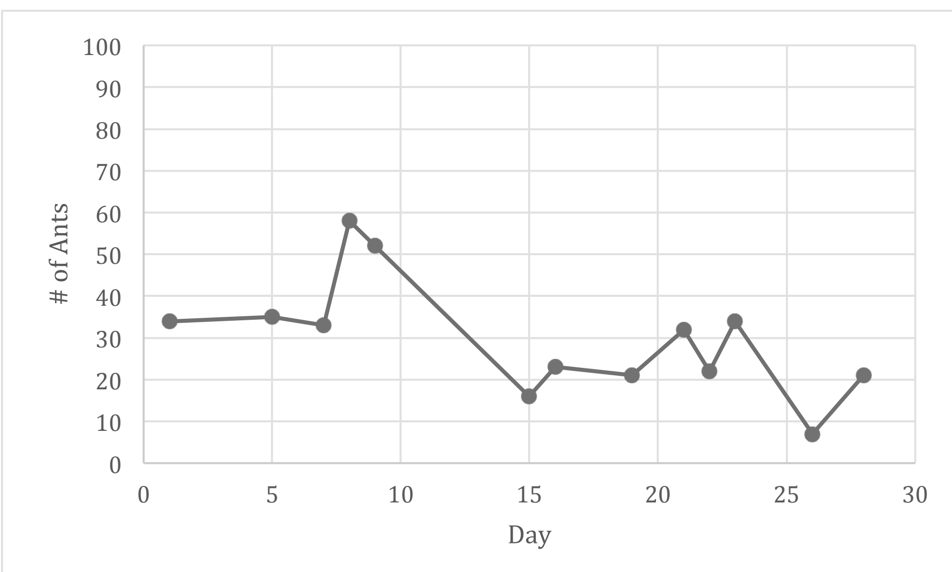

Figure 2. "Number of Ants Seen Daily on Senna Plants in the Nail Polish Treatment Group"

Initially, the amount of bees and other insects were recorded, but they showed no correlation in response to either of the treatments.

An unpaired t-test was utilized to verify the difference in ant numbers seen on the two treatment groups, $t(24)=3.73$, $p= 0.001$, with a significant difference found (Nail Polish M=29.85, Control M= 53.23).

Discussion

The data was found to be statistically significant ($p=.001$) and so it should be supportive of the prediction that ants would not visit the Senna plants with inaccessible extrafloral nectaries as much. This should also bear the implication that the extra benefit of easy prey on Senna does not play as big of a role as EFN ant attraction, but is still enough to keep ants returning. Yet, Several design flaws, a vague hypothesis, and uncontrolled variables draw into question the value of this implication.

The use of nail polish to form a barrier between ants and the extrafloral nectaries is a design flaw in this experiment, as the strong smell of the acetone that is found in nail polish may act as a repellent to certain insects. Bees and some other insects still came in almost equal numbers to both treatments, however, so how much of a repellent the nail polish was is up to debate.

That was not the only issue with this method of blocking extrafloral nectaries. The nail polish was a light purple color that sparkled, which is very unlikely to be seen in nature and could have confused the ants enough to keep some away. The addition of this unusual color added another unnecessary variable into this experiment, but one benefit of it was that it was easy to see if the Nail Polish faded away or if I missed a spot while applying it.

If this study were to be repeated, another method of “blocking” extrafloral nectaries should be used that would not be potentially as much of a repellent, such as glue, clear nail polish, or the cutting of the EFN bud to render the nectars inaccessible.

Although time of day was similar and weather conditions were mostly hot and humid, some storms did occur midway through or before my monitoring of the Senna plants. Rainy weather could explain in part the fluctuations seen in *Figure 1* and *Figure 2.*

One unexplained issue with this experiment was the fact that five of my specimens went missing over the course of this experiment. A controlled greenhouse for these plants many have been a solution that could have offset this issue and the ant number fluctuations due to weather.

Human error, is always another possibility, such as miscounting the number of ants on the plant, not fully covering every EFN with Nail Polish, or not reapplying it regularly enough.

Other variables, such as potentially stable trail pheromones, may have played an important role in this experiment for ants that continued to visit the Nail Polish treated Senna plants. Ants utilize pheromones to create a trail from a food source back to the nest to alert other ants of the opportunity. The durability of these trails can vary greatly among species of ants. In this study, I did not distinguish between different species of ants. For an idea of the variability between species, fire ants have a rapid response pheromone that lasts only minutes (Robinson et al., 2008), whereas the species *Tapinoma simrothi* has fairly stable trail pheromones with their half-life being 10- 19 days(Simon & Hefetz, 1991). But even the same species can have different decay rates of their trails depending on how the individual ant interprets their environment. Pharaoh's ants have four different pheromones used in trail making: one is a repellent, two are short lasting, and one is longer lasting (Robinson et al., 2008). A stable aphid population or the leaves of a tree might call for a longer lasting pheromone than a more temporary food source. The extrafloral nectaries on a Senna plant are somewhat permanent so ants may continue coming to the Senna plant for a time even if the EFNs were inaccessible. The time length of this experiment sook to control for this variable, yet it may not have been conducted long enough.

Between the variability of trail pheromone duration in ants and the sometimes worn down treatments, it is very difficult to know if the reason why a select number of ants could still be seen on the treatment specimens or if it was due to the benefit of easy prey.

Conclusion

Although a statistically significant difference in means between the control treatment, and the nail polish extrafloral nectary blocking treatment was observed in this study, far too many uncontrolled variables could be found for the actual data to be conclusive. This study merely attempted to test whether ants would continue to visit a Senna plant if the extrafloral nectaries were inaccessible. While the control group did exhibit a greater number of visiting ants than the nail polish treatment group, as predicted, more questions were raised from this experiment than answered.

Works Cited

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