Leaf-cutter ants (Genus: *Atta*) preference for leaves treated with secondary compounds in mid-elevation rainforest

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Abstract: Leaf-cutter ants collect pieces of vegetation to take back to the fungus garden being cultivated in within their colony, and many factors are considered when selecting a particular plant to be harvested. For this study, a 10% salt, a 10% sugar and a tap water solution were used to treat leaves collected from trails of ants, and then left out for ants to decide whether they picked them back up or not. After all trials were conducted for each trail, the data was analyzed and a p-values were calculated. Sugar treated leaves are more likely to be picked up by ants passing by when compared to the salt treated leaves. Leaf-cutter ants would take longer when investigating the salt treated leaves and ultimately deciding to not pick them up when compared to the sugar treated leaves. Ants did not prefer salt treated leaves, and because of this, salt can be used in future pesticide products to deter these insects.

Keywords: salt treatment, fungus, sugar treatment, preference, ants

Leaf-cutter ants cut and utilize various plant tissues to help culture the fungus (*Leucoagaricus gongylophorus*) garden found within the nest of these ants. When leaf-cutter ants were first discovered, Europeans assumed that the pieces of leaves being carried by the ants were used to build a roof to protect the colony from tropical rains (Naskrecki 2017). Some species of leaf-cutter ants show preference for grass to cultivate their fungus while other species specifically use eudicot plants (Da Silva Carmago *et al*. 2015).

Leaf-cutter ants are polyphagous, and the adult workers tend to eat the sap from the plant in which they are harvesting for their fungus garden to obtain a large amount of their energy. On the other hand, the symbiotic fungus serves as the main food source for their developing brood within the colony. Since these leaf-cutting ant species do not mainly harvest for themselves, various factors play a part in deciding which plant

would be beneficial for the symbiotic fungus, making it a complex process (Saverschek *et al.* 2010). Nutrient content, leaf toughness and presence of secondary compounds are some of the few factors that play a part in deciding whether the plant will be harvested for the fungus (Nichols-Orians *et al.* 1989; Herz *et al.* 2008). For this study, we decided to test these factors of plant selection by treating leaves with different kind of secondary compounds and seeing if salt or sugar would affect the choices made by the Costa Rican leaf-cutter ants. Leaves were also switched between trails to see if the ants would react differently to leaves that were not collected by ants of their own colony.

Materials and Methods.

Six trails of leaf-cutter ants were located within the Costa Rican rainforest, and forty-five leaves were collected from each individual trail, making sure to not mix the different trail leaves together. In the laboratory, 10% sugar, 10% salt, and tap water solutions were created to treat the leaves from each of the trails. Fifteen leaves from each group were submerged in water, fifteen were submerged in the 10% salt solution, and fifteen were submerged in the 10% sugar solution. The leaves were laid on a mesh net and allowed to dry overnight. The following day, the leaves were taken to their respective trails for testing. A Garmin GPSmap 60CSx (Garmin, Olathe, KS) was used to record the elevation and coordinates for each trail location before completing the different runs. An active part of the trail was found where three piles of five leaves from each treatment were placed, intersecting the trail. In order to reduce bias, three trials were conducted for each trail, and the treatments switched spots, so each treatment would be in each position (left, right or center) once. A timer on an iPhone was used to track when leaves were being taken, and eight minutes were allocated to each trial. The time and treatment of each leaf carried away was recorded. After the eight minutes were up, the remaining leaves (if any) were taken from the trail before starting the next trial. These trials were repeated for a total of three runs per trail, with six trails total.

In the second study, a test was conducted to test the willingness of leafcutter ants to select untreated leaves that were collected from a separate colony. The six trails from the previous study were used again and thirty leaves were collected from each trail. Each trail was paired with another, making three overall pairs, and the leaves were switched between those two trails. An active part of the trail was found, where a pile of five leaves of their own and a pile of five leaves from another trail were placed, intersecting the trail. Eight minutes were allocated for each trial, and three trials were conducted. The timing of selection and which leaf was carried away was recorded. After the eight minutes were up, the remaining leaves (if any) were taken from the trail before starting the next trial. This was conducted for all six trails, recording the results.

Results.

Leaf-cutter ants preferred sugar or water treated leaves from their own colonies when presented with a choice of three different treatments (Fig. 1). After an online Shapiro-Wilk Normality test and Kruskal-Wallis test was conducted, it can be concluded that sugar treated leaves were more likely to be collected when compared to the salt treated leaves ($p = 0.011$). When comparing the sugar and water treated leaves, no conclusion can be made between the two treatments as there was not significant difference in the data collected for both treatments. Time was also analyzed when comparing the three different treated leaves (Fig. 2). Salt treated leaves had a significantly longer time between leaves being taken when compared to both the sugar and water treated leaves (p

= 0.015). Again, there was no significant difference between the amount of time it took for sugar and water treated leaves ($p > 0.05$).

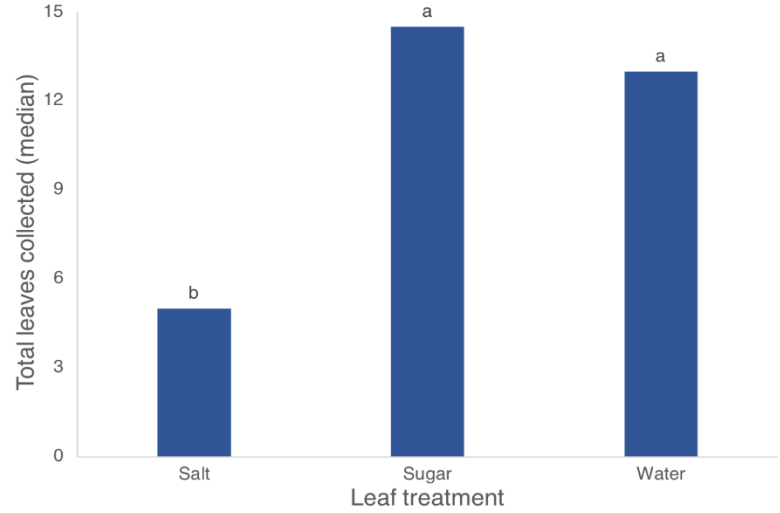

Figure 1. Three treatments of leaves were presented to six different leaf-cutter ant trails where the amount leaves taken for each treatment were recorded, showing a significant difference between the salt and sugar treated leaves ($p = 0.011$).

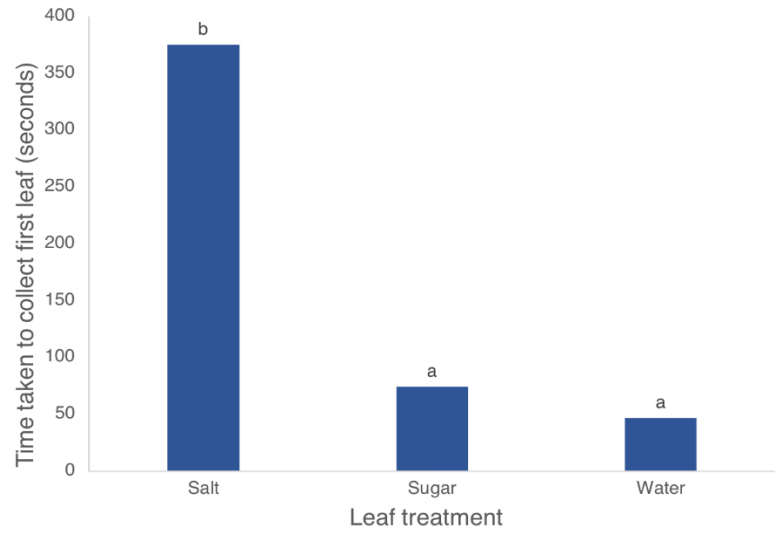

Figure 2. Three treatments of leaves were presented to six different leaf-cutter ant trails where the time it took for each treated leaf to be taken was recorded, showing a significant difference between the amount of time it took for salt and sugar leaves to be taken ($p = 0.015$).

A second study was conducted after the challenges of the original study which led to testing whether leaf-cutter ant trails showed preference to collecting leaves from their own trail or leaves from another neighboring colony's trail. Six trails were selected and paired, and two piles of leaves were placed within each trail and the results for three trials for each trail were recorded and analyzed. An online Wilcoxon Signed-Rank test was used to generate p-values between the two data sets to see whether the data collected was significant. With a p-value of 0.002, a conclusion can be made that leaf-cutter ant

trails prefer their own leaves when comparing to leaves collected from other colonies (Fig. 3).

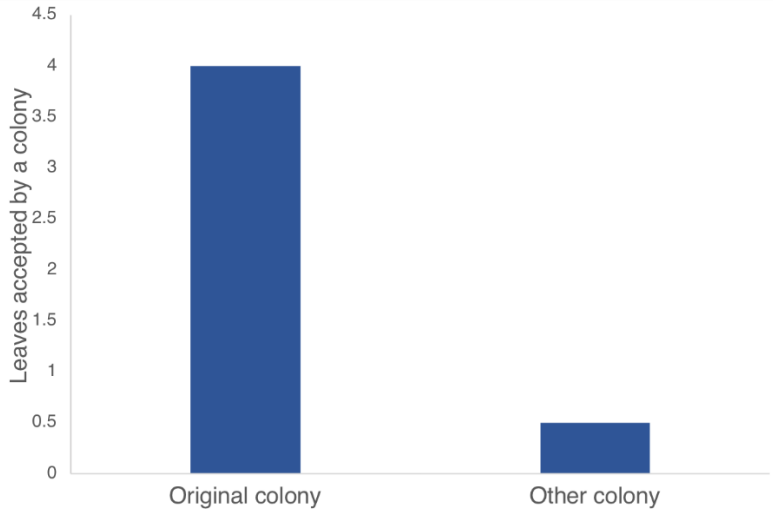

Figure 3. Leaves taken from the original colony and leaves taken from a neighboring colony were presented to a single leaf-cutter ant trail where which leaf taken was recorded, showing a significant difference between the two different types of leaves presented ($p = 0.002$).

Discussion.

Leafcutter ants cultivate a fungus by bringing it leaf fragments that the ants cut and collect from various plants. This serves as a mutualistic relationship where the ants bring the fungus leaves and in return, the fungus will hydrolyze the plants sugar to make food for the adult ants in the colony and the fungus tissue will serve as food to the ant brood (O'Donnell *et al.* 2010). Sodium is a key nutrient to animals, and it is said that herbivores are the most deprived of sodium when compared to carnivores. Therefore, leafcutters, who are herbivores, tend to forage for salt more than other species (Pizzaro *et al.* 2012).

In a previous study conducted in Costa Rica, O'Donnell et al. (2010) investigated whether leafcutter ants would harvest sodium chloride versus water and sucrose treated baits. This study resulted in the ants being attracted to the sucrose treated baits more than the other treatments, but showed the ants interacting with the salt treated baits more than the water (control). For this study instead of treating paper with the different solutions, leaf fragments were collected from ant trails and treated directly with salt, sugar and water solutions to show if there was a different reaction to leaves being directly treated then paper. Results from this study were proven to be non-parametric after conducting an online Shapiro-Wilk Normality test, and a Kruskal-Wallis method was used to analyze the leaf treatment and time to first leaf data. There was a significant difference between the sugar treated leaves and the salt treated leaves ($p=0.011$) whereas there was no significant difference between the water treated leaves and the sugar treated leaves ($p>0.05$). It was also noted that leafcutter ants took longer picking up salt treated leaves when compared to water or sugar treated leaves ($p=0.015$). Because of unpredictable weather changes, many trails were lost after collecting leaves and treating them. This arose the idea of a second study where leaves would be collected from two different trails and swapped. This test would show whether or not ants had preference for their own leaves

over other colonies. After many trials, an online Wilcoxon Signed-Rank Test was used to analyze the data, and the P and Z-values were generated. This test showed significant difference between leaves from their own colony versus leaves from a different colony ($p=0.002$). These results do not align with the results of the study conducted by O'Donnell et al. (2010), but this can be due to multiple factors. Leaves were used instead of paper, and the leafcutter ants might be more familiar with the leaves and will take more of the water treated leaves. Different concentrations for each solution were used, and this could have also provided different results than a previous study.

Many strengths and limitations were made apparent while conducting this research. Costa Rica is known to have an overabundance of insects at every turn while in the rainforest, making it not difficult to find many trails leading to different colonies of leaf-cutter ants. Many trails that were chosen to be studied were washed out due to unpredictable weather. This showed to be difficult as leaves were already collected fideas and assisting in the treated. There was also a thick canopy above majority of the trails being tested, therefore it was proven to be difficult to see the experiment due to poor lighting and cloudy skies. Dim flashlights were used so the trails could be seen, in hopes to not disturb the ants who were not used to bright lights.

In future studies, different concentrations of salt and sugar solutions could be tested to show a more significant difference between the treatments. Solutions in this experiment were drastically different, showing an obvious difference between treatments. Salt and sugar levels could also be tested directly on the fungus itself, where observations of the effects can made. This will need to be done in a laboratory setting with the proper tools which we did not have access to at this time. From the results of this study, salt could be used as a deterrent of colonies that are proven to be agricultural pests.

Forager leafcutter ants have a life devoted to cultivating the fungus that keeps their colonies afloat by providing food for both larval and adult stage ants. Different treatments of leaves have an effect on the ants themselves when deciding which leaf they will take. Salt treated leaves were not as appealing to the trails, and this can lead to a pesticide of these types of ants.

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