Preference of Leaf Cutter Ants for Leaf Toughness



Tu Huynh, Jessie Farris, Amanda Hale, Dean Williams, and Michael Misamore Department of Biology, Texas Christian University

Introduction

- Leaf cutter ants (LCA) facilitate plant growth and diversity by removing leaves from the trees and allowing sunlight to reach the lower forest levels.
- In the LCA-fungus mutualism, LCA actively cultivate the fungus as a food source. They collect plant material as substrate to feed the fungus (Fig. 4).
- LCA are selective in the plant material they collect⁵.
- The goal of this study is to determine whether LCA have a preference for soft leaves over tough leaves by examining the relationship between level of leaf herbivory and leaf toughness in different plant species.

Methods

- We obtained our data at the El Jamaical Field Station (Fig. 5) near San Ramon, Costa Rica.
- We collected 214 leaf samples with distinct LCA herbivory evidence (Fig. 1a) from 15 plant species along the foraging trails of two ant colonies.
- We obtained data for two variables:

1. <u>Herbivory proportion</u> is measured by the ratio between the area removed by LCA and the whole leaf area following these steps:

- We measured the remaining leaf area post LCA herbivory (Fig. 1c) using photos of leaf samples (Fig. 1a) in ImageJ.
- \circ We measured the whole leaf area (Fig. 1b) based on the remaining leaf.

• Calculate the proportion of herbivory area (Fig. 1d): Whole leaf area – Remaining area post herbivory *Herbivory proportion* = Whole leaf area

2. <u>Leaf toughness</u> was measured by the water volume required to tear the leaf apart using a designed device (Fig. 2) following these steps:

- \circ We hung the device on the leaf (Fig. 2).
- \circ We added water to the cup until the water weight tears the leaf apart.
- We measured this water volume as toughness score. Tougher leaves have higher toughness scores.



Figure 1. Leaf area measurements in ImageJ. The figures show a) the original photo of the remaining *leaf post LCA herbivory, b) whole leaf area, c)* remaining leaf area, and d) area removed by LCA (purple) compared to whole leaf area (green).



Figure 2. The designed device for quantifying leaf toughness as the water volume (in ml) required to tear the leaf apart.

- 1. Between 15 species
- slope=0.001, Fig. 3b).
- 2. <u>Within each species</u> (Table)



Figure 3. Regression of proportion of herbivory leaf area against leaf toughness a) with the outlier value and b) without the outlier value.

Species	n	Slope	Standard Error of Slope	P-value
Α	10	-0.0058	0.0020	0.0348
В	10	-0.0087	0.0036	0.0151
С	17	-0.0005	0.0008	0.2832
D	14	0.0011	0.0043	0.5453
E	8	-0.0055	0.0018	0.0391
F	15	0.0007	0.0021	0.7349
G	17	-0.0007	0.0003	0.0411
Н	20	-0.0047	0.0030	0.1775
I	27	-0.0006	0.0006	0.3157
J	9	-0.0093	0.0040	0.0428
К	17	0.0018	0.0010	0.0568
L	11	-0.0088	0.0046	0.0376
М	6	0.0039	0.0032	0.4013
Ν	20	-0.0024	0.0011	0.0443
0	13	-0.0019	0.0010	0.0490

Table. *Regression analyses of the relationship between leaf toughness and herbivory* proportion within a species.

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Results

We used regression analyses to examine the relationship at two levels:

• 1 of 15 species showed an exceptionally high value for mean leaf toughness. It was an outlier (Fig. 3a) that was excluded from the analysis. For the remaining 14 species, herbivory proportion did not differ among species of various leaf toughness (R²=0.0166, F_{1 12}=0.202, P=0.661,

11 of 15 species showed a negative relationship between leaf toughness and herbivory proportion. 8 of them had a significant slope (P<0.05). The remaining 4 species had a positive relationship but there was no correlation between leaf toughness and herbivory proportion (P>0.05).



Figure 4. LCA clear out vegetation to build a) foraging trails from b) the mound and c) harvest plant material (flower parts, leaves, fruits) along these trails as substrate for d) their fungus garden.

Discussion

1. Between 15 species

- LCA select plant material based on many factors other than toughness: defensive chemistry⁴, nutrient content¹, load mass⁶, secondary plant chemicals⁵, water content²...
- Different plant species differ in characteristics besides leaf toughness that potentially combine to create a complicated foraging pattern that does not solely rely on leaf toughness.
- There were significantly more mature leaves on the plants we sampled, so LCA were likely to encounter and cut mature leaves before new leaves ⁷.
- 2. Within each species
- LCA prefer young leaves over tough leaves in many plant species. • As young, soft leaves mature, they decrease in nutrients and water content and increase in defensive compounds, which makes them tougher³. LCA prefer young, soft leaves because they provide the most nutrients and water and discharge the least defensive chemicals to the fungus⁵. LCA cut more soft leaves, which have fast cutting speed⁸ in an attempt to maximize efficiency and energy conservation in foraging⁹.

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Figure 5. Map of the El Jamaical Fiela Station. The region marked in purple represents the data collecting site.



