Comparisons between strike and probe treatments were significantly different in both colonies, indicating that soldier ants behave more aggressively in response to the simulated anteater attack than to the more artificial stimulus of pounding.

Since all tests except one comparing patrol time among disturbance categories were insignificant, it seems likely that time could be an inappropriate measure of the aggressive behavioral response.

Morphological differences between the maxima of the two colonies (Colony 1 maxima were much larger) would be worth examining in more detail. A possible explanation for the different morphologies might be that in Colony 2, the mound structure resulted in the center of the colony being more protected while the center of 1 was closer to the surface. If this is the case, the disturbances to Colony 2 could have posed less of a threat than to Colony 1. The soldiers might have remained in the center, because they were not necessary for defense of the nest. The ants we observed could have been enlarged media.

Because of this possibility and the result that soldiers responded more aggressively to a larger disturbance, it seems that the colony is committing only the number of soldiers that is necessary for defense. The benefits of greater protection afforded by a greater allocation of soldiers might be outweighed by the costs of energy expenditure and predation.

INVESTIGATION OF A PREFERENCE FOR NEW OR OLD LEAVES IN *ATTA CEPHALOTES*

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Abstract. New leaves may have a higher nutritional value than old leaves as well as a higher concentration of defensive chemicals. We investigated which type of leaf *Atta cephalotes* prefers. We offered ants old and new leaf pieces. New leaf pieces were not taken back to the nest more frequently than the old pieces, although they were moved off the trail more frequently. (ALG)

INTRODUCTION (SAW)

Leafcutter ants (*Atta cephalotes*) accept a wide range of plant species (Lugo, et al. 1973, Haines 1978). They take leaves to the nest, chew them, defecate on them and feed on a fungus that grows on them. Many physical and chemical characteristics affect choice of plant species, including leaf toughness, tannin level and overall nutritional value (Feeny 1970, 1976). *A. cephalotes* prefer leaves from gap tree species over understory species, accepting high tannin levels as a trade off for higher nutritional value (Nichols-Orians 1991).

New leaves have more tannins and more nutritional value than old leaves (Coley 1983). We hypothesized that the same trade-off would result in a preference for new versus old leaves.

METHODS (JLB)

This study was conducted along the Sendero Rio Sirena and Sendero Ollas by the Sirena Station in Corcovado National Park, Puntarenas Province, Costa Rica. New and old leaves were obtained from four species of plants (*Heliconia*, a species of Marantaceae, *Aegiphila Martinicensis*, and *Ochroma lagopus*.) found on the Sendero Naranjo. All four species were assumed to be acceptable to leaf cutter ants as we had previously observed evidence of leaf cutter damage to each. New leaves were classified as those that had not yet unfurled or those at the apical meristem. Old leaves were chosen from those on the bottom branches which showed no evidence of herbivory.

Uniform sized disks were punched from the leaves using a standard paper punch. Disks were handled with tweezers and kept in plastic bags with damp napkins to retain freshness.

A four-species comparison was performed on the first nine active leafcutter trails encountered on the Sendero Rio Sirena. Four pairs of disks (one new, one old; one pair per species) were placed in the center of the trail at 50cm intervals. The order of the pairs along the trail was randomized. Within a pair, disks were placed 1cm apart. The order of new and old leaves along the trail was also randomized. As soon as a disk was removed by an ant it was replaced with a fresh disk. Trials were conducted for 20min periods. For each species, the numbers of new and old disks taken back to the nest and moved to the side of the trial were recorded.

After nine trials, fresh leaves were gathered for those species which

had been accepted (taken back to the nest) by any colonies. Two trials (using the same procedures as before) were conducted using two pairs of disks of each of these species, on the first two trials encountered on the Sendero Ollas.

We then returned to those trails on the Sendero Rio Sirena which had accepted leaves. One trial was performed on each of these trails, using four pairs of disks and the same procedures as before. On trails that had only accepted the species of Marantaceae, four pairs of disks of this species were used. On trails that had accepted both *Heliconia* and the species of Marantaceae, two pairs of disks of each species were used.

Results (JLB)

Only two species examined, *Heliconia* and a species of Marantaceae, were acceptable to leaf cutter ants. Although our sample size was not large enough for a statistical test, our data for *Heliconia* acceptability indicate a tendency for preference for old leaves over new leaves (Table 1). No preference was shown between old and new leaves of the species of Marantaceae ($p>0.05$). No preference was shown overall for new vs. old leaves ($p>0.05$).

Across species, ants removed greater numbers of new leaves than old leaves from the trail ($p<0.005$, Table 2). This trend was observed within species for *A. martinicensis* and *Heliconia* (Table 2). Although small sample size prevented statistical analysis of *O. lagopus*, the data suggest a slight trend toward more removal of new than old leaves, following the overall trend (Table 2). Only the species of Marantaceae showed no significant difference in removal of new vs. old leaves ($p>0.05$, Table 2).

Table 1. Acceptance and removal of new and old leaves by *Atta cephalotes*.

			Total # leaf disks taken back to nest		Total # leaf disks cleared to side of trail	
leaf species	# trials	# trails	New Leaves	Old Leaves	New Leaves	Old Leaves
Aegiphila martinicensis	9	9	0	0	49	21
Heliconia sp.	17	11	1	12	112	69
Marantaceae sp.	25	11	10	18	98	70
Ochroma lagopus	9	9	0	0	54	37

Table 2. Results of Wilcoxin matched pair signed rank test comparing responses to new and old leaf disks by *Atta Cephalotes*..

	Cleared to the side of the trail			Taken back to nest		
Leaf Species	n	t	p	n	t	p
Aegiphila Martinicensis	8	1	< 0.008	---	---	NS
Heliconia sp.	10	0	< 0.005	<5	---	NS
Marantaceae sp.	10	14	NS	5	5	NS
Ochroma lagopus	<5	---	NS	---	---	NS
All spp combined	28	34	0.005	---	---	---
*Heliconia sp.*and Marantaceae sp.	---	---	---	7	5	NS

Discussion (SLS)

We found no evidence to support the hypothesis that new leaves are preferred over old leaves as a substrate for fungal gardens in leaf cutter ants. The reverse trend (although not tested statistically) was found for *Heliconia*. Possibly the concentration of defense compounds in the young *Heliconia* leaves was so high as to outweigh their increased nutritional value. The ants should choose leaves of highest quality to maximize energy intake. Without analysis of the nutrient and toxin concentrations in the leaves, we are unable to examine the chemical basis of leaf preference by the leaf-cutter ants. Our finding that new pieces were moved to the side of the trail more often than old pieces may be explained by stronger chemical signals in young leaves. If young leaves did indeed contain more toxins, they may have been removed from the trail to remove any negative effects on the workers. Old leaf pieces, on the other hand, may be noticed but considered neither a threat nor a benefit to the colony and so less often removed.

We chose multiple species of leaves and multiple trails of ants to generalize our results over species and trails.

Further studies on this subject should include larger sample sizes of those species that were accepted. Improved procedures would include using new and old leaves of the tree species that was actually being carried by the ants on a specific trail. It would be interesting to examine the mechanisms responsible for the removal of more new than old leaves from the trail.

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