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Influence of biotic, chemical and mechanical plant defenses on the foraging pattern of the leaf-cutter ant (*Acromyrmex striatus*) in a subtropical forest

Patricia J. Folgarait[1,2], Alejandro G. Farji Brener[1,3] and Jorge J. Protomastro[1]
1 Depto. de Biología, FCEyN, Univ. de Buenos Aires, 1428 Buenos Aires, Argentina.
2 Department of Biology, Univ. of Utah, Salt Lake City, Utah 84112, U.S.A.
3 ECOTONO, Depto. de Ecología. CC 1336, Univ. del Comahue, 8400 Bariloche, Argentina.

Resumen. *Se estudió la dieta de la hormiga cortadora* Acromyrmex striatus *como evidencia indirecta de las actividades anthiherbívoras de la hormiga nectívora* Camponotus blandus *y de las defensas químicas y mecánicas presentes en las plantas. Se determinó la actividad de ambos tipos de hormigas y la abundancia vegetal de distintas especies de plantas y se cosechó material foliar para análisis de defensas químicas y físicas durante la primavera en un bosque subtropical del Chaco Argentino. Se encontró una relación inversa entre la proporción de visitas de* C. blandus *y la actividad recolectora de* A. striatus *para las especies vegetales que ofrecen néctar. Por otro lado, hallamos una correlación positiva entre la abundancia de las especies vegetales del bosque, y su proporción en la dieta de la hormiga cortadora. En general, las defensas físicas y químicas no mostraron correlación significativa cots la dieta de las hormigas cortadoras. Algunas especies mostraron poseer niveles de defensas superiores a las tolerables por* A. striatus. *Los resultados sugieren dos razones principales para explicar la ausencia o baja representación de las especies vegetales que ofrecen néctar en la dieta de* A. striatus*: actividad de hormigas nectívoras y baja abundancia de las especies vegetales.*

Abstract. *The diet of the leaf-cutter ant* Acromyrmex striatus *was used as indirect evidence for potential antiherbivore activity by the nectivorous ant* Camponotus blandus *and by chemical and mechanical plant defenses. Data on ant activity, plant abundance, and foliar material for analyses of chemical and mechanical plant defenses were collected during spring in a subtropical forest in the Argentinean Chaco. We found a negative relationship between the proportion of visits by* C. blandus *and leaf harvesting of* A. striatus *for the plant species that offer nectar. However, the most abundant plant species in the forest comprised the greatest part of the leaf-cutter diet. In general, no relationship was found between chemical or mechanical defenses and leaf-cutter diet for all the plant species of the forest. A few plant species with high levels of plant defenses suffered little or no harvesting by leaf-cutter ants. Our findings suggest two main reasons for the absence or low representation of nectar-offering plant species in the diet of* A. striatus*: nectivorous ant activity and low plant species abundances.*

Introduction

Nectivorous ants are known to have mutualistic relationships with plants (Beattie 1985). The plants “provide” nectar to the ants and “in return” the ants protect the plants against herbivores. However, not all ant species benefit plants. Leaf-cutter ants are among the most destructive herbivores in the Neotropics, a single colony can gather several kilograms of leaves per day (Weber 1969).

Protection by nectivorous ants may reduce damage to leaves (Bentley 1977, Koptur 1984), flowers (Keeler 1981), seeds and fruits (Inouye and Taylor 1979, Stephenson 1982) by removing the eggs and larvae of herbivore insects (Tilman 1978) or through aggressive behavior (Picket and Clark 1979). On the other hand, foraging by leaf-cutter ants seems to be affected by plant species identity and plant tissues (Rockwood 1977), leaf toughness (Stradling 1978), leaf water content (Bowers and Porter 1981), leaf nutrient content (Cherret 1968), and secondary compounds (Hubbell and Wiemer 1983, Howard 1987, 1988) but not by woody species abundance (Rockwood 1976).

Plant species with few or no mutualistic ants show increased chemical defense in their leaves (Janzen 1966, Rher et al. 1973, Koptur 1984). This trade-off in defenses suggests that defenses are costly and that resources to make them are limited (Rosenthal and Janzen 1979). Nectivorous and leaf-cutter ants visit similar types of plant tissues. However, the interaction between these two types of ants has hardly been investigated (Justum et al. 1981. This information could be particularly important since leaf-cutter ants have been shown to have a significant impact on a Subtropical Dry Forest in the Argentinean Chaco (Bucher 1982). In this study we investigated the diet of leaf-cutter ants in order to indirectly analyze the potential protective role of nectivorous ants and of chemical and mechanical plant defenses against herbivory.

Materials and Methods

Study site

This research was conducted in Copo's Forest Reserve in Santiago del Estero, Argentina (26° 15' S, 61° 55' W). The climate is subtropical and seasonal with 80% of annual rainfall (700 mm) concentrated in 6 months. The mean and absolute maximum temperatures are 27.7/44.8 °C in the summer and 15.5/24.5 °C in the winter. The site is an old secondary woodland that was selectively logged in 1950.

Plant abundance and species list

Seven 15 meter square plots were measured for plant abundance determinations. Basal area from 3 tree species and 14 shrub species were registered. Aspidosperma quebracho-blanco (AQB), Schinopsis lorentzii (SL), Zizyphus mistol (ZM), Capparis atamisquea (CAT), Capparis retusa (CR), Capparis salicifolia (CS), Achatocarpus praecox (AchP), Maytenus spinosa (MS), Celtis pallida (CP), Acacia furcatispina (AF), Acacia praecox (AP), Bouganvillea praecox (BP), Castela coccinea (CC), Schinus fasciculatus (SF), Mimosa detinens (MD) and Jodina rhombifolia (JR) respectively.

Ant activity

Sampling of ants was done during September-October of 1986 for four consecutive days, at the peak of nectar and leaf production. Nectivorous and leaf-cutter ants were sampled at the same time along the same 1 kilometer trail. In this paper we only report the activity of the most abundant nectivorous species, *Camponotus blandus*, and leaf-cutter species, *Acromyrmex striatus*. All *A. striatus* nests (n=18) and nectar-producing plant species (n=60) were tagged and sampled. Each leaf-cutter nest was sampled at random at six different times, for 5 minutes each during the four days. Ant leaf loads were collected near the nest, tagged according to species, separated into plant components, dried at 40°C, and weighed (error < 0.1 mg). The percentage of plant species in leaf-cutter's diet was calculated as the grams of each plant species divided by the grams of all species in the diet. Eight diets collected at random were chosen as replicates in order to calculate an average percentage of each item in the diet. This average percentage for each plant species was used in the figures.

Nectivorous ants were counted in the three branches per tree. Branches chosen were those with the highest number of ants per plant. The number of ants on each branch was summed for each individual plant. Each of the 60 plants was sampled once per day and the ant activity in each plant species was summed over the four day period. Ants were counted on the following plant species: *Capparis retusa* (n=16), *Acacia praecox* (n=13) both with foliage nectaries, *Castella coccinea* (n=14) with stem nectaries, *Capparis salicifolia* (n=6) and *Maytenus spinosa* (n=4) with floral nectaries and *Zizyphus mistol* (n=7) with homopterans highly attended by nectivorous ants. The first five plant species are shrubs, and the sixth is a tree. Percentage of visits of nectivorous ants for each

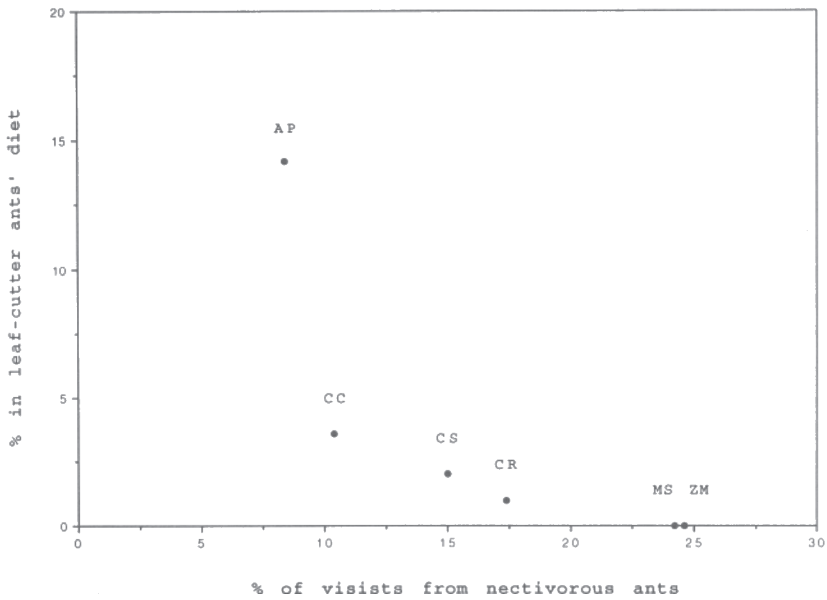

Figure 1. Correlation between percentage of visits by the nectivorous ant *C. blandus* in plants that offer nectar and its representation on the diet of the leaf-cutter ant *A. striatus*. R_s= -0.986; P < 0.01 . See text for plant species identification and sample sizes.

plant species, was calculated as the number of ants per plant divided by the summation of ants per plant in all the six plant species.

Chemical and mechanical plant defenses

Secondary compounds were studied in leaves immediately dried at 40°C after harvesting. Tannins were identified by measuring proanthocyanidins (Swain and Hillis, 1959) and related to commercial quebracho extract equivalents. Phenols were identified by Folin reagents. Alkaloids were qualitatively determined as in Culvenor and Fitzgerald (1973). Saponins and cyanogenic glycosides were qualitatively measured using the foam and pycrate test respectively (Gibbs, 1974). Toughness was measured with a punchameter (Feeny, 1970). For more details see Protomastro (1988).

Results

Plant species that were visited more frequently by nectivorous ants were less abundant in the diet of leaf cutter ants (Figure 1, n= 6, Spearman correlation r= -0.98, P< 0.01). There was no relationship between the percentage of each plant species in the diet, and the type of chemical or mechanical defense, in mature or young leaves (Toughness: mature leaves, n= 16, r= 0.382. Phenols: young and mature leaves respectively, n= 16, r= -0.051 and r= 0.16. Tannins: young and mature leaves respectively, n= 16, r= -0.289 and r = -0.366. Saponins: young and mature leaves respectively, Fisher exact test, P= 0.1177. Alkaloids: Fisher test, P= 0.543 for each type of leaf. Cyanogenetics: null production in all species). The single exception to this lack of correlation was a positive relationship between the toughness of young leaves and its representation in *A. striatus* diet (n=16, r= 0.535, P < 0.05).

Abundant plant species in the forest were also more common in the diet of leaf cutter ants (Figure 2, n= 16, Spearman correlation r= 0.44, P< 0.05).

The number of visits made by nectivorous ants was significantly different from the number expected if the visits were made at random (according to the plant sample sizes) for five of the six plant species. *C. retusa, M. spinosa* and *Z. mistol* were significantly more attended by nectivorous

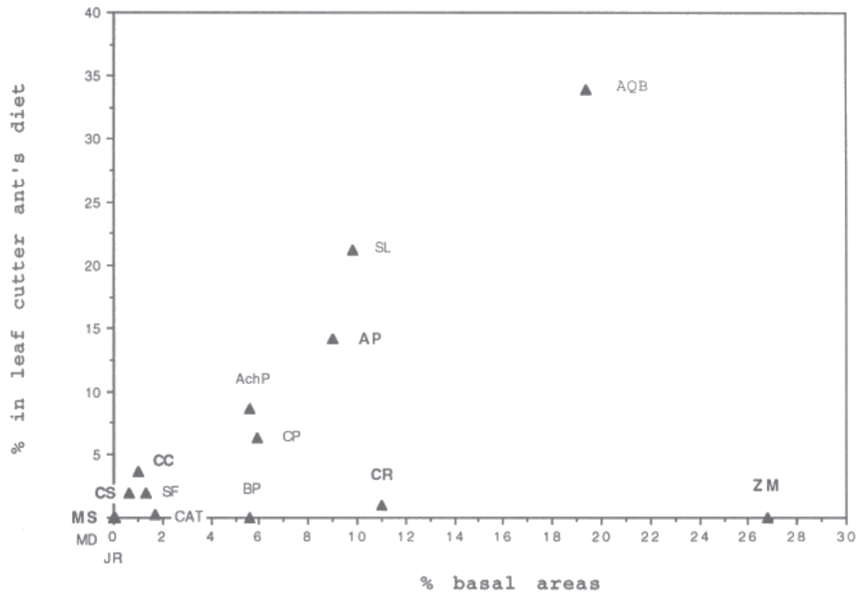

Figure 2. Correlation between plant species abundance in the forest and plant species abundance in the diet of the leaf-cutter ant *A. striatus* (both in percentages). Rs= 0.44; P < 0.05. Plant species that offer nectar are in bold and large font size. See text for plant species identification.

ants (X^2= 4, P < 0.05; X2= 14, P < 0.01; X2= 26.2, P < 0.01, respectively), *A. praecox* and *C. coccinea* were significantly less visited (X^2= 21.5, P < 0.01; X^2= 11.2, P < 0.01) and there was no significant difference for *C. salicifolia* (X^2= 0.0, P > 0.5).

Plant species that offer nectar also exhibited the production of, at least, one type of secondary chemical compound that could potentially act as a plant defense (Table 1). Of the six types of plant defenses analyzed, tannins and saponins in mature leaves, and toughness in young and mature leaves seemed to affect the foraging behavior of *A. striatus*. However, all the nectar-offering species studied produced levels of chemical and mechanical defenses that fall within the range tolerable by *A. striatus*. The only exception was for mature leaves of ZM. These leaves showed greater concentrations of tannins and saponins than this leaf-cutter ant seemed to tolerate (Table 1).

Discussion

This study had three main findings. First, we found that the diet of *A. striatus* was affected by nectivorous ant activity and by plant species abundance. Second, we did not find any correlation between herbivory by *A. striatus* and levels of chemical and mechanical plant defenses in either young or mature leaves. Third, we did not find any indirect evidence of trade-offs in plant defenses for the six plant species offering nectar. In the remainder of this paper, we discuss each of the findings in more detail.

Nectivorous ants and plant species abundance

In support for a potential protective role by nectivorous ants (Beattie 1985), we showed that plant species visited more frequently by nectivorous ants were less common in leaf-cutter ants' diet (Figure 1). Non-systematic field observations also support this suggestion. For example, fallen branches of *C. retusa* that had lost their nectivorous ants were heavily defoliated by *A. striatus*. Additional indirect evidence was provided by *Z. mistol*. The only branches that survived an outbreak of moths were those associated with homopterans, and therefore with nectivorous ants. Contrary to previous results (Rockwood 1976), this study showed that the more abundant a plant species in the forest, the more represented it was in the leaf-cutter ants' diet (Figure 2). In regard to both factors, five of the six

Table 1. Chemical and mechanical defenses for young (without brackets) and mature (in brackets) leaves and type of item eaten by leaf-cutter ants in six plant species that offer nectar-like substances †.

Species	Phenols	Tannins	Cyanogenetic	Alkaloids	Saponin	Toughness	Item eaten
Z. mistol	23.9	138.0	-	-	+ +	124.0	
	(61.8)	(750.8)	(-)	(-)	(+ +)	(340.0)	
C. retusa	9.6	0.0	-	-	+	279.0	
	(14.7)	(0.0)	(-)	(-)	(-)	(653.0)	young leaves
C. salicifolia	6.5	0.0	-	-	+	282.0	
	(14.8)	(0.0)	(-)	(-)	(-)	(625.0)	flowers
M. spinosa	121.0	409.5	-	+ +	+ +	133.0	
	(77.7)	(210.0)	(-)	(+ +)	(-)	(624.0)	
C. coccinea	138.6	0.0	-	-	-	202.0	
	(76.2)	(0.0)	(-)	(-)	(-)	(451.0)	flower
A. praecox	93.5	1055.7	-	+ +	+	147.0	young, mature
	(52.4)	(343.1)	(-)	(+)	(-)	(404.0)	leaves; flower and fruits
FV_{min}-FV_{max}	3.0-328.4	0.0-1055.7	-	- + +	- + +	100-914	
	(8.1-298.9)	(0.0-750.8)	(-)	(- + +)	(- + +)	(119-1399)	
DV_{min}-DV_{max}	3.0-328.4	0.0-1055.7	-	- + +	- + +	147-279	
	(8.1-298.9)	(0.0-343.1)	(-)	(- + +)	(-)	(232-957)	

† Phenolics: mg/g leaf dry weight as tanic acid equivalents. Tannins: mg/g leaf dry weight as commercial red quebracho equivalents. Cyanogenetics, alkaloids and saponins: -= absent, += present, ++=abundant. Toughness: as Feeny (1970). FV: range of values considering all plant species of the forest. DV: range of values only considering plant species present in the diet of leaf-cutter ants.

plant species offering nectar represented less than 4% of the diet (CR, CS, CC, ZM, MS). Three belonged to the group of plants that were significantly more visited by nectivorous ants (CR, ZM, MS) or that had extremely low abundance (less than or equal to 1 %) in the forest (CS, CC, MS).

Chemical and mechanical defenses

Leaf-cutter ant diets are known to be affected by plant secondary compounds and toughness (Howard 1987, 1988) but our results did not show any significant correlation between levels of secondary compounds and the herbivory data of *A. striatus.* The maximum level of chemical and mechanical defenses found to the plant species eaten by this leaf-cutter ant was used as a threshold tolerance level by *A. striatus* and we compared this value with the maximum level obtained for all the plant species in the forest (Table 1). For the six nectar-offering plant species, most of the values of chemical and mechanical defenses fell within the range of tolerable values (Table 1). Therefore in these nectar-offering species, chemical or mechanical defenses alone do not seem to be a barrier to this leaf-cutter ant species. However, the lack of significant negative correlations between diet and chemical/mechanical defenses can also be interpreted as a "none-all" response. For example, in mature leaves of AQB and young leaves of JR, toughness seemed to be high enough as to prevent herbivory by this leaf-cutter ant (mean toughness: 1399 and 914 respectively). In other species like BP, ACHP and MD, saponin levels in mature leaves seemed also to be too high (++) since *A. striatus* never eat them (probably their fungus might not be able to detoxify this chemical). The only significant correlation found in this study was the positive relationship between toughness and herbivory. We interpret this result as the leaf-cutter ants eating and testing leaves at the same time, presumably as a way to find out the relative concentration of the chemical defenses that are commonly present in young leaves (Coley 1983). However, above a certain threshold level of toughness, the physical barrier becomes difficult to overcome and the leaf-cutter ants do not eat these plants at all (as in AQB and JR).

Trade-offs between defenses

In our study, plant species that exhibited biotic defenses also invested in other type of defenses. Other authors have failed to find evidence for trade-offs between plant defenses (Seigler and Ebingler 1987, Stewart and Keller 1988). With a single exception, chemical and mechanical defenses in the six nectar-offering species fall within the range of acceptable food for leaf-cutter ants (Table 1). Interestingly, ZM - the only plant species offering nectar-like substances through homopterans- has tannin and saponin levels in mature leaves greater than what seems tolerable to leaf-cutter ants (Table 1). Since homopterans crowded in the tip of branches, the potential protection of ants may be restricted to young leaves. Another reason for not finding evidence of trade-offs could be that each type of defense is effective against types of herbivores that were not sampled in this study. However, in a few cases either nectivorous ants (i.e. CR) or low plant abundance (i.e. CS and CC) seemed to be responsible for the reduced foraging by leaf-cutter ants (Farji Brener et al. 1992).

The low representation of certain plant species in the diet of *A. striatus* might also be due to other reasons that the ones emphasized in this study. Interference with pollinators could also influence the low number of visits made by nectivorous and leaf-cutter ants to the flowers of CS. This species has its nectaries in floral structures, and its flowers are the only item ingested by leaf-cutter ants. Besides, ants are known to be poor pollinators (Hickman 1974). Thorns present in CC may have also prevented leaf-cutter ant access. Our data can not explain the presence of AP in the diet of leaf-cutter ants. However, AP is the only species offering nectar that was consistently visited by many species of nectivorous ants, possibly generating interference among them and therefore lowering visitation by the most effective nectivorous species, *C. blandus* (P. F. pers. obs.).

Conclusions

Our study suggests that nectivorous ants, low plant abundance and extremely high levels of chemical and mechanical defenses affect the pattern of herbivory by the leaf-cutter ant, *A. striatus.* These results, though preliminary, are relevant because leaf-cutter ants are very important herbivores in the Neotropics and their complete foraging behavior has never been documented in relation to the activities of nectivorous ants (but see Farji Brener 1992). Furthermore, the abundance of woody plants has not been reported before as a variable affecting leaf cutter ant's diet. However, the alternative hypothesis that leaf-cutter ants are influencing the distribution of nectivorous ants on plants should be tested (but see Justum et al. 1981). Future work should focus on direct observations of interactions between leaf-cutter ants and nectivorous ants as well as on direct experiments testing the protective role of nectivorous ants and plant species' preferences by leaf-cutter ants.

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