EVIDENCE OF INDIRECT PLANT DEFENSES IN TWO TROPICAL PLANT FAMILIES

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Abstract: Plants may use herbivore-induced chemicals to attract herbivore predators. The number and type of herbivore predators attracted may differ depending on plant family, time of day, amount of herbivory on leaf, and leaf age. We found no evidence of higher predation risk, either in the Melastomataceae or Arecaceae, for caterpillars on recently wounded leaves. Plants either were not producing volatiles that attracted predators, or we were unable to induce a response by our simulated herbivory. The type of predation on herbivores varied between night and day. In Arecaceae, the number of predators attracted decreased with leaf age. Perhaps the degree of herbivore-induced chemical production in plants is greater during seasons of high growth or herbivore pressure.

Key words: Arecaceae, clay caterpillars, La Selva, Melastomataceae, plant-induced herbivore predation, volatile chemicals

INTRODUCTION

Plants have a variety of methods of defending themselves against herbivores, including delayed greening, increased leaf toughness, rapid leaf expansion, synchronous leaf production, and secondary metabolites (Coley and Kursar 1988). An additional defense against herbivory is the release of volatile compounds in response to herbivory (Kessler and Baldwin 2001). These compounds attract herbivore predators including parasitoid wasps (De Moraes et al. 1998), ants, and rodents (Loiselle and Farji-Brener 2002) as a means of indirect defense.

By simulating herbivory on leaves from plants representing two tropical lowland rainforest families, we tested for evidence of indirect defenses of plants. Because plants often have diurnal patterns of volatile chemical production (Ryan 2001), we tested plants during both the day and night. Herbivory is more intense on young leaves, so plants may have patterns of induced responses based on leaf age. Conversely, when leaves are young, they may be physiologically incapable of synthesizing defense compounds. To evaluate these alternative possibilities, we included tests on both young and old leaves.

We predicted that predation on herbivores would be lower on Arecaceae than Melastomataceae because palms produce fewer secondary metabolites and have tougher leaves (Coley 2003). We predicted that type and amount of predation on herbivores would vary between night and day. Additionally, volatile chemical defense may be more prevalent in young leaves that lack lignification than in older leaves that are more difficult to damage (Coley and Aide 1991), thus we predicted that more predators would be attracted to herbivores on young leaves.

METHODS

On 14 February 2004, we conducted an overnight pilot study to determine if predators would attack clay caterpillars; for this, we damaged ten leaves with ~ twenty 1 mm puncture wounds (using forceps) in an assortment of plant families. We glued one clay caterpillar to each wounded leaf at dusk. Nine were recovered the next morning. Seven showed noticeable predation, usually consisting of the removal of large pieces of clay from the caterpillar body. Two caterpillars appeared untouched.

We then conducted an experiment to test for effects of previous leaf damage on caterpillar

predation risk. On 15 February 2004, we simulated herbivory on plants in the Arecaceae and Melastomataceae families at La Selva Biological Station, Costa Rica. We used previously undamaged leaves on 28 plants of each family. Trial plants were located at least 10 m apart along the Camino Circular Cercano and Camino Circular Lejano and chosen randomly. There were three morphospecies of Arecaceae and four morphospecies of Melastomataceae represented in our samples. We used young leaves on half the plants and old leaves on the other half. Half of the leaves in each age category (7) were controls, and on half we simulated herbivory by using scissors to remove four small sections of leaf. We estimated leaf age based on leaf size, color, and location on the plant. We made clay caterpillars simulating Sphingidae larvae and glued them on the center of each leaf in our study. Model caterpillars were 4 x 20 mm with painted yellow heads and green bodies (as described in Loiselle and Farji-Brener 2002).

We left caterpillar models out for 8 hours during the day, and repeated the experiment for 14 hours at night. Caterpillars were scored as depredated if they exhibited nicks or marks that appeared to come from mandibles, stingers, or bills. We performed a logistic contingency analysis using predation as the dependent variable comparing all main effects (time of day, family, age of leaf, and damaged treatment) and two-way interactions. We also collapsed types of predation into three categories based on the types of marks left on model caterpillars (Appendix 2) and tested for relationships with main effects.

Results

Model caterpillars did not suffer higher predation depending on time of day (55% vs. 48% for day vs. night), age of leaves (47% vs. 57% for old vs. young leaves), or control vs. experimental leaf damage (50% predations for both control and damaged leaves) (Table 1). Predation tended to be more probable for model caterpillars on Melastomataceae than on Arecaceae (59% vs. 44% predation), although the difference was not significant (Table 1).

No interactions were significant except family x age (Table 1). Among Melastomataceae, predation was highest for caterpillars on old leaves (56%), while among Arecaceae, predation was highest for caterpillars on young leaves (67%).

Table 1. Summary of results of logistic contingency analysis for predation. Summary includes all main effects and two-way interactions.

Effect	DF	Wald χ^2	P
Time of day	1	0.75	0.39
Family	1	2.60	0.11
Age	1	1.02	0.31
Damage	1	0.03	0.86
Time of day x family	1	1.59	0.21
Time of day x age	1	0.00	0.96
Time of day x damage	1	1.56	0.21
Family x age	1	6.41	0.01
Family x damage	1	2.77	0.10
Age x damage	1	0.39	0.53

Table 2. Summary of results for logistic contingency analysis of main effects for three functional categories of types of predation on caterpillars.

Effect	DF	Wald χ^2	P
Time of day	2	23.68	< 0.0001
Family	2	3.12	0.21
Age	2	2.92	0.23
Damage	2	0.51	0.77

The type of predation on caterpillars did not differ between plant families, age of leaves, or treatment (Table 2). However, type 2 predation occurred only at night, while type 1 and other types of predation occurred primarily during the day (Table 2, Appendix 2).

Discussion

Surprisingly, damaged leaves did not show higher predation rates on caterpillars than control leaves. This suggests that either leaves were not producing volatile chemicals that attract predators, or that they were producing the same amount of volatile chemicals prior to and independent of our treatments. It is also possible that our method of simulated herbivory did not induce a large enough plant response to create a difference in volatile chemical production between treatments. In our pilot study, we aggressively punctured and ripped leaf tissue to simulate herbivory, while in our experiment, we attempted to simulate herbivory more realistically by cutting small "bites" of leaf in our experiment. Additionally, torrential rain during the night portion of our study may have had an impact. Volatile chemical cues may be less effective when it is raining, and the number and type of caterpillar predators may also change with precipitation.

The trend towards higher predation on the Melastomataceae than the Arecaceae was in accordance with our prediction, but requires further sampling to confirm or reject. The frequency of predation did not differ with time of day, but the types of predation did, suggesting that plants produce different volatile chemicals during the day than at night, or that there is a temporal shift in the type of predators responding. While there was not an overall pattern of predation based on leaf age, there was a strong pattern within plant families. Young Arecaceae leaves attracted more predators than old leaves, while the highest amount of predation occurred on old Melastomataceae leaves. Arecaceae may produce fewer volatile chemicals as they age, allocating increased levels of resources to young leaves. Melastomataceae do not appear to allocate more resources to the defense of young leaves. They could be allocating more resources to growth, or the plants may be physiologically constrained, with only older leaves capable of synthesizing volatile chemicals.

Herbivore-induced plant chemical responses may differ between plant families and in the type of predators they attract. The magnitude of volatile chemical production in plants may be synchronized to peak during periods of rapid plant growth or higher herbivore pressure. Future studies may utilize similar methodology to examine indirect plant defenses in other plant families and with respect to other plant defense strategies.

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Appendix 1. Number of clay caterpillars depredated and not depredated in each treatment group.

Time of day	Family	Age	Damage	Predation	Number of occurrences
Day	Arecaceae	old	no	no	4
				yes	3
Day	Arecaceae	old	yes	no	4
				yes	3
Day	Arecaceae	young	no	no	3
				yes	4
Day	Arecaceae	young	yes	no	2
				yes	5
Day	Melastomataceae	old	no	no	4
				yes	3
Day	Melastomataceae	old	yes	no	2
				yes	5
Day	Melastomataceae	young	no	no	3
				yes	4
Day	Melastomataceae	young	yes	no	3
				yes	4
Night	Arecaceae	old	no	no	5
				yes	2
Night	Arecaceae	old	yes	no	7
				yes	0
Night	Arecaceae	young	no	no	1
				yes	5

Appendix 1 (Continued).

Time of day	Family	Age	Damage	Predation	Number of occurrences
Night	Arecaceae	young	yes	no	4
				yes	2
Night	Melastomataceae	old	no	no	1
				yes	6
Night	Melastomataceae	old	yes	no	2
				yes	4
Night	Melastomataceae	young	no	no	5
				yes	1
Night	Melastomataceae	young	yes	no	2
				yes	5

APPENDIX 2. Number of clay caterpillars suffering each predation type at day and night.

Predation type	Description	Number of occurrences	
		Day	Night
1	scratches, dents, or grooves	26	10
2	pairs of deep incisions	0	15
Other	includes flakes, gouges, or punctures	9	4