

The role of extrafloral nectaries in the herbivore defence of *Cassia fasciculata*

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ABSTRACT. 1. An experiment is presented that tests the hypothesis that the extrafloral nectaries of *Cassia fasciculata* reduce herbivory by attracting pugnacious ants that deter herbivores, and tests the equivalence of two methods of ant exclusion: treatment with Tanglefoot, and nectary excision.

2. The protectionist hypothesis is not supported; treatment plants produced as many pods as did control plants.

3. Treatment with Tanglefoot is equivalent to nectary excision in terms of pod production

4. A graphical model is presented that relates the efficacy of ant protection to plant density and ant activity.

Key words. Extrafloral nectaries, insect–plant interactions, ant–plant mutualism, *Cassia*, ants, plant defences.

Introduction

The hypothesis that extrafloral nectaries function to reduce herbivory through the agency of pugnacious ants has been debated by ecologists and naturalists since its inception over a century ago (e.g. Belt, 1874; Lloyd, 1901; Ihering, 1907; Wheeler, 1910; Wasmann, 1915; Janzen, 1966a, b; Bentley, 1977). The hypothesis, as stated, is testable but few experiments have addressed it. Nevertheless, confirmation of the protectionist hypothesis has been claimed for many ant–plant combinations. Proponents frequently have adduced patterns, such as negative regressions of leaf damage on distance from ant nests (Inouye & Taylor, 1979) or correlation of times of nectar secretion and ant activity (Elias *et al.*, 1975; Bentley, 1977; Tilman, 1978; Pickett & Clark, 1979), as demonstrations of protec-

tion by ants. Additional support for the protectionist hypothesis is based upon comparisons of seed set or leaf damage between plants that had attracted ants with unmanipulated plants that had not (Janzen, 1966a, 1972; McKey, 1974; Elias & Gelband, 1975; Bentley, 1977).

The strongest support for the protectionist hypothesis comes from experiments. Bentley (1976) artificially introduced 'nectar' onto plants which, compared to controls, experienced significant reductions in herbivory. Inouye & Taylor (1979) excluded ants with Tanglefoot and observed significant increases in seed predation on the unguarded plants. Koptur (1979) demonstrated significant increases in herbivory and significant reductions in seed set in plants from which she excised nectaries. Other experiments (Janzen, 1966b; Schemske, 1980; Stephenson, 1982) provide additional support for the protectionist hypothesis.

Experimental effects are directly measurable and repeatable but the inferences derived

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from them can be weakened by experimental artefacts and confounding variables (Underwood & Denley, 1984). For example, if extrafloral nectaries function in some necessary physiological capacity (Frey-Wyssling, 1955; Helder, 1958) then excision may produce an effect that is independent of the protection afforded by ants. In addition, experiments that use different treatments test different null hypotheses: an experiment that uses Tanglefoot tests the null hypothesis that Tanglefoot has no effect, one that uses nectary excision tests the null hypothesis that nectary excision has no effect. The experiments cited above use three different treatments and test three different null hypotheses. Whether the inferences derived from the rejection of these null hypotheses can be extended equivalently to the hypothesis that ants reduce herbivory will depend on whether the treatments themselves are equivalent. Unless the equivalence of these treatments is established, results from these experiments cannot be compared directly. The experiment reported here was designed to test both the protectionist hypothesis and the equality of the Tanglefoot and excision treatments. An inequality between the treatments may indicate an experimental artefact or some confounding variable.

Study sites and organisms

I conducted this experiment from June to October 1981 at Tall Timbers Research Station 35 km north of Tallahassee on the Florida–Georgia border. Tall Timbers is a mixture of pine–oak–hickory forests, pine savannahs, beech–magnolia hammocks, and agricultural fields. I selected two sites: the edge of a road bisecting a pine savannah (site 1) and a fire-break between two patches of pine–oak–hickory forest. Both sites were disturbance areas but differed in canopy cover with site 2 having the denser canopy.

Cassia fasciculata is an annual that is widely distributed throughout the Southeastern United States. It is common in disturbed areas such as roadsides and fire-breaks (Radford *et al.*, 1964). It flowers from June to September and the pods dehisce from July to October. The extrafloral nectaries are saucer-shaped and are supported by a small stalk. They are

located at the middle of each petiole. The stalked morphology of the nectaries allowed easy excision with a scalpel.

At both sites, the formicine ant, *Camponotus abdominalis floridanus* (Buckley), and the myrmicine ant, *Crematogaster ashmeadi* (Mayr), fed at the extrafloral nectaries. A qualitative estimate of ant activity at these two sites is zero to two ants on a 35 cm plant. *Camponotus abdominalis floridanus* is a large ant with powerful mandibles. It inflicts a painful bite by injecting acid into the wound. When excited, workers of *Crematogaster ashmeadi* bend their abdomens forward and protrude their stings (Leuthold, 1968). Both ants appeared to provide *Cassia fasciculata* with a formidable bodyguard against its principal herbivores, leaf hoppers and grasshopper nymphs.

Materials and Methods

At site 1, I randomly assigned 100 immature (<15 cm) individuals of *Cassia fasciculata* to four treatments: controls, extrafloral-nectary excision, coating the base of the stems with Tanglefoot, and excision plus Tanglefoot. At site 2, I randomly assigned forty-eight immature individuals to the four treatments. Tanglefoot is a sticky resinous substance that has been demonstrated to be an effective barrier to ants (Inouye & Taylor, 1979; Schemske, 1980). I removed all vegetation within a 10 cm radius about each plant, including the control and excision plants, to prevent ants from circumventing the Tanglefoot (see Keeler, 1977). The Tanglefoot was reapplied at 1–2 week intervals throughout the course of the experiment and appeared to be completely effective as no ants were seen on Tanglefoot-treated plants. The extrafloral nectaries were excised as soon as new leaves developed.

Statistical analysis

I selected three criterion variables: total number of pods attempted, number of mature pods, and the fraction of all pods to reach maturity. I considered a pod to be mature if it was at least 4 cm long and dehiscent; immature pods were typically less than 4 cm and did not dehisce. I did not measure herbivory *per se*, except in the case of total defoliation. I as-

sumed that herbivory would influence directly reproductive output in an annual such as *C. fasciculata*. This is a restrictive assumption, but if the ability to attract ants through nectar rewards is an adaptation to reduce herbivory, then the presence or absence of ants should affect reproductive output. Moreover, reproductive output is a better measure of plant fitness than is the level of herbivory.

The first two criterion variables are counts, likely to be distributed as Poisson random variables (Parzen, 1960). I transformed them by taking square roots (Box *et al.*, 1978). I transformed the final criterion variable by subtracting the natural logarithm of the total number of pods attempted from the natural logarithm of the number of mature pods. Expressing the fraction of pods to reach maturity as the difference of two logs alleviates distributional problems inherent with some ratios (Atchely *et al.*, 1976; Mosimann & James, 1979).

The experimental design is a 2×4 factorial ANOVA with sites and treatments as the main effects. This design allows independent assessment of the main effects as well as of their interaction. In addition to the ANOVA, I constructed orthogonal contrasts to test three additional hypotheses: (1) that the control group is equal to the average of the treatment groups; (2) that the excision group is equal to the Tanglefoot group; and (3) that the interaction group (both treatments applied) is equal to the average of the excision and Tanglefoot groups.

Results

The means of each treatment group were remarkably similar within a site for the number of pods attempted, the number of mature pods, and the fraction of pods to reach maturity (Table 1). The sites differed by a factor of 3 for the total number of pods attempted and the number of mature pods but were similar for the fraction of pods to reach maturity. The differences between sites was probably due to a difference in canopy cover; site 2 had a denser canopy. The control groups were highest at each site for the total number of pods attempted and the number of mature pods but were lowest at each site for the fraction of pods to reach maturity. However, these were not significant. Characteristic of all groups were high within-treatment variances relative to between-treatment differences. At site 1, the number of mature pods ranged from zero to twenty-four within treatments but the treatment means ranged only from 5.5 (Tanglefoot) to 7.4 (controls). Fig 1 illustrates the situation — the sites seem different but the treatment groups are quite variable and appear indistinguishable.

The high within-treatment variances were probably due to grasshopper feeding patterns as some plants, regardless of treatment group, were completely defoliated while other, often adjacent, plants were only partially defoliated. The control groups had six plants that were completely defoliated, the Tanglefoot groups

TABLE 1. Summary statistics for the three criterion variables.

	<i>N</i>	Total pods		Mature pods		Mature pods/ Total pods	
		<i>X</i>	<i>S</i>	<i>X</i>	<i>S</i>	<i>X</i>	<i>S</i>
Site 1							
Control	25	14.8	14.2	7.24	5.49	0.64	0.25
Tanglefoot	25	10.6	8.4	5.68	5.04	0.69	0.27
Excision	25	11.7	11.8	5.52	5.17	0.67	0.25
Tanglefoot plus excision	25	12.0	10.4	6.52	5.69	0.64	0.19
Site 2							
Control	12	4.8	3.7	2.2	2.1	0.64	0.28
Tanglefoot	12	4.7	4.8	1.9	1.8	0.65	0.28
Excision	12	3.0	3.7	1.6	2.3	0.79	0.24
Tanglefoot plus excision	12	4.2	3.7	1.9	2.0	0.65	0.22

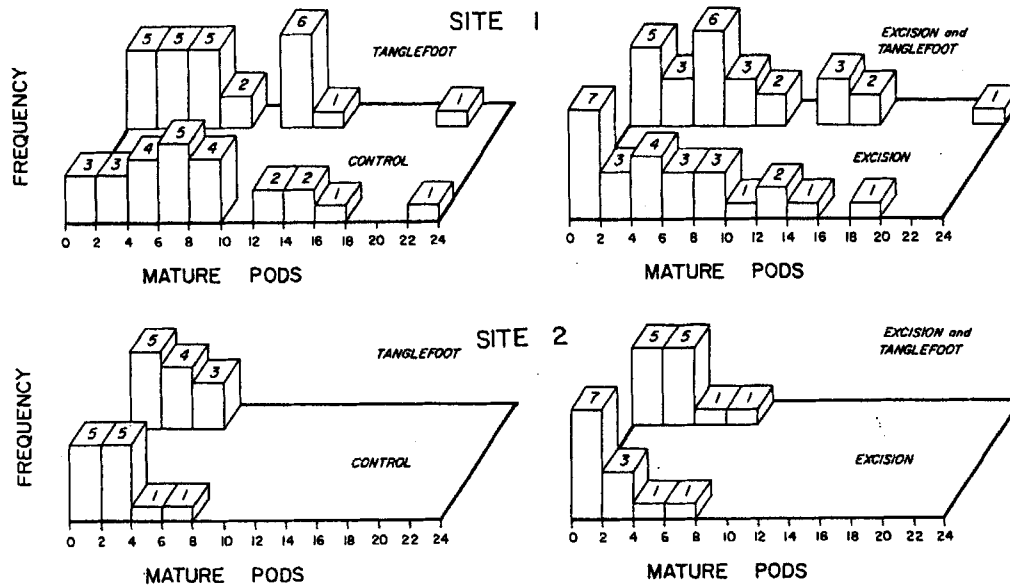


FIG. 1. Frequency histograms for the number of mature pods for both sites and each treatment.

nine, the excision groups thirteen, and the Tanglefoot plus excision groups six. However, the number of completely defoliated plants was not significantly different among the treatment groups ($\chi^2 = 2.1, P > 0.1$).

The small between-treatment differences relative to the high within treatment variances precluded significant treatment effects. No significant treatment effects were detected despite the fact that the factorial ANOVA design is powerful and the residual had 140 degrees of freedom. Only the site effect was significant for the total number of pods attempted (Table 2) and for the number of mature pods (Table 3). The treatment effect was not significant for either of these criterion variables. Furthermore, the two-way interaction was not significant for either variable.

TABLE 2. Summary of the analysis of variance for the total number of pods attempted.

Source	D.F.	SS	MS	F	Significance
Site	1	60.41	60.41	22.89	<0.001
Treatment	3	7.17	2.39	0.91	0.34
Site X Treatment	3	2.29	0.76	0.29	0.59
Residual	140	369.41	2.64		
Total	147	440.33			

TABLE 3. Summary of the analysis of variance for the number of mature pods.

Source	D.F.	SS	MS	F	Significance
Site	1	39.73	39.73	29.02	<0.001
Treatment	3	3.36	1.12	0.82	0.37
Site X Treatment	3	0.53	0.18	0.13	0.72
Residual	140	191.64	1.37		
Total	147	236.19			

The lack of a significant site-by-treatment interaction indicates that the differences between the sites simply added a constant to all treatment groups. The eight groups were indistinguishable with respect to the fraction of pods to reach maturity as neither the main effect nor the interaction was significant (Table 4).

The small between-treatment differences also precluded significant contrasts (Table 5). The first contrast, which compares the control groups to the average of the six treatment groups, is most specific in addressing the protectionist hypothesis. It was not significant for any of the criterion variables. The second contrast directly compares the Tanglefoot groups to the excision groups. Again, this contrast was not significant for any of the

TABLE 4. Summary of the analysis of variance for the fraction of pods to reach maturity.

Source	D.F.	SS	MS	F	Significance
Site	1	0.02	0.02	0.11	0.74
Treatment	3	0.44	0.15	0.84	0.36
Site X Treatment	3	0.33	0.11	0.63	0.43
Residual	140	24.68	0.18		
Total	147	25.33			

TABLE 5. Estimates and 95% confidence intervals for the orthogonal contrasts for total pods, mature pods, and the fraction of pods that reach maturity. The estimates are the values obtained for various combinations of the group means (see text for details).

	Estimate	95% confidence Interval
Total pods		
Contrast 1	0.42	(-5.13, 5.99)
Contrast 2	-0.31	(-3.67, 3.05)
Contrast 3	0.26	(-2.93, 3.44)
Mature pods		
Contrast 1	0.28	(-2.40, 2.96)
Contrast 2	-0.20	(-1.75, 1.35)
Contrast 3	0.22	(-1.72, 2.15)
Fraction of pods to reach maturity		
Contrast 1	0.09	(-0.22, 0.41)
Contrast 2	0.11	(-0.20, 0.42)
Contrast 3	-0.04	(-0.17, 0.09)

Contrast 1: Controls v. Tanglefoot, Excision, Tanglefoot and Excision.

Contrast 2: Tanglefoot v. Excision.

Contrast 3: Tanglefoot, Excision v. Tanglefoot and Excision.

criterion variables. This result is necessary to establish the equivalence of the Tanglefoot and excision treatments but is not sufficient because these treatments could produce different effects but of equal magnitude. If they do produce different effects of equal magnitude, then their combination in the interaction group (both treatments applied) should produce a still larger effect. The third contrast compares the interaction groups (both treatments applied) to the average of the Tanglefoot and excision groups. It was not significant for any of the criterion variables. This together with an insignificant second contrast suggests that the Tanglefoot and exci-

sion treatments are equivalent. In addition, the contrast estimates were close to zero for each criterion variable-by-contrast combination. For example, the contrast estimate for the number of mature pods, 0.28, represents less than a one pod difference between the control groups and the average of the treatment groups. This suggests that the treatment differences would remain insignificant even with a greatly increased sample size.

Discussion

Schimper (1903) suggests that the presence of extrafloral nectaries does not guarantee protection by ants. This experiment supports his contention that ant-plant interactions may be circumstance-dependent. The efficacy of an ant-mediated herbivore defence may depend on herbivore densities and ant activity (Fritz, 1983). Price *et al.* (1980) suggest that predator-mediated defences of plants may be influenced by characteristics of plant communities and populations. At both sites, *Cassia fasciculata* occurs in high density stands and may represent a superabundant resource to the ants. If this is so, the frequency and duration of ant visitation on individual plants will be reduced. The protection afforded to a plant by occasional visits may be negligible.

Fig. 2 is a graphical model that relates the efficacy of ant protection (benefit to a plant) to plant density and ant activity. The model

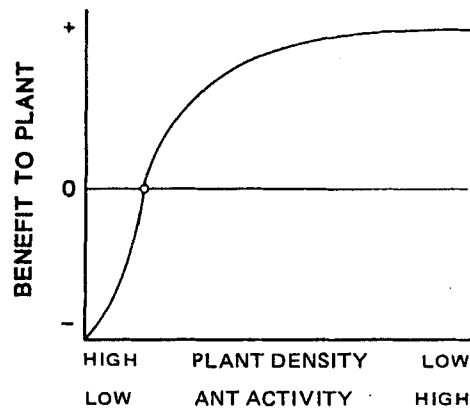


FIG. 2. Proposed relationship between the efficacy of ant protection (benefit to the plant) and plant density and ant activity for plants with active extrafloral nectaries. The open circle represents the point of neutral benefit.

assumes that herbivore density and overall ant abundance are constant and that the number of ants on individual plants is inversely related to plant density. At high plant densities or at low levels of ant activity, the benefit to plants with active extrafloral nectaries may actually be negative owing to the physiological costs of nectar production and secretion. These costs should be quickly compensated as plant density decreases and ant activity increases. At intermediate plant densities the net benefit should be positive and should approach an asymptote; not even the most aggressive ants at high densities will be 100% effective. Admittedly, it is a great leap from the two populations studied here to this general model but this experiment may have examined *Cassia* populations near the 'break-even' point of this model. However, an experiment that varies patch size, patch density, and ant exclusion will be necessary to test this hypothesis and this model.

Barton (1983) suggests that ants benefit *Cassia fasciculata* in areas of high herbivore pressure but are of little or no benefit in areas of low herbivore pressure. It seems reasonable that herbivore density could easily be substituted for the abscissa in Fig. 2.

The equivalence of treatment with Tanglefoot and nectary excision would best be established through experiments conducted in a controlled environment, such as a glasshouse. However, this experiment at least suggests that treatment with Tanglefoot is equivalent to nectary excision. Schimper (1888, cited in Schimper, 1903) excised nectaries from *Cassia neglecta*, found no change in the plants, and concluded that extrafloral nectaries did not have an important physiological function. However, this type of evidence is insufficient to eliminate a physiological hypothesis (Wheeler, 1910) or to accept the protectionist hypothesis by default. It does suggest that studies using either treatment can be compared directly.

The general hypothesis that extrafloral nectaries have evolved in conjunction with ants as an anti-herbivore device is not testable. This experiment has tested and refuted the more restrictive hypothesis that the extrafloral nectaries of *Cassia fasciculata* function to reduce herbivory through the agency of pugnacious ants. Entertaining this hypothesis

establishes a theatre in which the general hypothesis can be addressed in ecological time. It does this by allowing an explicit null hypothesis (no treatment effect) to be formulated, tested experimentally, and contrasted against the alternative hypothesis (protection by ants). Strong inferences from this experiment are parochial; their strength lessens as these results are extended to other sites, years and species. The inferences are especially weak in an evolutionary context. However, only through an accumulation of many restrictive studies, both that support and refute the protectionist hypothesis, can the necessary conditions for effective ant protection be identified and the general hypothesis be accurately evaluated.

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