Extrafloral nectaries: ants, herbivores and fecundity in *Cassia fasciculata*

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Summary. Extrafloral nectaries of *Cassia fasciculata* attract nectar feeding ants which protect the plant against leaf herbivores. High ant visitation in late July coincided with high herbivore densities at two sites in east central Iowa. The highest level of leaf herbivory occurred during the time of flowering and early fruit filling, just after the peak of herbivore and ant activity. Results of ant exclusion experiments at the two sites showed that ant visitation resulted in decreased herbivore numbers, decreased leaf area loss, increased growth, and at one site decreased plant mortality. However, this reduction in leaf area loss and increase in growth did not translate into seed set differences between plants with and without ants at either site. Initial plant size was more important than the presence or absence of ants in determining fecundity for this temperate annual during a year of summer drought.

Recent investigations of plant-ant interactions have centered on the degree to which extrafloral nectaries provide plants with protection against leaf herbivores and seed predators by attracting nectar feeding ants. Ant visitors can provide the plants with a defense against leaf herbivores (Deuth 1980; Koptur 1979) and/or seed predators (Keeler 1981; Inouye and Taylor 1979) through their aggressive behavior (Janzen 1966) or by removing the herbivore eggs from the plant (Letourneau 1983; Schemske 1980). The assumed benefit of this protection is that plant fitness is increased either indirectly because reduced leaf herbivory leads to increased seed production, or directly through decreased seed predation.

Much of the data supporting the role of extrafloral nectaries as a plant defense have been of a correlative nature. For example, studies have demonstrated low leaf herbivory (Beckman and Stucky 1981; Deuth 1980; O'Dowd 1979; Pickett and Clark 1979) and/or high nectar production (Bentley 1977) to be associated with high ant visitation rates. In contrast, there has been little experimental demonstration of the role of ants as protective agents. Keeler (1981), Schemske (1980) and Schemske and Horvitz (1984) demonstrated that ants increase plant fecundity by warding off seed predators. Koptur (1979) and Stephenson (1982) experimentally demonstrated that ant protection reduced

leaf damage levels, and so doing, resulted in an increase in seed production for the plant.

However, not all experimental studies support the ant protection theory. Tempel (1983) and O'Dowd and Catchpole (1983) present evidence that extrafloral nectaries are not providing protection against leaf herbivores and seed predators, respectively. Tempel (1983) demonstrated that the pattern of nectar production and ant visitation in bracken fern (*Pteridium aquilinum*) were synchronous but that herbivory levels and herbivore numbers did not coincide with the timing of nectar production and ant visitation. O'Dowd and Catchpole (1983) found that ant excluded plants of the genus *Helichrysum* had higher numbers of insects than those plants with ants present, but found no difference in the numbers of specialist seed predators visiting the plant. Thus, there were no differences in the number of mature seeds produced. These conflicting results and the sparsity of experimental data leave in question the importance of extrafloral nectaries as a defense mechanism.

I chose the annual *Cassia fasciculata* Michx. (Leguminosae: Caeselpinoideae) to ask three questions concerning the role of extrafloral nectaries as a plant defense. First, do ants reduce the numbers of herbivores feeding on *C. fasciculata*? Second, does reduction in herbivore numbers result in increased fitness as measured by plant fecundity? Third, since the efficacy of ant protection has been shown to vary among sites (Bentley 1976; Inouye and Taylor 1979), does the amount of protection provided to *Cassia* vary over a small geographic distance?

Methods

Cassia fasciculata is a temperate annual legume which grows in open and often times disturbed areas (Foote and Jackobs 1966). In eastern Iowa, seeds germinate in early June. The leaves are pinnately compound, with 4–26 leaflets at sites examined. Extrafloral nectaries are borne at the base of each leaf petiole with the exception of the first three or four seedling leaves. Nectar is produced both day and night and throughout the growing season (pers. obs.). Flowers are produced on racemes in the leaf axils from late July into September. Fruits mature throughout August and September and contain between one and 16 seeds.

I performed ant exclusion experiments on *C. fasciculata* during the summer of 1983 at two different sites in Johnson County, Iowa separated by 15 miles. The Jolly Roger's Road site (JR) was located 13 miles N of Iowa City (Ely Quadrangle) on a gravel road. Williams Prairie (WP) was

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located 16 miles W of Iowa City (Amana Quadrangle) on a high portion of wet prairie. During the last week of June and the first week of July, eighty seedlings, approximately each 0.5 m apart, were marked at each site along a transect. These plants were then alternately assigned to either a control or experimental group. Experimental plants had ants excluded by applying Tanglefoot to the base of the stem. Tanglefoot prevented ants from gaining access to the extrafloral nectaries of the plants while allowing flying and/or jumping herbivores, parasitoids and seeds predators to utilize the leaves and fruits. Tanglefoot was reapplied throughout the summer as needed. Vegetation around all plants was clipped periodically to prevent ants from using it as a bridge to the nectaries.

Plants were censused twice a week from early July to late September for survivorship and for ant, herbivore, and herbivore egg densities. Herbivores censused were lepidopteran larvae, coleopteran beetles and acridid grasshoppers. Plant size was measured four times throughout the growing season, beginning with the initially marked plants in late June. The total number of leaflets present on a plant was used as an indicator of plant size. Initial plant size was defined as the size of the plant at the beginning of the first census period. Plants at this stage had a total of 18–78 leaflets at WP and 26–130 leaflets at JR.

Leaf herbivory was also measured four times throughout the season. Percent leaf area was calculated as the number of 1/4 leaflets missing per plant divided by plant size. Since leaf scars of senescent leaves are evident along the base of the stem, I was able to follow the damage record for each leaf and leaflet throughout its life on the plant. Because leaves senesce and abscise as the plant grows, I considered a leaf to have been totally eaten only when the petiole stub remained or the whole plant had been eaten. Otherwise, the leaf was considered senesced and, accordingly, excluded from damage estimates. Thus my estimates of leaf herbivory are conservative. Analysis of herbivore eggs, herbivore numbers and leaf damage was done with nonparametric statistics since these data were not normally distributed.

Growth was calculated as the number of leaflets added between two census periods. Analysis of covariance (ANCOVA) was used to examine for potential differences in growth between control and experimental plants while controlling for the influence of initial plant size. Height or height increment was not used as a measure of size or growth, respectively, since plants can lose their apical meristems to herbivores and send out axial branches in two or three directions.

Only those plants producing flowers were considered in the seed analysis. Fruits were collected throughout the fall as they matured. I considered seed production to be the sum of all seeds produced for each plant. The effect of ant exclusions on seed production was analyzed by ANCOVA, again using plant size as the covariate. The square root transformation of the variable, seed production, was used to achieve normality. Weighted least squares were used for seed data from JR to correct for an increasing variance in seed production with increasing initial plant size. Weighted least squares was obtained by grouping the plants into two size classes, those larger than the initial size mean and those smaller than the initial size mean. Variances for seed production were than computed for the two size classes separately. Each plant's seed set score (Y observed-Y ex-

pected) was then weighted by the inverse of the size class variance to which it belonged (Neter and Wasserman 1974).

The site data are analyzed separately. My initial intention was to consider site differences as well as their interactions in the analysis. However, I was unable to perform this analysis with parametric statistics since the variances of the two sites were heterogenous for seed production (Cochran's test, $P < 0.01$) (Kirk 1982). Although nonparametric methods would have allowed me to make this comparison, these methods would not allow me the use of the covariate which analysis showed to be a significant factor.

Results

The extrafloral nectaries of *Cassia fasciculata* are visited by several ant species (Table 1). At the JR site ant visitation fluctuated greatly, with peaks in late July, late August and late September, but at WP it remained at a low, constant level throughout the season (Fig. 1). Between plant variability in ant visitation was high at both sites, but greater at JR (Fig. 1). For example, the widest range in the number of ants per plant at JR was 0 to 33 on August 8, whereas

Table 1. Ant species visiting nectaries of *C. fasciculata* at the two study sites during the summer of 1983

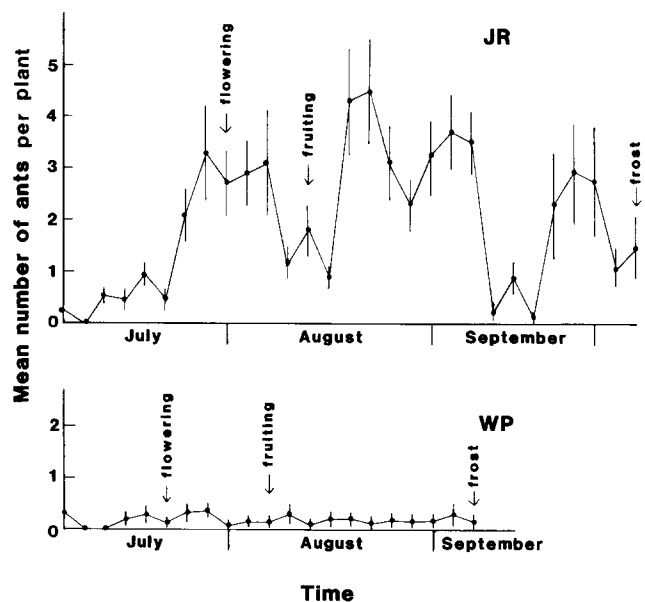|	
WP	*Myrmica americana* Weber
	Lasius alienus Foerster
	Formica fusia L.
	Leptothorax curvispinosus Mayr
	Crematogaster lineolata Soy
	Lasius niger L.
	Paratrechina parvula Mayr
JR	*Monomorium minimum* Buckley
	Leptothorax curvispinosus Mayr
	Crematogaster lineolata Soy
	Aphaenogaster rudis Emory

Fig. 1. Ant abundance throughout the summer of 1983 for control plants at the two study sites. Bars represent plus and minus one standard error. Flowering and fruiting represent the initiation of these processes
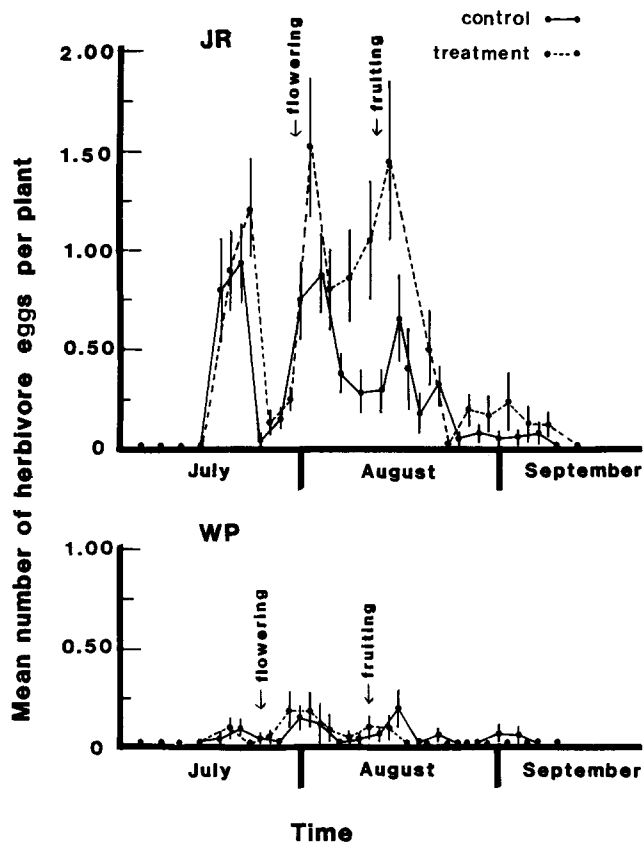
Fig. 2. Mean number (± one standard error) of eggs of *Eurema lisa* on control and experimental plants of *C. fasciculata* at the two study sites throughout the summer of 1983. Flowering, fruiting and frost represent the initiation of these processes
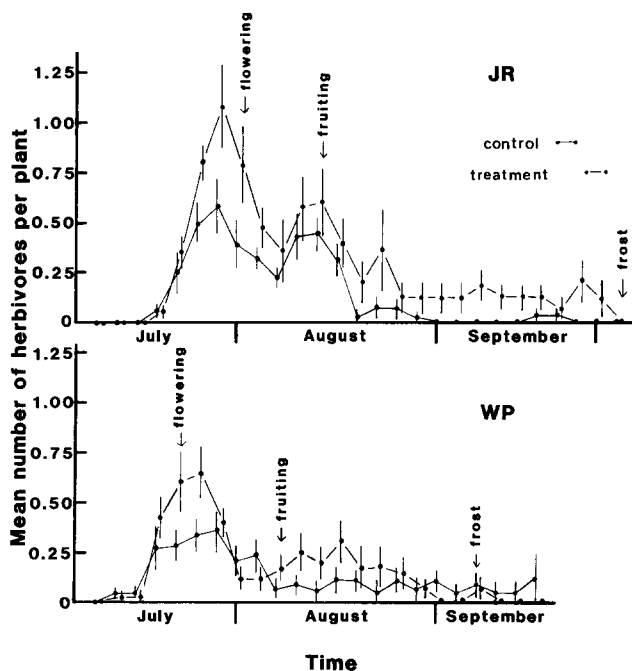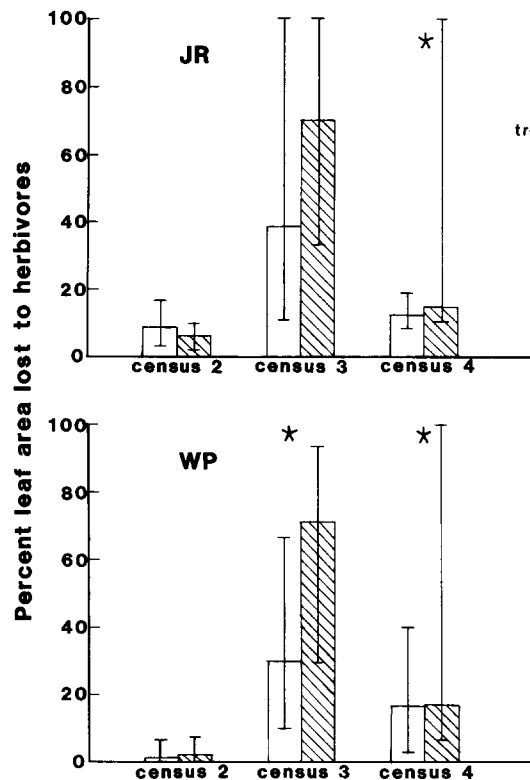

Fig. 4. Medians with first and third quartiles of percent leaf area lost to herbivores during the summer of 1983 for control and experimental plants. Area lost was measured at three dates for each of two study sites. At *JR*: census 2(July 15), census 3(August 13), census 4(September 17). At *WP*: census 2(July 13), census 3(August 3), census 4(August 28) *$P<0.05$

Fig. 3. Mean number (± one standard error) of herbivores throughout the summer of 1983 for control and treatment plants at the two study sites. Flowering, fruiting and frost represent the initiation of these processes

at WP it was 0 to 3 on July 25th. The Tanglefoot treated plants were always free of ants.

Several herbivores utilize *C. fasciculata* at the two sites studied. The larvae of *Eurema lisa* (Pieridae: Lepidoptera) were the major defoliators of *C. fasciculata* at both sites and they often consumed entire plants. Damage due to grasshoppers was observed but these insects never defoliated an entire plant. Likewise, tortricid moth larvae were capable of severely damaging a leaf or a branch but never the whole plant. Parasitoids were uncommon around the plants at both sites. Of the 20 pierid larvae reared throughout the summer of 1983, none were parasitized.

Ant exclusions resulted in increased numbers of lepidopteran eggs and herbivores and, in turn, higher leaf damage. At JR the mean number of lepidopteran eggs per plant was consistantly higher on experimental than on control plants throughout the season (Fig. 2), but not at WP. At JR there were significant differences between control and treatment plants in the number of eggs per plant in mid August (Mann-Whitney U, $P<0.05$). At both sites herbivore levels peaked in late July and gradually decreased toward the end of the growing season (Fig. 3). At JR, the mean number of herbivores was consistantly greater on experimental versus control plants throughout the season (Fig. 3). These differences were significant at peak herbivore abundance in late July (Mann-Whitney U-test, $P<0.05$) (Fig. 3). Likewise, at the peak herbivore densities in late July at WP, there was a strong treatment effect on the number of herbivores present on plants (Mann-Whitney

Table 2. Analysis of covariance for the effect of treatment and initial plant size (the covariate) on subsequent growth. Means $(\bar{X})$ and standard deviations are given for the number of new leaflets added between census periods during the summer of 1983. The means adjusted for the covariate are given when the covariate is significant (Pedhauzer 1982)

| Census	Control $\bar{X} \pm$ SD	$\bar{X}_{adj.}$	N	Treatment $\bar{X} \pm$ SD	$\bar{X}_{adj.}$	N	F	P
JR								
1–2	77.0± 48.1	74.6	39	70.2± 36.3	72.7	37	0.0	0.948
				Initial plant size			21.85	0.0001
2–3	249.0±318.5	221.4	36	113.4±144.2	140.4	37	8.68	0.087
				Initial plant size			40.54	0.0001
3–4	57.2± 50.8		18	17.3± 41.2		12	3.96	0.056
				Initial plant size			0.2	0.66
WP								
1–2	64.1± 25.5	64.6	34	64.5± 28.9	63.6	33	0.02	0.896
				Initial plant size			10.13	0.002
2–3	87.0± 51.6	87.46	32	61.8± 66.8	61.4	31	3.19	0.079
				Initial plant size			4.85	0.031
3–4	20.5± 23.5	21.2	28	33.4± 35.3	32.3	18	1.73	0.195
				Initial plant size			4.16	0.047
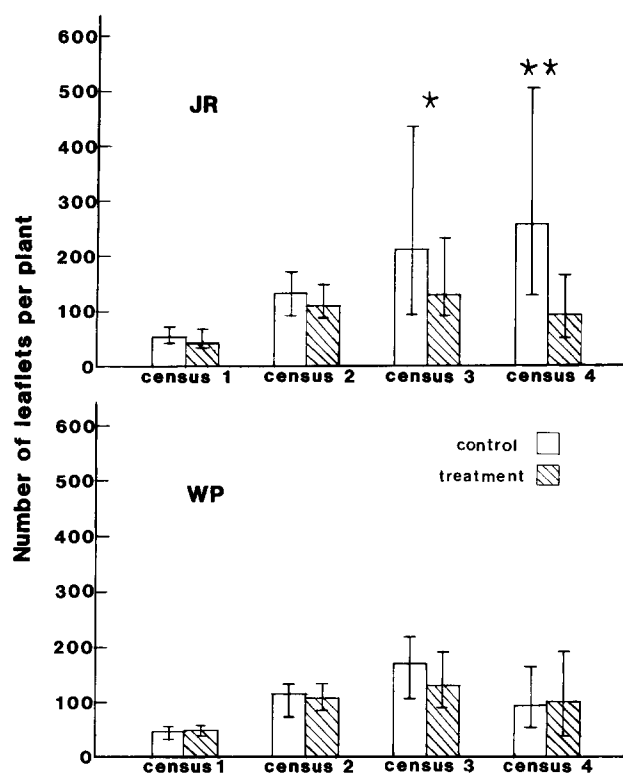
Fig. 5. Medians with first and third quartiles of plant size as measured by the total number of leaflets. Measurements are for control and experimental plants during the summer of 1983 at both study sites. At *JR*: census 1(July 2), census 2(July 15), census 3(August 13), census 4(September 17). At *WP*: census 1(June 24), census 2(July 13), census 3(August 3), census 4(August 28). $^{*}P<0.05$, $^{**}P<0.01$
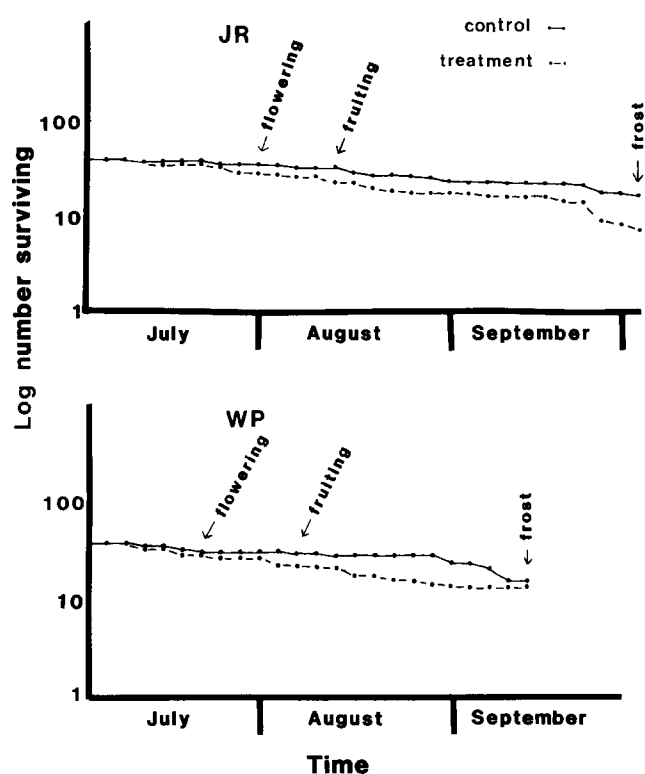

Fig. 6. Survivorship of control and experimental plants is shown for the summer of 1983 at the two study sites. Flowering, fruiting and frost represent the initiation of these processes

U-test, $P<0.05$) (Fig. 3). Ant exclusions resulted in a greater per cent leaf area loss incurred by plants between censuses 3 and 4 at JR (Mann-Whitney U-test, $P<0.05$) (Fig. 4) and at WP between censuses 2 and 3, and censuses 3 and 4 (Mann-Whitney U-test, $P<0.05$) (Fig. 4).

At both sites, growth was influenced by initial plant size, as well as by the presence of ants. Results of the growth ANCOVA (Table 2) indicate that initial plant size in early July was a significant predictor of future growth between censuses 1 and 2, and 2 and 3 at both sites. In addition to the initial size effect, ant-excluded plants tended to grow less between censuses 2 and 3 ($P<0.087$), and 3 and 4

Table 3. Analysis of covariance for the effects of treatment and the covariate, initial plant size, on seed production at the two study sites. Actual variable used in the analysis was $\sqrt{\text{seed number}}$. The means adjusted for the covariate are given (Pedhauzer 1982)

Site		$\bar{X} \pm SD$	N	$\bar{X}_{adj.}$	F	P
JR	Control	11.92 ± 6.7	21	11.1	1.14	0.292
	Treatment	6.36 ± 4.8	19	7.3		
	Initial plant size				22.31	0.0001
WP	Control	2.2 ± 2.4	26	2.3	2.38	0.131
	Treatment	3.4 ± 3.3	16	3.3		
	Initial plant size				4.9	0.032

$(P<0.057)$ at JR and between censuses 2 and 3 at WP $(P<0.079)$. Thus those plants which were initially larger grew more then smaller plants, and those plants with ants grew more than those plants without ants. These increases in growth translated into plant size differences at censuses 3 and 4 at JR but not at WP (Fig. 5).

For both sites, survivorship was consistantly higher for plants visited by ants than in those from which ants had been excluded (Fig. 6). While there was no significant difference in survivorship prior to reproduction between controls and ant-excluded plants at JR, those plants at WP with no ants suffered significantly higher mortality (Kolmogorov-Smirnov two sample test, $P<0.05$) than those plants with ants present. The largest increase in mortality for plants at WP with ants excluded occurred in mid August when heat and drought conditions were most extreme (NOAA 1983). Seed production was not affected by the treatment of excluding ants. The ANCOVA for seed production showed that initial plant size was a significant predictor of seed producion at both sites while the exclusion treatment was not so at either site (Table 3).

Discussion

If extrafloral nectaries serve as a plant defense against herbivores, ant visitation should decrease herbivore damage. Furthermore, if herbivory acts as a selective force for the maintenance of extrafloral nectaries, the decrease in herbivory associated with these nectaries should result in increased fecundity. This means that it is not only necessary for ants to be attracted to plants, but their presence must coincide with that of the participating herbivores which affect plant fecundity.

In this study the potential for extrafloral nectaries to act as a defense mechanism exists due to the coincidence of herbivores and ants. Ants were present on plants at both sites in late July and August when herbivores were active. Ants were also present on the plants when the greatest amount of leaf damage was measured at both sites. In addition, the majority of leaf area loss occurred during the flowering and fruit filling period when one would suspect this loss to have the greatest effect on fecundity. Finally, ant presence resulted in significant decreases in leaf area loss at both study sites during this time.

Despite the coincidence of ant visitation, and herbivore visitation and damage, the results of the ant exclusion experiments do not show that these ant-herbivore interactions affect seed set. At JR the increase in herbivore numbers, the trend for greater leaf herbivory, decreased growth and decreased size associated with ant-excluded plants, all support the ant protection theory. Likewise, at WP increased herbivore numbers, increased herbivory, decreased growth, and decreased probability of survival to reproduction were associated with the ant excluded plants. However, for both sites these differences did not depress seed set in treatment plants. Thus, ant presence affected the probability of survival to reproduction and the amount of leaf area a plant had at that time. It did not however, affect how many seeds were produced during that reproductive period.

Studies of other systems have demonstrated that ant attraction does not necessarily translate into increased fitness. Tempel (1983) presented incongruities in the timing of ant-herbivore activity and O'Dowd and Catchpole (1983) found no effect of ants in deterring an important seed predator. The present study presents a different incongruity. Why is the correct timing of ant-herbivore interactions and the positive effect of ant deterrence on important herbivores not translated into increased fecundity for surviving plants? Several factors may have contributed. First, since high variation among control plants existed in the amount of ant visitation, certain control plants with low numbers of ants may have resembled the experimental plants more than they did other control plants with higher numbers of ants. Thus, ants could affect seed production but this experiment was unable to show it due to unclear boundaries between the two groups. However, this is unlikely because of the significant differences between the two groups in leaf herbivory and growth.

A second potential explanation is that herbivory may not depress seed set in C. fasciculata because a large portion of the photosynthate needed to mature seeds is provided initially by the green fruits. Flinn et al. (1977) found that illuminated pea fruits required 19% less carbon from the parent plant than pea fruits grown in darkness. Thus, since C. fasciculata fruits are green in early stages of development, they may be supplying some of the photosynthate needed to mature the seeds.

Finally, the lack of relationship between greater herbivory, decreased growth, and decreased seed production may be due to depressed seed production for all plants. The study season (1983) was a drought summer in Iowa, as precipitation was 50% and 60% below average for July and August, respectively (NOAA 1983). Lee and Bazzaz (1982) have shown that C. fasciculata grows less and produces fewer flowers under water stress. If the study plants were under water stress, the differences in herbivore damage may not have affected seed set to any large degree because the plants may not have had the resources for greater seed output, regardless of whether ants were present. The plants at WP appeared to be under severe water stress since they were not only much smaller than those at JR but were also two to three times smaller than other Cassia plants growing in wetter, more shaded areas at the same site. Plants at WP with ants did not convert increased growth and decreased herbivory into increased size because leaf senescence at the bottom matched leaf production at the top. Thus, the presence of ants may not have had any influence on plant fitness due to the limitation of water. Positive effects of ants may be easier to show under more favorable water conditions.

An interesting result of the seed analysis was that initial plant size was a more important determinant of seed set for this annual than ant visitation. Larger plants grew more and finally produced more seed due to their initial size advantage. Correlation analysis within the control plants showed a positive relationship ($r=0.847$, $P<0.0001$) between the number of ants on a plant and seed set at JR. Thus ants may be initially attracted to larger seedlings. Because of this, larger seedlings were able to grow more and produce more seed due to the effect of ant protection against herbivores.

The *Cassia*-ant-herbivore interaction differed at the two study sites in two important aspects. First, control plants were significantly smaller at WP than at JR at all four census periods (Fig. 5) and attracted fewer ants (Fig. 1). This is despite the fact that initial baiting experiments in 1983 as well as personal observations in 1984 indicated large numbers of nectar feeding ants at both sites. Perhaps the plants at WP were not large enough to attract as many ants as those at JR. Second, there were fewer herbivores on experimental plants at WP than at JR. These inter site differences provide evidence that interactions of this type can vary spatially. Thus, there can be variability in the numbers of herbivores which act as a selective force for the maintenance of old or the evolution of new defenses, and variability in the number of ants acting as a plant defense.

I conclude from this study that extrafloral nectaries have the potential to increase plant fitness for *Cassia fasciculata* by attracting ants which protect the plants against herbivores. Plants visited by ants had lower numbers of herbivores, suffered less herbivory, produced more leaf area, and at one site suffered less pre-reproductive mortality. Whether this potential is actually translated into increased fecundity could be a function of seasonal weather patterns and available resources. In this study, drought conditions were associated with a lack of an effect of ant visitation on seed production. As a result, initial plant size was of greater importance in determining fecundity than was the amount of ant protection against herbivores. Since resources and weather patterns can vary greatly over geographical distances and through time, the effect of ant visitation on the fecundity of *C. fasciculata* undoubtedly varies between years and sites. Consequently, the strength of the selective pressure to maintain this facultative plant-ant mutualism must vary both spatially and temporally.

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