

J. H. Ness

***Catalpa bignonioides* alters extrafloral nectar production after herbivory and attracts ant bodyguards**

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Abstract Inducible anti-herbivore defenses are found within many plant taxa, but there are fewer examples of inducible indirect defenses that incorporate the third trophic level. This study links caterpillar foraging, herbivore-induced changes in extrafloral nectar production, and the attraction of ants to vulnerable leaves and plants. *Catalpa bignonioides* Walter (Bignoniaceae) uses extrafloral nectar to attract ant (*Forelius pruinosus* (Roger)) bodyguards in response to *Ceratomia catalpae* (Boisduval)(Lepidoptera: Sphingidae) herbivory. Ant density per leaf increased with the sugar content of extrafloral nectar excreted by sampled leaves, suggesting that increased nectar production could attract or retain beneficial arthropods. The masses of sucrose, fructose, glucose and all three sugars combined in the extrafloral nectar increased two- to three-fold on attacked leaves within 36 h of the experimental addition of caterpillars. Production rates for neighboring non-attacked leaves and non-attacked leaves on adjacent plants did not differ over the same time period. Ant attendance at caterpillar-attacked leaves increased two- to three-fold within 24 h of herbivory, relative to attendance at neighboring, undamaged leaves. These attacked leaves attracted the fewest ants prior to the onset of herbivory, suggesting the specialist caterpillar may avoid or be excluded from leaves with more bodyguards. The removal of leaf tissue with scissors did not alter ant attendance at damaged leaves. Mean ant attendance per leaf on attacked plants increased 6- to 10-fold after caterpillar introduction, relative to adjacent unattacked plants. The plant's biotic defense thus operates at two scales; the number of bodyguards (ant workers) on the plant increases after attack, and this increased workforce is biased towards

attacked leaves within plants. Fewer caterpillars remained on plants that attracted greater numbers of ants, suggesting these bodyguards benefit the plant.

Keywords Indirect plant defense · Induction · Mutualism · Rewards · Tritrophic interactions

Introduction

Induced defenses are responses activated through a previous encounter with a consumer or competitor that confer some degree of resistance to subsequent attacks. Inducible defenses are distinguished from constitutive defenses by their activation in response to environmental stimuli (Harvell 1990). Induced defenses are observed within a range of taxa and frequently include the induction of new or increased physical and chemical defenses (e.g., trichomes, spines, and secondary compounds in foliage, see Karban and Baldwin 1997). These defenses are known as “direct defenses” because they act directly against the herbivore/consumer. The abundance and impact of herbivores can also be limited by higher trophic levels (e.g., predators, parasitoids and pathogens), and traits that enhance the performance of these agents are considered “indirect” plant defenses (Price et al. 1980; Dicke 1999). As with chemical and structural defenses, there is growing evidence that indirect biotic defenses may be inducible (e.g., Turlings et al. 1995; DeMoraes et al. 1998; Dicke 1999; Thaler 1999; Agrawal 1998).

Carnivorous insects, and ants in particular, are excellent candidates to act as inducible, biotic plant defenses due to their mobility, capacity for recruitment, and sensory capacity to detect disturbance or chemical cues (Agrawal and Rutter 1998; Agrawal and Dubin-Thaler 1999). Many plants offer extrafloral nectar containing valuable sugars and amino acids (Koptur 1992) and herbivore-induced changes in nectar production could benefit the plant by attracting new nectary visitors (carnivores) or by steering visitors towards attacked portions of the plant (Koptur 1989; Smith et al. 1990;

J.H. Ness (✉)

Institute of Ecology, University of Georgia, Athens,
GA 30602–2601, USA
e-mail: jness@email.arizona.edu
Fax: +1-520-6219190

Present address:

J.H. Ness, Department of Ecology and Evolutionary Biology,
University of Arizona, Tucson, AZ 85721, USA

Agrawal and Rutter 1998). Some ants track seasonal and diurnal changes in extrafloral nectar production (Tilman 1978; Stephenson 1982; Gaume and McKey 1999), exhibit preferences for nectars or honeydews on the basis of sugar and/or amino acid composition (Lanza et al. 1993; Völkl et al. 1999), and stay longer in patches with more sugar (Bonser et al. 1998). Similarly, nectar-satiated parasitoids stay in herbivore-occupied patches longer and attack more herbivores (Stapel et al. 1997). These observations suggest that plants with increased nectar production could attract or retain more bodyguards, thereby receiving greater protection against herbivores.

Although changes in nectar production subsequent to leaf damage have been reported in seven plant species, the link between cause (herbivory) and effect (altered nectar production resulting in recruitment or retention of plant bodyguards) is tenuous. Two studies failed to compare treatment and control leaves prior to the damage (*Catalpa speciosa*: Stephenson 1982, *Campsis radicans*: Stevens 1990). Four greenhouse experiments have shown induced changes in nectar production in response to varied stimuli. Scissor damage induced increases in extrafloral nectar volume and amino acid concentrations in *Vicia sativa* (Koptur 1989) and *Impatiens sultani* (Smith et al. 1990), respectively. Herbivory induced a localized increase in nectar production rates in castor bean (*Ricinus communis*) and both a localized and weakly systemic response in cotton (*Gossypium herbaceum*) (Wäckers et al. 2001). None of these studies, however, causally linked these responses to bodyguard recruitment or plant protection, although Lanza et al. (1993) found that laboratory colonies of *Solenopsis geminata* (but not *S. invicta*) preferred solutions that mimicked the post-defoliation composition of *I. sultani* to pre-defoliation mimics. One author cautioned that alterations in nectar production may be obscured in the field due to coincident changes in ambient humidity, wind, and other factors (Koptur 1989). Only one study has successfully linked herbivory as an elicitor of extrafloral nectar production with subsequent predator recruitment and some form of plant protection (*Macaranga tanarius*, Heil et al. 2001a).

Here, experiments that explore the recruitment of beneficial arthropods by *Catalpa bignonioides* Walter (Bignoniaceae) after the plant is exposed to herbivory by *Ceratomia catalpae* (Boisduval) (Lepidoptera: Spingidae) are described. The direct defenses of *Catalpa bignonioides* include iridoid glycosides sequestered in the foliage, although these compounds act as a feeding stimulant for the specialist *Ceratomia catalpae* (Nayar and Fraenkel 1963). The plant's indirect defenses include extrafloral nectaries, and this study focuses on visitation by *Forelius pruinosus* (Roger) (Formicidae: Dolichoderinae), the most common nectary visitor at the study site. The following questions are addressed: (1) are ants found in greater abundance on leaves with more productive nectaries; (2) does herbivory induce changes in extrafloral nectar production; (3) do ants aggregate on leaves damaged by caterpillars; (4) does ant attendance on plants increase after caterpillar attack, and (5) do

caterpillar disappearance rates increase on plants visited by more ants?

Materials and methods

Organisms and site

The southern catalpa, *Catalpa bignonioides*, often occupies disturbed mesic habitats. Extrafloral nectaries are found at the intersection of major veins within leaves; 4–9 active nectaries are generally found on a mature leaf (mean \pm SD: 6.2 \pm 1.7, $n=72$ leaves). Leaf lifespan averages about 5–6 months, and young leaves (<1 month old) do not secrete nectar. *Ceratomia catalpae* is the only known defoliator of the northern and southern catalpas, *Catalpa speciosa* and *C. bignonioides*, respectively (Baerg 1935). Moths lay more than 100 eggs on a single leaf, and caterpillars feed gregariously until the third and fourth instar, at which time solitary individuals become common. Beyond these caterpillars, the *Catalpa* herbivore community is depauperate. Stephenson (1982) recorded an agromyzid leaf miner attacking *C. speciosa* in Michigan, and I witnessed a brief scale outbreak limited to three *C. bignonioides* trees in Georgia (species unknown, Ness, personal observation).

Research was conducted at the University of Georgia Horticulture farm, Clarke County, Georgia, USA, from June to November, 1999–2000. The Horticulture farm includes a planted stand of six *Catalpa* trees bordering an artificial pond. These trees are approximately 15 years old, 3–5 m in height, and surrounded by fifty 2- to 4-year-old *Catalpa* seedlings 1–3 m in height. The surrounding landscape includes thinned secondary forest and mixed agricultural plots.

A variety of arthropods visit *Catalpa* nectaries at this and other sites [e.g. *Camponotus*, *Prenolepis*, *Crematogaster*, and *Formica* ants, coccinellids, and vespid wasps (Stephenson 1982; Ness, personal observation)], although *F. pruinosus* was by far the most frequently observed visitor at the study site (i.e. >95% of arthropods observed). This widespread, common ant is a diurnal forager, acting as a predator, scavenger, and collector of honeydew and extrafloral nectar (Holl Dobler 1982). The second most common ant, *Formica schaufussi*, was ~2 orders of magnitude less abundant on the foliage and no competitive interactions between the two ant populations were observed. *Catalpa bignonioides*, *Ceratomia catalpae* and *F. pruinosus* are all native to the southeastern United States. Granular pesticides (Amdro) were used to suppress exotic fire ant (*Solenopsis invicta*) colonies within 20 m of the *Catalpa* grove.

All statistical analyses were performed with SAS version 6.12.

Methods

Nectar and ants

To test whether ant attendance at a leaf increases with extrafloral sugar production, ant visitation was compared among leaves with different production rates. Four mature (i.e. fully expanded, 2–4 months old, non-senesced) leaves were randomly selected from each of 15 sunlit branches ($n=60$), and the number of ants on the blade and petiole of each leaf were counted eight times over a 36-h period (1500, 1600, 1700, 1800 and 2000 hours EDST on 16 September 2000, and 1100, 1200 and 1500 hours on 17 September). Ant attendance was recorded in a “snap-shot” fashion; the number of ants on the leaf was recorded at that moment, rather than over a defined time (e.g. ants leaf⁻¹ min⁻¹). The 15 branches were from 11 plants, including four trees and seven seedlings. One branch was selected from each seedling; two from opposite sides of larger trees. Subsequent to the surveys, each focal leaf and branch were encircled with Tanglefoot adhesive (Tanglefoot Company, Cedar Rapids, Mich.) to exclude the crawling arthropods that consume

extrafloral nectar. All crawling arthropods were then removed from the branches by hand. Leaves were not bagged, because (1) bags can alter sucrose production rates at the enclosed nectaries (Wyatt et al. 1992), and (2) ants were observed at nectaries much more frequently than were flying nectivores. After 36 h, extrafloral nectar was collected from the seven largest nectaries on each of the 60 leaves using 5 μ l microcapillary tubes. Viscous nectar was diluted with drops of distilled water prior to collection. As the addition of water made it impossible to estimate sugar concentrations or nectar volume, analyses focused on the mass of sugar produced over a 36-h period. Nectar was stored in the freezer in 0.5 ml of methanol to discourage post-collection microbial growth.

Nectar composition (preliminary identification of sugars and amino acids) was determined using thin layer chromatography. Subsequent analyses focused on sugars, as they were found in much greater quantities within the nectar. Gas chromatographic analysis of the sugars was performed on a Hewlett-Packard 5890 Gas Chromatograph fitted with a DB-5 (25 m \times 0.32 mm i.d.) capillary column (0.52 μ m film thickness) (J.W. Scientific, Folsom, Calif.); injector 250°C, detector 350°C. The GC was operated in the splitless mode of injection. Phenylglucose (30.66 μ g in a methanol solution) was added to each sample as an internal standard. After mixing, a 250 μ l aliquot of each nectar solution was placed in an auto-injector vial, and water was removed under a stream of nitrogen. Two drops of acetonitrile were added to each sample to insure complete removal of water during the drying process. Sugars were analyzed as their trimethylsilylated derivatives. *N*,*O*-bis-(trimethylsilyl) trifluoroacetamide and dimethylformamide were added in the solution in a 1:1 ratio (usually 20 μ l each, although 100 μ l were added to the most concentrated nectars). This solution was heated at 75°C for 30 min, and a 1- μ l subsample was then injected into the GC. GC oven temperature increased from 100–320°C at 8°/min. Peaks corresponding to α - and β -glucose were summed for quantification, and total sugars consisted of summation of all peaks in the fructose, sucrose and glucose retention ranges of each chromatogram. Linear regressions were used to correlate mean ant attendance per leaf for the 36-h period prior to Tanglefoot application with the production of fructose, sucrose, glucose and all three sugars combined after Tanglefoot application, as 36-h production rates at undamaged leaves are consistent over short time periods (i.e. days, see below). The sugars were analyzed separately because they are differentially attractive to some ants (Koptur and Truong 1998). General Linear Models (GLM) compared production rates and ant attendance among branches and plants.

Caterpillars and nectar production

To test whether herbivory influenced extrafloral nectar production, caterpillars were added to 20 of the leaves described above (Nectar and ants) and nectar was collected from all 60 leaves 36 h later (19–20 September 2000). Four large trees and two seedlings were designated as caterpillar-attacked, and the remaining five seedlings as unattacked. A fifth instar caterpillar was added to two of the four focal leaves within each focal branch on the attacked plants. All 60 leaves remained encircled in Tanglefoot (see above), and the caterpillars were removed 24 h after their introduction. Late instars were used because they can cause considerable leaf damage within this short duration (e.g., 10–30% of total leaf area removed in 24 h). The change in 36-h production rates of fructose, glucose, sucrose, and all three sugars combined for each leaf were computed as follows:

$$\Delta_{\text{production}} = \text{Production}_{\text{post-treatment mass}} - \text{Production}_{\text{pre-treatment mass}}$$

This design permits comparisons among caterpillar-damaged leaves, neighboring undamaged leaves, and undamaged leaves on unattacked control plants. One-sided nonparametric Wilcoxon tests, using individual leaves as replicates, tested the a priori hypotheses that damaged leaves increased production relative to undamaged leaves. Two-sided tests compared the changes in production among the two undamaged leaf groups, as attacked plants may exhibit a systemic response at undamaged leaves. The preceding analyses treat individual leaves and sugars as if they were independent data,

ignoring that some leaves are from the same plants and the physiological links between the sugars. To address these complications, an additional analysis included the treatment means from individual plants as replicates and total sugar production (all three sugars combined) as the dependent variable.

Caterpillars and ants: intra-plant comparisons

To test the hypothesis that ant attendance at caterpillar-damaged leaves differs from attendance at undamaged leaves, attendance at the two types of leaves were compared before and after attack. On 23 July 2000, 30 leaves on each of 15 seedlings ($n=450$) were marked with a permanent marking pen. The number of ants on these leaves were surveyed 24 h later (see above). All leaves were mature, all or nearly intact (>90% original leaf area remaining), and exposed to direct sunlight, although otherwise randomly selected. There was no evidence that marking affected either the leaves or the ants. After this initial ant survey, 12 fifth instar caterpillars were added to each seedling. Caterpillars were added to randomly selected leaves and the main stem and were removed after 24 h. Caterpillar movement and feeding were unconfined within the plants. Defoliation was estimated by eye, and leaves with >10% total leaf area missing were classified as attacked. New damage was distinguished from old by the absence of browned edges on the damaged portions. The number of ants on the 450 leaves were surveyed 1, 2, 3, 5, 7, and 12 days after caterpillar introduction, and all leaves were surveyed within the same 30-min period. No feral caterpillars (or other herbivores) were on the plants during this experiment, as any naturally occurring *C. catalpae* egg masses were collected prior to eclosion.

The ratio of ant attendance at damaged leaves to undamaged leaves within each plant was calculated as: Relative Attendance (RA) = (mean no. ants per damaged leaf on a plant) / (mean no. ants per undamaged leaf on that plant). RA>1 indicates ant attendance biased towards leaves attacked by caterpillars. One-tailed, paired *t*-tests were used to test two a-priori hypotheses: (1) caterpillars first occupy and feed on leaves with lower ant densities (RA<1 for day 0), and (2) ant densities are higher on attacked leaves than on unattacked leaves after attack (RA>1 for days 1, 2, 3, 5, 7 and 12). Individual *t*-tests were performed for each day ant attendance was measured.

To test whether an ant response could be elicited by mechanical damage, ant attendance was compared among leaves damaged by scissors and undamaged control leaves. The number of ants were counted on each of 75 randomly selected, mature, sunlit leaves on a tree known to attract ants after experiencing herbivory. Twenty-five leaves were then randomly designated as undamaged controls, and leaf tissue was removed from the remaining 50 leaves with scissors. *Ceratomia catalpae* consumes leaf material the leaf edges inward, and the degree of defoliation differs among leaves. This removal was grossly simulated by cutting with scissors in a similar edge-inward fashion [albeit at a greatly accelerated rate (~60 s)], and alternating removing 30 and 60% of the total area of each treatment leaf. To minimize the deposition of any contaminants on the scissors, the blades were washed with distilled water and 50 strokes were cut on *Catalpa bignonioides* leaves from another plant prior to the experiment. Ant attendance at each leaf was surveyed 2 h, and 1, 2, 3, 4, and 5 days later, with all leaves surveyed during the same 30-min periods. A repeated measures analysis was used to test whether the pattern in ant attendance at the two leaf groups (damaged and control) differed over time.

Caterpillars and ants: inter-plant comparisons

To test the hypothesis that caterpillar attack induces an increase in the total number of ants on the host plant, nine fourth instar caterpillars were added to each of 15 seedlings, and mean number of ants per leaf on these plants was compared with 15 plants that lacked caterpillars. To limit inter-group differences in the proximity to ant colonies, adjacent plants were assigned to control and

experimental treatments. The caterpillars were added to randomly selected leaves, were unconfined, and were removed after 4 days. During each survey, the number of ants was counted on 25 randomly selected leaves (irrespective of caterpillar occupation or leaf damage). Plants were surveyed 7, 6, and 0 days prior to caterpillar addition to confirm that pre-treatment ant attendance levels were comparable between the control and experimental plants. The 30 plants were surveyed 24 and 36 h, and 2, 3, 4, 5, 6, 7, 8, 12, 15, and 17 days after caterpillar introduction. Pre- and post-treatment surveys were performed between 1700 and 1900 hours, from 3 September to 5 October 2000, with the second post-attack survey (36 h) performed at 0900 hours. All plants were surveyed during a 1-hour period.

Repeated measures analysis was used to evaluate the treatment effects on a plant's mean ant attendance per leaf over time. A *t*-test was used to confirm that the mean ant attendance of plants in the two groups did not differ prior to the experiment.

Ant density and caterpillar deterrence

To test the hypothesis that plants with greater densities of ants per leaf are better protected against herbivores, caterpillar disappearance rates (mortality + emigration) were compared among plants. Seventeen second-instar larvae were added to each of 30 seedlings on 6 July 2000, and all plants were surveyed for caterpillars 48 h later. Larvae molted to the third instar during this period, and this density is within the range observed in naturally occurring aggregations for these instars (mean aggregation size and SD: second instar = 17.2 ± 16.1 ($n=20$); third instar = 6.2 ± 6.2 ($n=38$)). Caterpillars were added to randomly-selected leaves and the main stem. Plants were inspected from top to bottom, and the total number of larvae remaining on each plant was recorded. The numbers of ants on 15 randomly-selected leaves were also counted on each plant at that time (1900 hours). Using these ant counts, the 30 plants were divided into three groups: low (mean ant density per leaf < 0.07 , $n=10$), intermediate (mean ant density per leaf $\pm SE = 0.28 \pm 0.3$, range: 0.13–0.53 ants leaf⁻¹, $n=15$) and high ant density (mean: 1.09 ± 1.5 ; range: 0.8–1.67, $n=5$). A General Linear Model (GLM) was used to compare caterpillar disappearance rates among these three groups.

Fifth instar larvae were not used in this experiment because they abandon the host plant to pupate (Baerg 1935), and count data could not distinguish this absence from predation. Third instars originally damage leaves with low ant attendance, and ant attendance at damaged leaves and plants increases within 24 h after attack (Ness 2001). These responses are qualitatively similar to those of, and elicited by, later instars (see below).

Results

Nectar and ants

The amount of sugar produced in the extrafloral nectar varied greatly among leaves (mean \pm SD = 259 ± 522 $\mu\text{g}/36\text{h}$, range: 0–2,495 μg), as did the mean number of ants observed on each leaf (mean \pm SD = 0.95 ± 1.23 , range: 0–6.28). Glucose, fructose and sucrose accounted for 31% \pm 10, 19% \pm 10, and 23% \pm 14 (mean \pm SD), respectively, of the total sugars by mass. The remaining sugars included rutinose (sporadically) and a variety of unidentified trisaccharides. Thin layer chromatography suggested that trace amounts of alanine, glycine, glutamine, serine, threonine, valine, and aspartic acid were also present in the nectars. Mean ant attendance per leaf among all leaves was positively correlated with production of total sugars ($r^2=0.45$, $n=60$, $P<0.0001$), sucrose ($r^2=0.43$, $P<0.0001$),

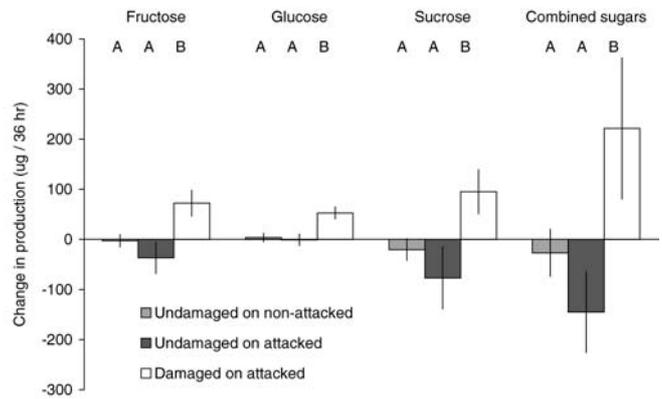


Fig. 1 The effect of herbivory on sugar production rates in extrafloral nectar (fructose, glucose, sucrose and all three sugars combined). The y-axis indicates the differences in sugar production ($\mu\text{g}/36\text{h}/\text{leaf}$) before and after treatments. Undamaged leaves on non-attacked plants are untreated controls. Undamaged leaves on attacked plants were protected from herbivory, although caterpillars attacked adjacent leaves. Damaged leaves on attacked plants were exposed to caterpillars for 24 h. Values are mean \pm SE, and significantly different values among treatments are indicated by different letters (Wilcoxon tests, $P<0.05$). Fructose, sucrose and glucose values are the means of all individual leaves; the combined sugar values are the means of individual plants (see text)

fructose ($r^2=0.40$, $P<0.0001$) and glucose ($r^2=0.28$, $P<0.0001$). Ants were approximately twice as abundant in the late afternoon as at noon, although the relative abundance of ants among individual leaves was consistent. There was a strong positive correlation between the number of ants observed on a leaf in any one survey with the mean number of ants observed on that leaf during the other surveys (mean $r^2 \pm SD = 0.63 \pm 0.12$, range: 0.43–0.76, $P<0.0001$ in all cases), suggesting the “snap-shots” provided an accurate estimate of relative ant attendance over longer time periods.

Comparisons among all sampled leaves may be misleading, because sugar production rates per leaf varied among branches (GLM, $F=4.56$, $df=14, 37$, $P<0.0001$) and plants ($F=2.32$, $df=10, 42$, $P=0.028$) and branches differ in their proximity to ant colonies. Within the 15 branches, mean ant attendance at the leaves with higher total sugar production rates was significantly greater than that of the leaves with lower rates (paired *t*-test: $t = 3.03$, $df=14$, $P<0.005$). The difference in total combined sugar production rates per leaf between seedlings and large trees, however, was not statistically significant (two-sided *t*-test: $t = 1.52$, $df=3,6$, $P=0.61$).

Caterpillars and nectar

Changes in nectar production over time were estimated for 51 of the 60 leaves, due to leaf abscission and/or poorly capped collection tubes. Changes in the rate of fructose, glucose, and sucrose production between the first and second collections differed across the three leaf groups (Fig. 1). The 36-h production rates of glucose, fructose, and

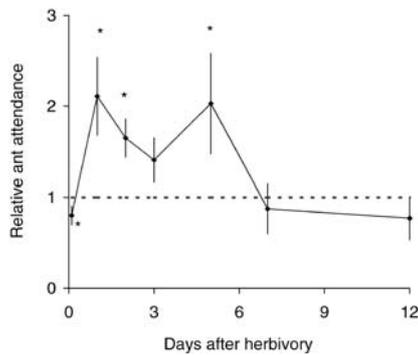


Fig. 2 The ratio of ant attendance at damaged leaves to undamaged leaves within caterpillar-attacked plants (mean \pm SE shown). Relative attendance (RA) was calculated as: (mean no. of ants per damaged leaf on a plant) / (mean no. ants per undamaged leaf on that plant). RA > 1 indicates ant attendance biased towards leaves attacked by caterpillars. Values > 1 indicate ant attendance biased towards damaged leaves. Values significantly different than 1 are indicated by an asterisk (paired *t*-tests, $P < 0.05$). Caterpillars were removed after 24 h. Day 0 indicates relative ant attendance prior to caterpillar occupation, day 1 indicates relative ant attendance while caterpillars occupied plants, and all days thereafter compare undamaged leaves with leaves previously damaged by caterpillars

sucrose increased on caterpillar-damaged leaves ($n=14$), a response not observed in neighboring undamaged leaves on attacked plants ($n=19$) (one-sided Wilcoxon test: $\chi^2=10.04$, $P=0.0007$; $\chi^2=8.07$, $P=0.003$; $\chi^2=8.705$, $P=0.002$, respectively). Fig. 1 hints that increased sugar excretion at damaged leaves could come at the expense of decreased excretion at neighboring undamaged leaves. The mean changes in sugar production on damaged leaves within a branch, however, were not significantly correlated with mean changes in production in neighboring undamaged leaves on those same branches (regression analyses; sucrose: $F=1.54$, $n=10$, $P=0.261$; glucose: $F=1.23$, $P=0.309$; fructose: $F=0.598$, $P=0.469$). The non-significant trends were positive for all three sugars (i.e., the greatest increases in production at damaged leaves were associated with increases at neighboring leaves, rather than decreases). The increases in glucose, fructose and sucrose production rates at damaged leaves also differed from those observed in undamaged leaves on undamaged plants ($n=18$) (one-sided Wilcoxon test: $\chi^2=11.43$, $P=0.00035$;

$\chi^2=8.336$, $P=0.002$; $\chi^2=10.182$, $P=0.0007$, respectively). The changes in sugar production in undamaged leaves on attacked and unattacked plants were not significantly different (glucose: $\chi^2=0.023$, $P=0.879$; fructose: $\chi^2=0.101$, $P=0.749$; sucrose: $\chi^2=0.1809$, $P=0.671$). The relative ratios of these three sugars did not change appreciably in any of the three treatment groups.

Results were similar when the masses of three sugars were summed and the treatment means from individual plants used as replicates (Fig. 1). The mean change in production was greater in damaged leaves (mean \pm SE = 222 ± 141 μg , $n=5$) than in undamaged leaves on attacked plants (-145 ± 81 μg , $n=6$) and undamaged leaves on unattacked plants (-27 ± 47 μg , $n=5$). Comparisons of damaged leaves with undamaged leaves on attacked and unattacked plants indicated these differences were significant (one-sided Wilcoxon test: $\chi^2=4.82$, $P=0.014$ and $\chi^2=3.15$, $P=0.038$, respectively), although the latter groups did not differ ($\chi^2=0.536$, $P=0.464$).

Caterpillars and ants: intra-plant comparisons

Caterpillars fed upon, and were found on, slightly less than half the marked leaves within individual plants (mean \pm SD: $0.47 \pm 0.12\%$ of marked leaves). These leaves had lower levels of ant attendance prior to caterpillar introduction (day 0, Fig. 2). Ant attendance on leaves previously damaged by caterpillars increased within 24 h of the arrival of caterpillars, and at that point exceeded the attendance observed on unoccupied leaves. RA was significantly < 1 prior to caterpillar occupation (day 0), and > 1 on days 1, 2, and 5 ($P < 0.05$ in all 4 cases). The difference between previously occupied/damaged and unoccupied leaves was marginally significant on day 3 ($P=0.059$) and non-significant by days 7 and 12 ($P < 0.10$ for both days) (Fig. 2). By days 7 and 12, RA had returned to levels statistically indistinguishable from those observed prior to the caterpillar introduction.

Ant attendance at scissor-damaged and control leaves was similar during each of the seven surveys (one pre-treatment + six post-treatment), as indicated by a non-significant treatment effect and treatment by time interaction terms (Table 1). The mean ratio of ant attendance

Table 1 Repeated measures analyses contrasting two experiments of ant attendance at (1) scissor-damaged leaves and (2) caterpillar-damaged plants with undamaged control leaves and undamaged control plants, respectively. Additional probabilities corrected for sphericity are provided using the Greenhouse-Geisser (*G-G*) and Huynh-Feldt (*H-F*) corrections

Source	<i>df</i>	MS	<i>F</i>	<i>P</i>	G-G	H-F
Contrasting ant attendance at scissor-damaged and control leaves						
Treatment	1	0.115	0.05	0.828		
Error (treatment)	73	2.434				
Time	6	21.999	17.51	0.0001	0.0001	0.0001
Treatment \times time	6	5.308	0.42	0.8641	0.7463	0.7585
Error (treatment \times time)	438	1.256				
Contrasting mean ant attendance per leaf at caterpillar-attacked and control plants						
Treatment	1	42.038	23.43	0.0001		
Error (treatment)	25	1.794				
Time	12	2.56	12.79	0.0001	0.0001	0.0001
Treatment \times time	12	2.349	11.75	0.0001	0.0001	0.0001
Error (treatment \times time)	300	0.199				

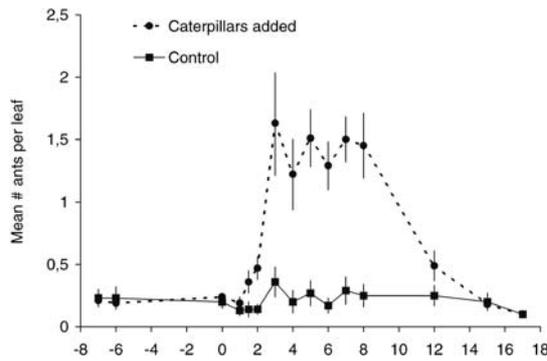


Fig. 3 The effect of caterpillar occupation and herbivory on mean ant attendance per leaf. Caterpillars were added to treatment plants after the day 0 survey, and removed after the day 4 survey. Control plants lacked caterpillars. Values are mean \pm SE

at scissor-damaged versus control leaves (RA) over the six post-treatment surveys was 1.19 ± 0.24 (mean \pm SE) (non-significantly favoring damaged leaves). Ant attendance was greatly increased on both leaf groups on the fifth day after leaf removal, as indicated by the significant time effect (Table 1). This was the only rainy day of the five.

Caterpillars and ants: inter-plant comparisons

Control and treatment plants did not differ in mean ant densities per leaf prior to the caterpillar addition (t -test, $P > 0.05$), and most leaves (>50%) on attacked plants were not damaged by caterpillars. Within 48 h of caterpillar addition, mean ant density per leaf on caterpillar-infested plants was greater than the mean densities observed on control plants (Fig. 3). Within 72 h, ant attendance per leaf on infested plants had increased 6- to 10-fold, relative to control plants. Ant attendance at attacked plants remained at these high levels more than 4 days after caterpillars were removed (days 4–8). Significant differences remained apparent for at least 12 days after the caterpillar introduction, and attendance on attacked plants returned to pre-treatment levels within approximately 14 days (Fig. 3). The change in ant attendance at attacked plants was not observed on control plants, as indicated by the significant treatment by time interaction (Table 1). Ant attendance on attacked plants was biased towards damaged leaves, although attendance on undamaged leaves also increased, relative to leaves on unattacked plants.

Ant density and caterpillar deterrence

Caterpillar disappearance rates, attributable to mortality and/or emigration, differed among the three ant density groups ($F = 4.07$, $df = 2, 27$, $P = 0.028$). A greater percentage of the original larvae were found on plants with low ant densities (mean \pm SE: $65\% \pm 5$) than on intermediate

($54\% \pm 5$) and high ($38\% \pm 7$) ant density plants (pairwise contrasts, $F = 8.12$, $P = 0.008$ and $F = 4.57$, $P = 0.042$, respectively). Caterpillar retention rates on intermediate- and high-ant density plants did not differ significantly ($F = 1.25$, $P = 0.274$), a result possibly attributable to low sample size for the latter group ($n = 5$).

Discussion

Each of the three participants in this system (plants, caterpillars and ants) influenced, and was influenced by, the other two. Caterpillars occupied and damaged leaves with lower ant attendance, possibly reflecting avoidance of ant-occupied leaves or predation / eviction on and from leaves with higher ant attendance. In spite of any selective foraging, both ant attendance and extrafloral nectar production increased at caterpillar-occupied leaves, strongly suggesting a causal link between herbivory, nectar production, and bodyguard recruitment. An alternative explanation is that ants are recruited to leaf volatiles, as occurs in some obligate ant-plant systems (Fiala and Maschwitz 1990; Agrawal 1998; Agrawal and Dubin-Thaler 1999; Brouat et al. 2000). Given that ant attendance and sugar excretion increased by similar magnitudes (2- to 3-fold) over the same duration of time (within 36 h of damage), and ants are more abundant on productive leaves, recruitment to food rewards is a better-supported, and parsimonious, explanation. Furthermore, ants did not aggregate at mechanically-damaged leaves, an obvious source of volatiles. This noteworthy result also suggests the cues that induce ant attraction (and perhaps nectar production) may be herbivore-specific (e.g., saliva). Other studies demonstrated that nectar production could be induced by mechanical damage (Koptur 1989; Smith et al. 1990), that herbivory and mechanical damage induced similar responses (Wäckers and Wunderlin 1999), or that mechanical damage induced a greater response than herbivory (Heil et al. 2001a). Furthermore, the two studies that challenged plants with herbivores found that nectar induction was limited to young leaves with the most productive nectaries (Heil et al. 2000; Wäckers et al. 2001), and gave no indication of whether these leaves are naturally preferred or avoided by herbivores. The present study demonstrates that herbivory induces nectar production in mature leaves, that herbivores readily attack these leaves, and that plants and/or ants respond to legitimate threats (herbivory) but not “false alarms” (mechanical damage).

The reorganization of ant attendance among leaves within plants (biased towards newly damaged leaves) was accompanied by the attraction of additional bodyguards on a larger spatial and temporal scale. This is the first demonstration of a damaged plant increasing extrafloral nectar production and attracting new bodyguards, although a localized increase in the number of “plant-defending” insects at young induced leaves has been shown (Heil et al. 2001a). It is difficult to assign a cause to the substantial increase in ant attendance at damaged

plants over this long time period (weeks), as nectar production at damaged and undamaged leaves was only measured over a 36-h duration (and on other plants). Herbivory induces increases in nectar production at both damaged and undamaged leaves in cotton, although the latter response was delayed (Wäckers et al. 2001). Induction in *Catalpa bignonioides* may occur in a similar fashion, albeit with a delay greater than 36 h after herbivory.

Fewer herbivores remained on plants that attracted more ants. Smaller caterpillars (1–3 instars) are attacked by *F. pruinus* ants and carried back to the nest, although later instars (4–5) are harassed but rarely killed (J. Ness, personal observation). Plant protection and bodyguard density have been linked in both ant-plant systems (Rocha and Bergallo 1992; Madden and Young 1992; Heil et al. 2001b) and plant-mite systems (Dicke 1999). This is the first study to link induced extrafloral nectar production, increased bodyguard density, and herbivore removal at the whole plant scale. In the only comparable study, Heil et al. (2001a) found that more “defender” insects and fewer herbivores were observed on experimentally induced *Macranga tanarius* leaves, relative to leaves on control plants, and that induced leaves suffered less herbivory. Nectar induction in that system is confined to young damaged leaves, however, and that study did not address whether these defenders and herbivores merely immigrated from / emigrated to other leaves on the host plant (respectively), as occurs in other systems (Rocha and Bergallo 1992). This study demonstrates that bodyguard recruitment need not be a zero-sum game, wherein the greater protection afforded one site engenders greater vulnerability at another.

Catalpa bignonioides and myrmecophytic plants utilize bodyguards differently. The ants associated with myrmecophytic plants exhibit an immediate, swarming recruitment to damaged or herbivore-occupied leaves (Fiala and Maschwitz 1990; Rocha and Bergallo 1992; Agrawal 1998; Federle et al. 1998; Agrawal and Dubin-Thaler 1999; Gaume and McKey 1999). This response is short-lived (minutes to hours), with no indication of new or greater rewards offered by the host plant during that time span. Such rewards are perhaps unnecessary in these obligate plant-ant systems, wherein the participants are codependent (Agrawal and Rutter 1998). By contrast, ant “induction” (aggregation) in the facultative (myrmecophilic) *C. bignonioides* system is slower (within 24 h of attack), of greater duration (days to weeks), attracts an ant workforce 5- to 10-fold greater than that typically supported by the plant, and is mediated by the augmentation of food rewards when the plant is in greatest need of protection. The production of this reward offers an explanation for both the delay between herbivore attack and ant response, and the relatively modest ant response. Ant attendance follows nectar production (both approximately doubling within 36 h), although it is no greater. Nonetheless, the localized increase in the food rewards attracts or retains visitors at those sites where protection is required, presumably benefiting the host plant.

The production of food rewards may also help *C. bignonioides* avoid some of the costs incurred by plants that attract carnivores (e.g., parasitoids, predatory mites) with volatiles. One limitation of the latter strategy is that the carnivores may also protect neighboring conspecifics that avoided both herbivory and/or the costs associated with producing the volatiles (Bruin et al. 1995; van der Meijden and Klinkhamer 2000). By contrast, visitors must remain on the reward-producing plant to ingest that reward. Plants with the most productive nectaries (e.g. induced plants) should also be stronger competitors for ant visitation. Where ants are limited, food producers are more likely to “steal” bodyguards from neighbors than attract them to the benefit of all (Cushman and Whitham 1991). There were striking differences in the density of ants per leaf on different plants in the present study, although no direct evidence for competition (i.e., attendance did not decrease at neighboring unattacked plants).

This study was performed with a range of caterpillar instars, on seedlings and mature plants, in an ant community dominated by a single species. By virtue of an unbalanced design, the effects of herbivory on nectar production were measured largely with leaves from mature plants. Mean nectar production rates per leaf on mature trees and seedlings was indistinguishable, however, and the attraction of ants to attacked seedlings, relative to undamaged control plants, suggests herbivore-induced nectar production occurs in seedlings as in adults. Both early and late instar caterpillars damage leaves with fewer ants first, and these leaves subsequently attract greater numbers of ants than do undamaged neighboring leaves (present study; Ness 2001). That these ants can provide protection was demonstrated with *F. pruinus* and early instars. Whether the larger, more mobile, late instars avoid *Catalpa*'s biotic defense is perhaps contingent upon the duration of time a caterpillar occupies a newly damaged leaf, as well as the aggressiveness, foraging intensity, and dietary preferences of the local ant community. Studies that compare the level of protection provided by different arthropod communities would thus be of interest.

Claims that a trait acts as an inducible defense should be supported by the following evidence: (1) the trait increases in magnitude in response to an appropriate stimulus; (2) the efficacy increases with magnitude (more is better), at least over some range of values; and (3) the trait acts as defense. These standards are applicable to both direct and indirect defenses. The dynamic interactions between the extrafloral nectaries of *Catalpa bignonioides*, the plant's herbivore, and *F. pruinus* workers satisfy these first two criteria. The sugar content of the extrafloral nectar produced by damaged leaves increased within 36 h of herbivory, and, as a likely result, ant attendance at attacked leaves and plants increased after caterpillar attack. Caterpillar disappearance (mortality + emigration) rates were higher on plants that attracted more ants per unit leaf area. These results indicate the ant-plant interaction provides inducible *resistance* (sensu Karban and Baldwin 1997) against the herbivore. Iden-

tifying this interaction as an inducible plant *defense* may be premature, as the effect of ants on caterpillars were demonstrated by a natural, rather than controlled, experiment, and the effects of caterpillars or ants on plant fitness were not measured in this study. Caterpillar mortality rates are approximately twice as great on branches accessible to *F. prunosus*, relative to those branches from which ants have been experimentally excluded (Ness 2001), and there is evidence that herbivory does impact plant fitness. *Ceratonia catalpa* caterpillars are the only significant defoliator of *Catalpa speciosa* and *C. bignonioides* (Baerg 1935), dry mass per fruit and per seed decreases with defoliation of *C. speciosa* branches (Stephenson 1982), and caterpillars feed on the fruits of defoliated plants (Ness, personal observation). Taken together, these observations suggest that caterpillars can have significant, detrimental effects on plant fecundity (at least within a year), and that ant recruitment can diminish these impacts on plant fitness.

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