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PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

# Is extrafloral nectar production induced by herbivores or ants in a tropical facultative ant–plant mutualism?

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**Abstract** Many plants use induced defenses to reduce the costs of antiherbivore defense. These plants invest energy in growth when herbivores are absent but shunt energy to defense when herbivores are present. In contrast, constitutive defenses are expressed continuously regardless of herbivore presence. Induction has been widely documented in temperate plants but has not been reported from tropical plants. Most tropical plants have higher, more constant herbivore pressure than temperate plants. In this situation, it is hypothesized that constitutive defenses rather than induced defense would be favored. Using natural herbivores of four species of *Inga* saplings on Barro Colorado Island, Panama, herbivore presence was crossed with ant presence to determine their effects on extrafloral nectar production. Analysis of nectar samples revealed that *Inga* species do not induce nectar production in response to herbivores. This result is not due to an inability of the plants to respond, as the plants in this study increased nectar production in response to light and ant presence. Contrary to most induction experiments with temperate ecosystem plants, these results demonstrate that tropical plants do not induce one type of defense, and they suggest that the most adaptive defense strategies are different for the two ecosystems.

**Keywords** Constitutive defense · *Inga* · Facultative mutualism · Plant–insect interaction · Herbivory

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## Introduction

Plants have evolved a battery of antiherbivore defenses in response to the selective pressure exerted by herbivore damage. Defenses such as toxic chemicals, physical structures, and rewards that attract enemies of herbivores are believed to be costly for plants to produce, and the resources used for defense usurp resources from other plant activities such as growth and reproduction. Thus, plants are faced with the classic dilemma of investing in growth or in defense (Herms and Mattson 1992). The optimal defense hypothesis addresses this dilemma by predicting that plants will invest in defenses only when they are cost-effective and will reduce investment in redundant defenses (Stamp 2003).

Induced defenses have received considerable attention in the past decade as a cost-efficient defense strategy. This defense strategy permits plants to maintain a low level of defense that can be upregulated in response to environmental cues (biotic or abiotic) in order to reduce future herbivore damage. Induced defenses are predicted to evolve only when reducing the defense would enhance fitness in the absence of herbivores and when the risk of herbivore damage is variable and a reliable cue exists to trigger the induced response (Karban et al. 1999; Rohde and Wahl 2008). In contrast, constitutive defenses (defenses that are continuously expressed regardless of herbivore presence) are predicted to be advantageous over induced defenses when herbivore pressure is constantly high.

Most examples of defenses induced by herbivores have been in plants from temperate ecosystems (Boege 2004). Temperate environments experience seasonal herbivore loads that may predict future herbivore pressure and act as a reliable cue for plants. There is, however, a lack of evidence demonstrating herbivore-induced defenses from tropical forests. Tropical plants, especially young leaves, experience higher percent herbivore damage than temperate plants (Coley and Aide 1991). Young leaves of tropical plants are the most susceptible to herbivore consumption because they are softer and have higher relative nutrient and water contents than mature leaves (Coley and Kursar 1996). Roughly 70% of a tropical leaf's lifetime damage occurs in this short period of intense growth (Coley and Barone 1996). Thus, young leaves in tropical forests are under high, almost constant herbivore pressure, which should favor constitutive defenses over induced defenses.

Facultative ant–plant mutualisms are common antiherbivore defenses in tropical forests (Schupp and Feener 1991). In return for extrafloral nectar, ants serve as bodyguards and patrol young leaf surfaces, removing or deterring unwanted pests. In contrast to obligate ant–plant mutualisms, facultative mutualisms provide food but not housing for ants, which can result in rapid changes in the abundance and identity of the ant partners (Heil and McKey 2003). Consequently, we predict that extrafloral nectar in facultative ant–plants should respond to ant presence by changing the nectar production rate.

In this paper we examine the effects of experimentally manipulated herbivore and ant presence on the production of extrafloral nectar in four Neotropical tree species in the genus *Inga* (Fabaceae) on Barro Colorado Island, Panama. At the genus level, *Inga* has a diverse array of chemical and phenological defenses (Brenes-Arguedas et al. 2006; Coley et al. 2005; Lokvam et al. 2006; Lokvam et al. 2004; Lokvam and Kursar 2005). Chemical defenses are toxic to herbivores and phenological defenses, such as rapid leaf expansion, allow the leaves to spend less time in the vulnerable young leaf state (Coley et al. 2005). Most species of *Inga* also have extrafloral nectaries that produce nectar and use ant protection to varying degrees (Brenes-Arguedas et al. 2006; Koptur 1984; Koptur 1985). Extrafloral nectaries in *Inga* are located on the rachis between leaflet pairs and only secrete nectar when the leaves are young and expanding. It has been shown that extrafloral nectary traits are heritable and that the quantity and quality of nectar can influence the patrolling behavior of ants (Apple and Feener 2001; Bluthgen et al. 2004; Bronstein et al. 2006; Koptur 1992; Koptur 1994; Koptur 2005; Rudgers 2004; Rudgers and Gardener 2004). We therefore conducted a survey of extrafloral nectar production in *Inga* and how changes in this production rate influenced ant patrolling behavior. There is, however, a possible feedback loop in some systems between ant presence and nectar production. Heil et al. (2000) have shown that ant presence itself induced the production of extrafloral nectar. Therefore, we manipulated herbivore presence and ant presence to determine if either induces extrafloral nectar production on the young leaves of *Inga*.

## Methods

Field research was conducted on Barro Colorado Island (BCI), Panama from May 2007 to November 2007, and again in the same months in 2008. BCI is located in the Panama Canal (9°N80°W) and is maintained by the Smithsonian Tropical Research Institute. The island is a tropical moist lowland forest that experiences a 4-month dry season (January to April) (Croat 1978; Holdridge et al. 1971; Leigh 1999). For *Inga*, most young leaves are produced during the rainy season (Coley and Kursar, pers. observ.). This is also the season that herbivores and their predators (ants) are more abundant in both gaps and understories (Richards and Windsor 2007).

### Ant visitation and nectar quality and quantity

Nectar surveys were conducted from May to August of 2007 and 2008. Six *Inga* species along the trails on BCI were selected: *I. acuminata* ($n = 3$), *I. marginata* ($n = 44$), *I. multijuga* ($n = 23$), *I. pezizifera* ($n = 22$), *I. umbellifera* ($n = 40$), and *I. vera* ($n = 5$). One branch per individual tree between 1 and 4 m tall was sampled at one time. Most branches had only one young leaf. When multiple young leaves per branch were sampled, the average nectar production of those leaves was used as a response for the individual tree. To collect nectar from the young leaves, nectaries were washed with distilled water to remove accumulated nectar. Then the entire leaf was placed in a plastic bag to prevent rain or insects from removing nectar. We did not use mesh bags because they would not have prevented disturbance by rain. While plastic bags may influence temperature and humidity, the bags were not airtight and samples were collected mostly in the shaded understory and during the rainy season when air temperatures were cooler. In addition, condensation on the inside of the bags was rarely observed in either habitat (gap or understory), indicating no significant heat difference between the inside and outside of the bag. After 24 h, the nectar was collected and its volume measured using microcapillary tubes (initial collection). To collect any residual nectar, one microliter drops of distilled water were placed on nectaries, collected, and added to the initial collection. The nectar was collected into glass GC vials, dried under vacuum, and frozen at −50°C until analysis.

An HP 6890 gas chromatograph with a DB-1 capillary column and FID was used to identify and quantify the sugars in the nectar. The majority of the components in the nectar were the sugars fructose, glucose, and sucrose. The dried nectar samples were dissolved in 50 μL of pyridine. Due to the polarity of the sugars, the samples were derivatized by adding 50 μL of BSTFA with 1% TCMS to the pyridine solution and allowed to sit for 10 h. External standards and calibration curves were used to identify and quantify the three sugars in the nectar. The total mass of the three sugars was summed for each sample and corrected by the number of nectaries per leaf over a 24 h period (μg sugar $\text{nectary}^{-1}$ 24 $\text{h}^{-1}$).

Based on the survey of natural nectar production, artificial nectar solutions were prepared and placed on adult leaves of four *Inga* species: *I. marginata*, *I. multijuga*, *I. pezizifera*, and *I. umbellifera*. Adult leaves were used as they no longer produce nectar naturally. Solutions maintained a sugar ratio of one [i.e., 1 mg sucrose/(0.5 mg fructose + 0.5 mg glucose)]. Five separate concentrations were prepared: 0 μg $\mu\text{L}^{-1}$ ($n = 30$); 2 μg $\mu\text{L}^{-1}$ ($n = 29$); 20 μg $\mu\text{L}^{-1}$ ($n = 27$); 180 μg $\mu\text{L}^{-1}$ ($n = 28$); and 950 μg $\mu\text{L}^{-1}$ ($n = 28$). A concentration was randomly chosen for each plant included. A plant was only used once and only one leaf on the plant was observed. One microliter of the selected solution was placed on the two proximal nectaries on the rachis. After an hour, the ants on leaf surfaces and at nectaries were counted.

### Induction

To assess if herbivores could induce defenses in young leaves, we randomly assigned plants along the trails of BCI to either an herbivore damage treatment (plus-caterpillar) or an herbivore-free treatment in which herbivores were removed and they received no herbivore damage (minus-caterpillar). Previous research has shown that mechanical wounding alone does not mimic herbivore damage (Arimura et al. 2005); therefore, natural herbivores were used to elicit an induction response in young leaves of four species of *Inga*: *I. marginata* ($n = 33$), *I. pezizifera* ($n = 20$), *I. multijuga* ($n = 25$), and *I. umbellifera* ($n = 27$). The most common herbivore on *Inga* species on BCI is a black-headed leaf roller (Gelechiidae, species not identified) (Kursar et al. 2006). Second- and third-instar gelechiids were collected from non-focal *Inga* plants and moved to plus-herbivore plants.

To test if ants affect expression of defenses by plants, individual plants in each of the two herbivore treatments were assigned to either a treatment with normal ant visitation to leaves (plus-ants) or to a treatment where ant access to leaves was restricted by the addition of a sticky barrier (Tanglefoot) on the branch (minus-ants). In addition, plants were sampled in gaps and in the understory, and measures were taken to ensure that each treatment was evenly crossed with these two light categories for each species. These three factors—herbivore treatment, light and ant treatment—were fully crossed, and an individual plant only experienced one level of each treatment.

All of the treatments were applied to young leaves. Plants were incorporated into the experiment before their young leaves reached 15% of their average adult leaf area. If there was any pre-existing damage, plants were not included and plants were not reused. Treatments were maintained until the leaves reached 80% of their adult size (the size when the majority of herbivory has occurred and defense chemicals are high; Kursar and Coley 2003) or over half of their leaf tissue was damaged. At these terminal time points, nectar was collected for laboratory analysis (see above for the collection method).

Individual plants were treated as units of replication. When a plant had nectar collected from multiple leaves, the values for the multiple collections were averaged and used for an individual plant. Only values from leaves that were in the targeted size range (60–80% of adult size) were used. An analysis of variance was run with all three factors (herbivore presence, ant presence, and light) using plant species as a blocking variable and an alpha level of 0.05 (Table 1).

## Results

### Ant visitation and nectar quality and quantity

The predominant components of extrafloral nectar of *Inga* species were sucrose, fructose and glucose. The ratio of these three sugars is typically expressed as the sucrose-to-hexose ratio [mg sucrose/(mg fructose + mg glucose)]

**Table 1** ANOVA summary table for the effects of induced responses by herbivores, ants, light, and their interactions

| Source | DF | Mean square | *F* value |
|---|---|---|---|
| Ant presence | 1 | 437,839 | 4.2679* |
| Caterpillar presence | 1 | 17,352 | 0.1691 |
| Light | 1 | 1,096,620 | 10.6894** |
| *Inga* species (block) | 3 | 331,142 | 3.2278* |
| Ant × caterpillar | 1 | 19,411 | 0.1892 |
| Ant × light | 1 | 67,271 | 0.6557 |
| Caterpillar × light | 1 | 176,381 | 1.7193 |
| Ant × *Inga* spp. | 3 | 48,476 | 0.4725 |
| Caterpillar × *Inga* spp. | 3 | 101,631 | 0.9907 |
| Light × *Inga* spp. | 3 | 592,899 | 5.7793** |
| Ant × caterpillar × light | 1 | 72,688 | 0.7085 |
| Ant × caterpillar × *Inga* spp. | 3 | 29,792 | 0.2904 |
| Ant × light × *Inga* spp. | 3 | 55,736 | 0.5433 |
| Caterpillar × light × *Inga* spp. | 2 | 13,581 | 0.1324 |
| Ant × caterpillar × light × *Inga* spp. | 2 | 401 | 0.0039 |
| Residuals | 75 | 102,589 | |

Plant species was used as a blocking variable to remove variation due to different natural history traits

* $P < 0.05$, ** $P < 0.01$

(Baker and Baker 1982; Baker and Baker 1983). The average sugar ratio for the seven study species was 0.48 (when outliers were removed), with no significant difference among species. We were unable to positively identify and quantify any additional components of the nectar, such as amino acids.

There was no significant correlation between the volume of nectar produced and the concentration of the nectar. There was, however, a significant positive relationship between the volume of nectar produced and the total mass of sugar produced (slope = 0.85, $r^2 = 0.72$, $P < 0.001$). Thus, we chose to use total mass of sugar per nectary per 24 h period (µg nectary$^{-1}$ 24 h$^{-1}$) rather than volume for all further comparisons, as it was less prone to environmental fluctuation and is the component of the nectar that is most attractive to ants (Gonzalez-Teuber and Heil 2009). On average, the amount of sugar produced was 168 µg nectary$^{-1}$ 24 h$^{-1}$. However, this varied among plant species. *Inga acuminata* produced no nectar (0.0 µg nectary$^{-1}$ 24 h$^{-1}$) while *Inga marginata* produced the most nectar (277 µg nectary$^{-1}$ 24 h$^{-1}$; Fig. 1).

Increases in natural and artificial nectar rewards significantly increased ant visitation to leaf surfaces. Natural nectar production for seven species of *Inga* was positively correlated with natural ant visitation ($r^2 = 0.62$, $P = 0.035$, Fig. 2). Furthermore, when artificial nectar was placed in the inactive extrafloral nectaries of mature leaves, the number of ants increased as the amount of sugar increased ($P < 0.001$, Fig. 3). In addition, there was a significant interaction between concentration and observed ant location ($P = 0.0045$): at low concentrations, an equal number of ants visited nectaries as visited leaf surfaces, but at high concentrations, more ants visited nectaries than visited leaf surfaces (Fig. 3).

## Induction

We examined the extent to which nectar traits were induced by herbivore presence, ant visitation, and light availability. For this experiment we focused on four species: *I. marginata*, *I. multijuga*, *I. pezizifera*, and *I. umbellifera*.

As in the nectar survey, concentration did not change significantly among the treatments; nor was it correlated with the volume of nectar produced. Sugar ratios showed no significant differences among the treatments or species. Overall, the average was 0.46, indicating there were similar amounts of sucrose to the two monomers, fructose and glucose. However, total mass of sugar was positively correlated with volume (slope = 0.74, $r^2 = 0.55$, $P < 0.01$). Thus, mass per nectary per 24 h period was used for the statistical analysis.

On average across all treatments, *I. multijuga* produced the most nectar in a 24 h period (358 µg nectary$^{-1}$ 24 h$^{-1}$), followed by *I. pezizifera* (167 µg nectary$^{-1}$ 24 h$^{-1}$) and *I. marginata* (147 µg nectary$^{-1}$ 24 h$^{-1}$), with *I. umbellifera* producing the smallest amount (103 µg nectary$^{-1}$ 24 h$^{-1}$). Therefore, plant species was used as a blocking variable for analysis of variance. Despite a large amount of damage (21% on plus-herbivore plants), herbivore presence did not induce an increase in nectar production (Fig. 4a). However, ant presence and high light did induce an increase in nectar production ($P = 0.042$ and 0.0016, respectively) (Fig. 4b, c).

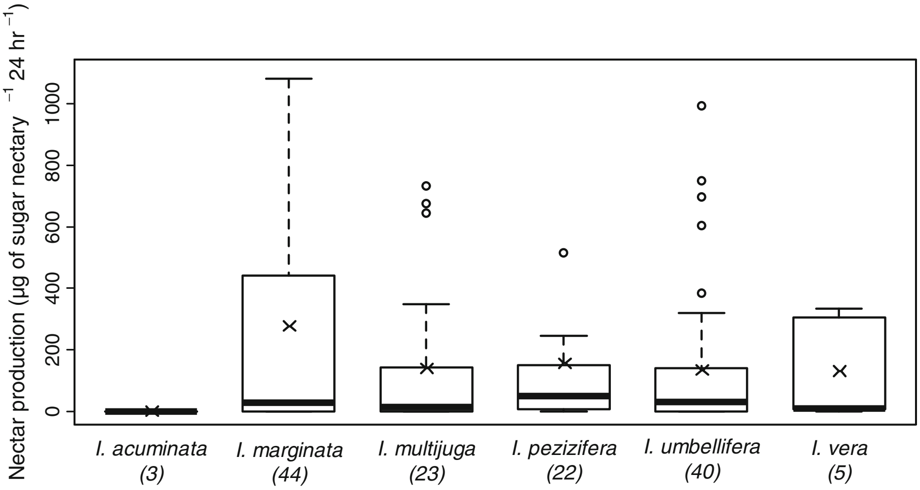

**Fig. 1** The natural variation in nectar production among six *Inga* species: *I. acuminata* ($n = 3$), *I. marginata* ($n = 44$), *I. multijuga* ($n = 23$), *I. pezizifera* ($n = 22$), *I. umbellifera* ($n = 40$), and *I. vera* ($n = 5$). The *black bars* in the middle of each *box* represent the median value for the species, the *ends of the box* represent the upper and lower quartiles, and the *whiskers* represent the upper and lower non-outlier values. The *crosses* represent the mean value for each species. Sample sizes are in *parentheses* below each species name

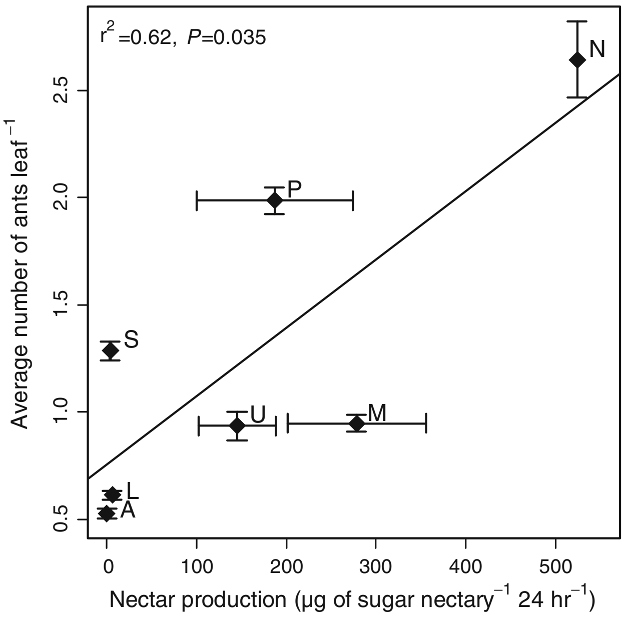

**Fig. 2** The average number of protective ants on leaf surfaces in relation to a species' investment in extrafloral nectar production ($r^2 = 0.62$, $P = 0.035$). *Points* in the graph represent the average number of naturally occurring ants observed during spot counts and the average mass of sugar produced per nectary in a 24 h period for seven species: *I. acuminata* (*A*), *I. laurina* (*L*), *I. sapindoides* (*S*), *I. umbellifera* (*U*), *I. pezizifera* (*P*), *I. marginata* (*M*), *I. nobilis* (*N*). *Letters* next to each point represent the species code, and the *error bars* are standard errors of the mean for both axes

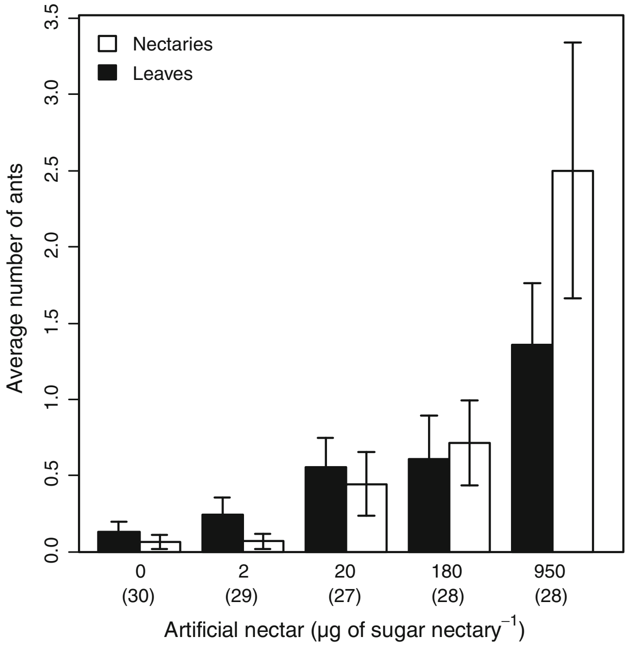

**Fig. 3** The average number of ants on leaf surfaces and at nectaries in relation to artificial nectar concentration. One microliter of artificial nectar of five different concentrations was placed on extrafloral nectaries (sample sizes appear in *parentheses* below each concentration). After an hour, the ants were counted on the leaf surfaces (*black bars*, mean ± SEM) and at nectaries (*white bars*, mean ± SEM). Artificial nectar of higher concentrations attracted significantly more ants than lower concentrations ($P < 0.0001$). In addition, there was a significant interaction between concentration and observed location of ants ($P = 0.0045$). At concentrations below 200 µg sugar nectary$^{-1}$ 24 h$^{-1}$, ants did not preferentially visit leaf surfaces or extrafloral nectaries. However, at concentrations above 200 µg sugar nectary$^{-1}$ 24 h$^{-1}$, ants preferentially visited extrafloral nectaries (*white bars*)

## Discussion

### Ant visitation and nectar quality and quantity

The number of ants on leaf surfaces increased when natural nectar production increased and when sugar concentration in the artificial nectar trials increased. Natural nectar production was most often less than 500 µg nectary$^{-1}$ 24 h$^{-1}$ and ranged from no measurable nectar produced in 24 h to 2,400 µg nectary$^{-1}$ 24 h$^{-1}$. The artificial nectar trials ranged from 0 to 950 µg nectary$^{-1}$ and confirmed the results obtained from observations of natural nectar production: increases in nectar production lead to increases in ant visitation. Even at low nectar levels, 20 µg nectary$^{-1}$, more ants visited leaf surfaces than when water was presented. Ant visitation continued to increase as sugar concentration increased (Fig. 3), further suggesting that nectar quantity on leaf surfaces influences ant visitation. Other authors have also demonstrated that increases in both natural nectar and artificial nectar can increase ant presence and that an increase in ant presence leads to decreased damage to host plant tissue (Bentley 1976; Bentley 1977; Kost and Heil 2005). Bentley (1977), for example, demonstrated that increased nectar production around flower buds of *Bixa orellana* shrubs attracted more ants during certain periods of flower and fruit development, and that plants with more ants had a higher number of healthy fruits. Koptur (1984) also showed a decrease in herbivore damage when ants were present on *Inga*.

Despite the continued increase in ant visitation with increased artificial nectar, *Inga* plants did not commonly present natural nectar rewards that were as concentrated as our artificial rewards. When higher concentrations of artificial nectar were presented, the number of ants on a leaf increased, however the part of a leaf the ants visited was influenced by the nectar concentration. Ants first visited extrafloral nectaries with sugar rewards before patrolling leaf surfaces (Fig. 5). For moderate and low concentrations of artificial nectar (below 200 µg sugar nectary$^{-1}$), as many ants or more were on leaf surfaces as were at nectaries. However, when the nectar concentration was above 200 µg sugar nectary$^{-1}$, ants tended to stay at the extrafloral nectaries instead of patrolling leaf surfaces (Fig. 3). This may be why plants do not commonly produce more than 500 µg sugar nectary$^{-1}$ 24 h$^{-1}$: at higher nectar

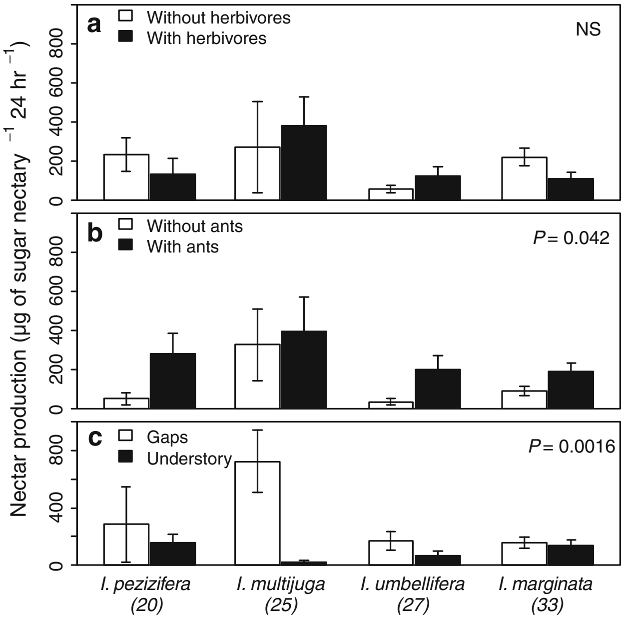

**Fig. 4** Nectar production (mean ± SEM) in response to **a** herbivore presence, **b** ant presence, and **c** light. None of the four *Inga* species significantly increased their nectar production when herbivores were present (**a** *NS*). Nectar was induced when ants were present (**b** $P = 0.042$) and when the plants were in gaps (**c** $P = 0.0016$). The $P$ value in each panel (**a**–**c**) represents the value for each of the main effects in an A × B × C ANOVA with species as a blocking variable. There was a significant interaction between light and *Inga* species ($P = 0.0013$). Sample size appears in *parentheses* below each species

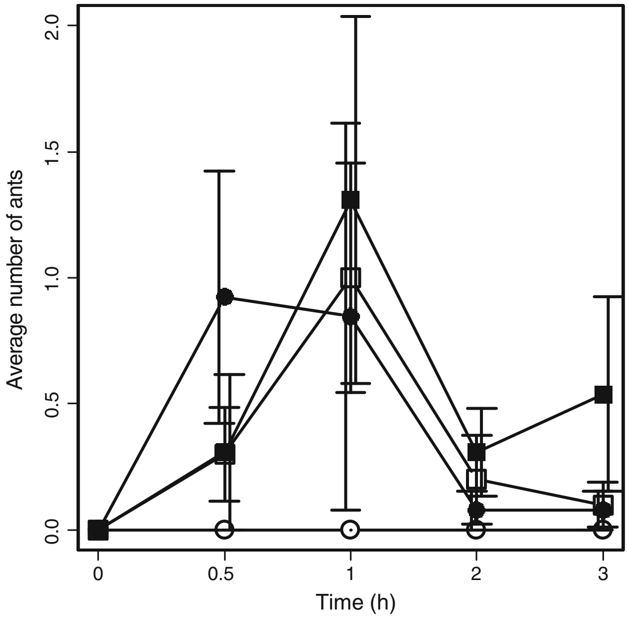

**Fig. 5** The timing of ant visits to nectaries and leaf surfaces. Artificial nectar (200 µg $\mu L^{-1}$) was placed on two of a leaf's nectaries at time 0. Ants were counted at half an hour, 1 h, 2 h, and at 3 h. Separate observations were made for nectaries with artificial nectar (*filled circles*), nectaries left empty (*open circles*), leaves attached to nectaries with nectar (*filled squares*) and leaves attached to nectaries without nectar (*open squares*). Ants first visit nectaries with artificial nectar (0.5 h), then spread out over leaf surfaces (1 h) before departing the leaves (2 h). Ants spent no time at empty nectaries (*open circles*). Standard errors of the mean are offset to make them more visible at each time point

production rates ants would gorge themselves at nectaries instead of patrolling leaves. This suggests that an intermediate level of nectar production may be most beneficial for plants. This is similar to hypothesized floral nectar production strategies in other systems where intermediate levels of nectar production encouraged continued pollinator foraging and enhanced pollen movement (Zimmerman 1988).

Nectar is principally derived from phloem. Phloem components are "filtered" through a layer of cells below the nectary before being presented as nectar. In these layers, sugars and other essential materials such as amino acids are thought to be added, concentrated or removed in order to transform phloem into nectar (Pacini and Nepi 2007). However, we found no significant difference in sugar concentration among *Inga* species. This suggests that rather than change the concentration of their nectar, plants control the volume of the nectar and thus the mass of sugar produced.

Although amino acids have been reported in the extrafloral nectar of *Inga* (Koptur 1994), these were not analyzed in our study. Baker and Baker (1973) suggested that additional components such as amino acids in floral nectar are likely to influence pollinator visitation. Some studies have shown that ants can have preferences for amino acids in nectar and that herbivory can change amino acid concentrations in nectar (Lanza 1988; Lanza et al. 1993; Smith et al. 1990). However, the same studies also showed that ant preferences can be ant-species specific and that not all plant species responded the same when damaged (Lanza 1988; Lanza et al. 1993; Smith et al. 1990). In addition, Gonzalez-Teuber and Heil (2009) showed that amino acids only affected nectar attractiveness when they were at very high concentrations, and that facultative ant partners in ant–plant mutualisms did not have a preference for changes in amino acid composition. Their results suggested that sugar was more influential in facultative ant–plant mutualisms. In *Inga*, ants visit extrafloral nectaries opportunistically and the identity of a visiting ant species may not be the same from day to day. Thus, plants need a general reward to attract the "passerby" of the day. Heil et al. (2005) demonstrated that qualitative differences in nectar sugar composition can attract specific ants, and that sucrose is more attractive than fructose and glucose to generalist ant bodyguards. Given the above observations from the literature—that sucrose, glucose and fructose were the dominant components of *Inga* nectar, and that there was no difference in the suite of ant-species that visited the different *Inga* species—we hypothesize that sugars were the principal nutritional components that influenced ant behavior.

## Induction

### Effect of ants

The increase in nectar production by all species in high light or with ants present demonstrates that nectar production is not strictly constitutive, and that plants are capable of changing their investment in nectar in response to biotic and abiotic signals. We hypothesize that both light and ant presence increase nectar production through a source–sink mechanism. We define nectaries as sinks and photosynthate from the phloem as the source. Photosynthate is most likely imported to young leaves from storage tissues (in mature leaves, roots or stems) due to the high demands in young leaves, a lack of sufficient photosynthetic machinery in young leaves, and, in the understory, insufficient light (Kursar and Coley 1992). As ants remove nectar from the nectary, the sink is emptied then refilled by the phloem. In *Macaranga*, plants that had nectar removed regularly with pipettes produced more nectar than plants that had their nectar collected at longer intervals (Heil et al. 2000). In this study, nectaries were isolated from ants for 24 h before nectar collections were made. During this period, nectaries that had received normal ant visitation produced more nectar despite being isolated from ants for 24 h.

### Effect of light

Increased nectar production also occurred when plants were in higher light environments, such as light gaps. There was a significant interaction effect between light and plant species ($P = 0.0013$, Table 1), but all plant species had the same trend of increased nectar production in high light (Fig. 4c). Plants in the understory can be limited by light, as typically less than 2% of full sunlight penetrates through to the rainforest understory (Leigh 1999). The increased flow into the extrafloral nectaries when plants are found in the higher light of gaps could be due to the increased photosynthate in the phloem from storage tissue or photosynthesizing leaves. Similar shifts in carbon-based ant rewards have been observed in *Cecropia* plants. In high light, these plants increased their investment in Mullerian bodies, which are predominately glycogen (Folgarait and Davidson 1994). In addition, when *Inga vera* individuals were grown in the light, they received better protection from their mutualistic ant partners, suggesting increased nectar production (Kersch and Fonseca 2005).

While the greater availability of photosynthate stands out as the predominant reason for increased nectar production in high light, it has also been found that on BCI there is a higher abundance of herbivores and predators of herbivores in gaps (Richards and Coley 2007). So investment in ant defense in gaps would be more likely to attract a defender, and it is an environment where more protection is needed.

### Effect of herbivores

Herbivore-induced production of secondary metabolites is regulated by jasmonic acid (JA) and the octadecanoid pathway (Arimura et al. 2005). We showed that one species of herbivore does not induce nectar production in the genus *Inga*. This may be because nectar production (1) is constitutive and therefore not under JA pathway regulation, (2) does not respond either to the amount of damage inflicted by the experimental treatment or to the species of herbivore that we used, or (3) does not respond to short-term changes in herbivore presence. With respect to (1), we have shown in this study that nectar production is not constitutive and can in fact be altered by both biotic and abiotic factors. Heil and Silva Bueno (2007) showed that leaves exposed to volatile organic compounds increased their nectar production through JA signaling. Additionally, Heil et al. (2001) showed that extrafloral nectar is induced by exogenous JA and that this increased ant visitation in *Macaranga tanarius*. This suggests that nectar production can be controlled by JA and is not constitutive in *Inga*.

With respect to (2) above, the larval gelechiids used in this study are the most common herbivores on *Inga* on BCI (Kursar et al. 2006), and, in the plus-herbivore treatment of the current study, they consumed 21% of a young leaf on average. Karban and Baldwin (1997) reported that many other genera show induced responses to “minute” amounts of damage, often well below 20% of the leaf area. These data suggest that if nectar production in the genus *Inga* is responsive to herbivore damage, the experimental treatment was sufficient to elicit an induced response and that an appropriate herbivore was used. However, gelechiid caterpillars do build leaf “tents” to hide from predators. Therefore, because more ant bodyguards may not be more effective against these concealed enemies, this herbivore may not induce nectar production and consequently ant protection.

The final hypothesis, (3) above, suggests that plants would not benefit from relaxing their defenses because herbivore pressure in tropical habitats is high and herbivore-free periods are brief. That is, because herbivore pressure in tropical forests does not vary between high and low periods as in temperate habitats, there may not be a benefit for defenses to respond to short-term changes in herbivore presence on individual plants. The pattern of induced nectar production in temperate plants but not in tropical plants has been suggested by Koptur (1989), who showed induced nectar production in a temperate annual (*Vicia sativa*) but not in tropical perennial plants (*Ipomoea carnea*, *Inga brenesii*, and *Inga punctata*). While these results support the proposed hypothesis, Koptur points out that the difference could be due to the plant origin (i.e., temperate or tropical) or to growth form (i.e., perennial or annual), and that further research needs to be pursued. Other authors have shown that defenses can be induced in temperate perennial plants (Berenbaum and Zangerl 1994; Bjorkman et al. 2008; Dalin and Bjorkman 2003; Darrow and Bowers 1999; Litvak and Monson 1998; Ness 2003; Scutareanu et al. 2003; Shiojiri and Karban 2006; Wold and Marquis 1997; Wooley et al. 2007), which suggests that the differences found in this study are due to the selective pressures associated with a plant's native habitat and not to its growth form.

Currently, plant–herbivore defense theory emphasizes the advantages of induction. We suggest, however, that induction in response to herbivores may not be adaptive for all environments. The lack of evidence supporting induced defenses in tropical plants suggests that the selective forces are different across a latitudinal gradient and that selection favors constitutive defenses in tropical plants. Furthermore, it suggests that constitutive defenses are adaptive under certain conditions and not necessarily a relic of an ancestral state.

In contrast to the predictably high risk of herbivore attack to young leaves, ant visitation in a facultative mutualism is more variable. If ants are present, it is adaptive to invest in nectar. However, if ants are not present, then the cost of nectar production would not be balanced by the benefits of protection. Thus, we suggest that selection has favored plastic responses of nectar production to ants but not herbivores in tropical forests.

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