American Journal of Botany 102(1): 58–66, 2015.

DYNAMIC EXTRAFLORAL NECTAR PRODUCTION: THE TIMING OF LEAF DAMAGE AFFECTS THE DEFENSIVE RESPONSE IN *SENNA MEXICANA* VAR. *CHAPMANII* (FABACEAE)[1]

IAN M. JONES[2] AND SUZANNE KOPTUR

Department of Biological Sciences, Florida International University 11200 S.W. 8th Street, Miami, Florida 33199 USA

- *Premise of the study:* Extrafloral nectar (EFN) mediates food for protection mutualisms between plants and defensive insects. Understanding sources of variation in EFN production is important because such variations may affect the number and identity of visitors and the effectiveness of plant defense. We investigated the influence of plant developmental stage, time of day, leaf age, and leaf damage on EFN production in *Senna mexicana* var. *chapmanii*. The observed patterns of variation in EFN production were compared with those predicted by optimal defense theory.
- *Methods:* Greenhouse experiments with potted plants were conducted to determine how plant age, time of day, and leaf damage affected EFN production. A subsequent field study was conducted to determine how leaf damage, and the resulting increase in EFN production, affected ant visitation in *S. chapmanii.*
- *Key results:* More nectar was produced at night and by older plants. Leaf damage resulted in increased EFN production, and the magnitude of the response was greater in plants damaged in the morning than those damaged at night. Damage to young leaves elicited a stronger defensive response than damage to older leaves, in line with optimal defense theory. Damage to the leaves of *S. chapmanii* also resulted in significantly higher ant visitation in the field.
- *Conclusions:* Extrafloral nectar is an inducible defense in *S. chapmanii*. Developmental variations in its production support the growth differentiation balance hypothesis, while within-plant variations and damage responses support optimal defense theory.

Key words: extrafloral nectar; Fabaceae; optimal defense theory; plant defense; *Senna mexicana* var. *chapmanii*.

Extrafloral nectaries (EFNs) are glands that secrete nectar (solutions of sugar and other compounds) located outside of flowers and have been reported on species belonging to 93 families and 332 genera (Koptur, 1992; Marazzi et al., 2013). One can find EFNs on almost any vegetative or reproductive plant structure (Bentley, 1977; Inouye and Taylor, 1979; Koptur, 1992), and these nectaries may serve diverse ecological functions (Baker et al., 1978; Becerra and Venable, 1989; Wagner and Kay, 2002; González-Teuber and Heil, 2009; Heil, 2011). Extrafloral nectar (EFN) may be consumed by a broad spectrum of arthropods, but its discovery by ants, in particular, is known to benefit many plants by providing indirect defense against herbivores (Bentley, 1977; Koptur, 1992; Rosumek et al., 2009). Uncovering the factors that affect EFN production can help us to understand how plants regulate their investment in defense and how they manage and maintain interactions with beneficial insects.

A host of studies have identified food for protection mutualisms between ants and plants (Koptur, 1992; Rosumek et al., 2009). In many cases, plants provide domatia and food bodies as well as EFN, and the resulting interactions may be obligate. Janzen (1966) famously observed that *Acacia cornigera* plants succumbed to herbivory when resident ants (*Pseudomyrmex ferruginea*) were experimentally removed. Plants that provide only EFN are normally involved only in facultative interactions with ants; however, significant fitness benefits have been reported nonetheless (Koptur, 1979, 1984; Oliveira, 1997; Rudgers, 2004; Koptur et al., 2013).

Although indirect defenses are thought to be metabolically inexpensive in comparison with direct defenses, the secretion of nectar can undoubtedly be costly. Floral nectar production in *Asclepias syriaca*, for example, can consume up to 37% of daily assimilated carbon (Southwick, 1984). It is not surprising, therefore, that plants adjust nectar production over time and in response to herbivory (Heil et al., 2000). Several studies addressing EFN secretion have demonstrated increased production in response to mechanical leaf damage (e.g., Stephenson, 1982; Koptur, 1989; Engel et al., 2001) and herbivory (Koptur, 1989; Agrawal and Rutter, 1998; Heil et al., 2001; Mondor and Addicott, 2003). The volume of nectar produced by each nectary (Heil et al., 2001) and the number of nectaries (Mondor and Addicott, 2003) have been seen to increase in damaged or herbivore infested plants. The ability to express defensive traits plastically confers several key evolutionary benefits to plants. First, metabolic costs are reduced in cases where production is low, and second, more variable expression of defensive traits provides a barrier against the evolution of insect resistance (Heil, 2010).

In addition to responses to leaf damage, temporal variations in EFN production have also been observed, with different

---

[1] Manuscript received 29 August 2014; revision accepted 11 November 2014.

The authors thank A. Sinanan and Y. Lago for help caring for plants and collecting data. Special thanks also go to B. Barrios, J. Clayborne, J. Gehring, C. Pimienta, and S. Zona for constructive input. Thanks also to S. Zona for his generosity with greenhouse facilities. Finally, the authors thank S. Kephart, M. Heil, and an anonymous reviewer for helpful feedback on earlier versions of this manuscript. This work benefited from financial support from the Lloyd Kelly Foundation for Tropical Botany. This is contribution number 291to the Florida International University Program in Tropical Biology.

[2] Author for correspondence (e-mail: ijone002@fiu.edu)

doi:10.3732/ajb.1400381

species exhibiting peak production at different times of day (Wickers, 1997; Heil et al., 2000; Raine et al., 2002; Lucas-Barbosa et al., 2011). Such marked differences suggest that production patterns are not the result of a general physiological mechanism, but may be determined by particular selection pressures acting on each species (Tilman, 1978; Corbet and Delfosse, 1984; Kuo and Pate, 1985; Heil et al., 2000). The EFNs of *Macaranga tanarius*, for example, are dominated by ants during the night, and nectar robbing flies during the day (Heil et al., 2004b). The availability of suitable mutualists, as well as changing herbivore pressures, are likely factors in any cost-benefit analysis for EFN production and inevitably affect the outcome of ant–plant interactions. For example, Koptur (1979) removed EFNs from the common vetch, *Vicia sativa*, growing naturalized in California and found that nectariless plants attracted fewer ants, suffered greater herbivore damage, and produced fewer fruit. The same species revealed a more complex situation in its native England, however, where ants visiting nectaries effectively protected the internally feeding pod-dwelling herbivores from their natural enemies (Koptur and Lawton, 1988). Such findings remind us that the costs and benefits of EFN production can only be truly understood in the context of the community.

In addition to diurnal variations, changes in EFN production occur over the course of plant development (Quintero et al., 2013). Investment in defensive traits should aim to maximize fitness benefits, while minimizing costs (McKey, 1974). As plants age, the balance between costs and benefits may shift, and changes in the expression of defensive traits are predicted to occur (Elger et al., 2009). The effects of these ontogenetic variations on the dynamics of ant–plant interactions have only recently been explored (Boege and Marquis, 2005; Kwok and Laird, 2012; Quintero et al., 2013). Ontogenetic variations in plant indirect defenses are expected to be shaped by plant limitations (resource allocation, architectural requirements, and anatomical constraints), as well as external drivers (herbivore pressures and the availability of suitable mutualists) (Quintero et al., 2013). Predicting such variations has relied on two prominent plant defense hypotheses: optimal defense theory (ODT) (McKey, 1974) and the growth differentiation balance hypothesis (GDBH) (Herms and Mattson, 1992). The ODT predicts that plant parts or developmental stages that are of particularly high value or that are highly vulnerable to herbivory should exhibit heightened defenses. The GDBH predicts that defenses should be heightened in tissues or life stages in which the nutrient requirements for growth have been met. Only then will excess carbon from photosynthesis be allocated to the differentiation processes required for traits such as induced defenses (Stamp, 2003). Predictions made using these hypotheses may be contradictory in some cases. For example, the seedling stage of many plants is particularly vulnerable to herbivory (Clark et al., 2012), and any loss of tissue results in a relatively high fitness cost (Coley and Barone, 1996; Heil et al., 2004a; Lambdon and Hassall, 2005; Radhika et al., 2008). Seedlings are also subject to high levels of competition and are often carbon limited. As a result, ODT predicts an increased investment in defense at the seedling stage, while the GDBH predicts the reverse.

Developmental changes in indirect defenses may also be related to the types of interactions that they mediate. Myrmecophytic plants, providing both food and shelter for their insect mutualists, tend to produce these resources only once they reach a threshold size or stage, and they produce them in greater abundance as they age (Young et al., 1997; Itino et al., 2001). A meta-analysis conducted by Quintero et al. (2013) supports this pattern and lends support to the GDBH. Conversely, in myrmecophylic plants, which do not provide nesting sites for their mutualists, no consistent ontogenetic patterns have been observed. Doak et al. (2007) observed a marked decrease in EFN production with age in trembling aspen (*Populus tremuloides*, Salicaceae), while others have found EFN production to be increased in, or even limited to, mature stages (Koptur, 1979; Falcao et al., 2003; Kobayashi et al., 2008; Holland et al., 2009). More studies of myrmecophylic plants are required to understand the factors that affect plant resource allocation to defense.

Temporal changes in the type and intensity of plant defenses are predicted, not only over the development of the plant, but also within individual leaves. Young leaves are nutrient rich (Heil et al., 2004a; Lambdon and Hassall, 2005; Radhika et al., 2008) and are necessarily soft to allow for cell expansion (Yamawo et al., 2012). As a result, young leaves are particularly susceptible to herbivory and should invest more heavily in defense according to ODT. Indeed, the leaves of *Mallotus japonicus* have been observed to shift from more costly direct defenses, such as trichomes, toxins, and secondary compounds, to relatively “cheap” indirect defenses, such as EFN or food bodies, as they age (Yamawo et al., 2012). Any such changes in defensive strategy, however, may be influenced by plant life histories. Fast-growing plants are expected to concentrate defensive investment in young leaves (McKey, 1974; van Dam et al., 1994), while slow-growing plants, with greater leaf longevity, are likely to place equal importance on the defense of mature leaves (van Dam et al., 1996). Several studies have shown EFN production to vary with leaf age, and peak production most often occurs in young to middle aged leaves (Folgarait and Davidson, 1995; Heil et al., 2000; Kobayashi et al., 2008; Radhika et al., 2008).

Over the last few decades, a rich literature has emerged on the role of EFN, the factors that affect its production, and the insect–plant interactions that it mediates. Despite how taxonomically widespread EFN is and its importance as an indirect defense against herbivores, there are surprisingly few species for which the dynamics of its production are understood. Particularly little attention has been paid to temporal variations in EFN secretion, knowledge of which is imperative if we aim to understand how and to what extent plants manipulate their mutualists.

Here we conducted two greenhouse experiments and one field study to determine the dynamics of EFN production in *Senna mexicana* var. *chapmannii* (Jacq.) (hereafter referred to as *S. chapmanii*), a legume native to the pine rocklands of South Florida and the Caribbean (Lee and West, 2011). *Senna* is a genus within the subfamily Caesalpiniodeae, comprising around 350 species. Substantial diversification is thought to have occurred in the genus coinciding with the evolution of EFNs (Marazzi and Sanderson, 2010). *Senna chapmanii* is threatened in South Florida and grows primarily in the pine rocklands, themselves under pressure from saltwater intrusion and a host of anthropogenic factors (Noss, 2011). The plants bear single globe-shaped EFNs on the rachis between the first pair of leaflets (Fig. 1), as well as on the pedicels, which are commonly patrolled by ants (I. Jones, Florida International University, personal observation). Only nectaries on the rachis were sampled here, as none of the plants flowered during the study. Perennial legumes have often been used in experimental systems

Fig. 1. Extrafloral nectary of *Senna chapmanii* with nectar droplet. Nectary diameter = 1.4 mm.

to investigate the ecological role of EFN (Heil, 2004; Choh et al., 2006; Rios et al., 2008; Jezorek et al., 2011). Harnessing biotic plant defenses in these systems may represent an opportunity to increase agricultural production or to decrease the use of harmful and expensive pesticides.

Greenhouse experiments were conducted at Florida International University (FIU) to determine how factors such as plant age and time of day affect EFN production and whether EFN production is inducible by leaf damage in *S. chapmanii*. In addition, experiments were designed to address two questions that have not previously been answered for any species: (1) Does the time of day at which leaf damage occurs affect the defensive response? (2) Does damage to young and old leaves elicit the same degree of EFN induction?

Plants response to leaf damage may be influenced by changes in resource availability during the day. The timing of leaf damage may also inform the plants response by providing information regarding the source of the threat. We know that plants can manipulate EFN production in response to the presence of consumers (Heil et al., 2000, 2009), and that different types of damage can elicit different defensive responses (Schmidt et al., 2009; Sotelo et al., 2014); however, the effect of damage timing on inducible defenses has never been studied.

Rates of EFN production are known to vary as leaves age (Folgarait and Davidson, 1995; Heil et al., 2000; Kobayashi et al., 2008; Radhika et al., 2008), and in some cases, developmental patterns of investment have been used to support ODT (Radhika et al., 2008; Holland et al., 2009). Damage to leaves is widely known to elicit increased EFN production in many plants (Stephenson, 1982; Koptur, 1989; Engel et al., 2001); however, responses to damage to leaves of different ages have never been compared. Greater EFN production in response to damage to young leaves would provide further support for ODT.

In this study, we report EFN production as the mean mass of sugar (mg) produced by each plant, as this provides the best representation of defensive investment. Previous studies have generally reported only nectar volume or concentration, both of which are affected by extraneous factors such as temperature and humidity. Where we refer to EFN production in the discussion, we refer to mean sugar production unless otherwise stated.

In addition to greenhouse experiments, we conducted a field study to determine whether leaf damage and any subsequent rise in EFN production actually lead to increased ant attendance in *S. chapmanii*. Though any increase in EFN production is assumed to confer greater defense, the effects of EFN on herbivory rates and plant reproductive fitness have largely been observed through the complete exclusion of ants or the removal of EFN (Rosumek et al., 2009). Few studies have showed that the degree of ant defense is actually proportional to the quantity of EFN produced (but see Heil et al., 2001; Kost and Heil, 2005), and more evidence is required, particularly for species that mediate ant defense solely through food rewards.

MATERIALS AND METHODS

Experiment 1: Identifying temporal and developmental patterns of EFN secretion in* S. chapmanii *and determining the effects of leaf damage and damage timing on EFN production*—Senna chapmanii* plants were grown from seeds in a greenhouse at Florida International University (FIU). After 3 wk, seedlings were transplanted into 0.6-L pots, and 1.5 g of slow release fertilizer (Nutricote NPK; Florikan, Sarasota, Florida, USA) was added to each plant. Plants were maintained in the greenhouse until they reached one of two developmental stages: 1, young seedlings with 5–7 mature leaves; 2, older seedlings with 10–12 mature leaves. Older plants were allowed to grow for 3–4 wk longer (posttransplantation) than younger plants before experimentation. Using differently aged plants, within specific size ranges, allowed us to control for differences in plant growth rate; however, variation in growth rates appeared low.

Thirty young plants were divided at random into treatment and control groups. Mechanical leaf damage (50%) was inflicted on leaves 1–5 of the treatment plants (leaf 1 being the most apical mature leaf) by cutting each leaflet in half horizontally using scissors.

Extrafloral nectar production from leaves 1–5 of each plant was measured at 07:00 hours and 19:00 hours every day for 4 d (8 measurements). After each measurement, and 12 h before the first measurement, plants were washed to remove any residual nectar and dried with paper towels to prevent dilution of subsequent samples. Each measurement, therefore, represented 12 h of nectar production either during the day (19:00 hours), or during the night (07:00 hours).

Nectar volume was measured using 1, 2, and 10 μL micropipettes, and its concentration determined using a handheld refractometer. Total sugar production by each plant was then calculated as described below. To determine baseline nectar production, measurements began at 07:00 hours, immediately before damage treatments. The experiment was later repeated (for a total of 60 plants) with initial nectar measurements, and subsequent damage treatments at 19:00 hours. As a result, combined results could be used to observe the response to leaf damage, controlling for natural diurnal variations in EFN production. Additionally, the two sets of experiments could be analyzed to compare the response to damage occurring at 07:00 hours and 19:00 hours. The experiment was repeated a further two times (60 plants) using older seedlings.

***Experiment 2: Determining the effects of damage to young vs. old leaves. Does EFN production in* S. chapmanii *support optimal defense theory?*—**Ninety plants of *S. chapmanii* were grown from seeds as in experiment 1 and were left to grow until they had at least 10–12 mature leaves.

Thirty plants were divided at random into three treatment groups. In group 1, leaves 1–5 (the five youngest open leaves) were mechanically damaged (50%). In group 2, mechanical leaf damage (50%) was inflicted on leaves 6–10 (older leaves farther from the apical meristem). In group 3, the control group, no leaf damage was inflicted. Leaf damage was inflicted in groups 1 and 2 by removing 50% of each leaflet using scissors. Damage occurred at 07:00 hours on day 1 of the experiment.

Extrafloral nectar production, by each plant, was measured every 12 h (07:00 and 19:00 hours) for 48 h post treatment. Nectar was measured as in experiment 1, recording total nectar volume, concentration, and sugar production from the nectaries of leaves 1–10.

***Experiment 3: Determining the effects of leaf damage on ant attendance in* S. chapmanii**—One hundred plants of *S. chapmanii* were grown from seeds as in experiment 1. Twenty plants with at least 10–12 mature leaves were then divided at random into two treatment groups, damaged and undamaged. In group 1, leaf damage was inflicted on leaves 1–5 as in experiment 1. Damage was inflicted at 19:00 hours on day 1 of the experiment. Plants in treatment group 2 remained undamaged.

Immediately after leaf damage, the 20 plants were placed in pine rockland habitat within the FIU nature preserve. Pairs of plants (one from each treatment group) were placed side by side underneath fine mesh cages, which excluded most insects but not ants. These cages served to prevent herbivory and protect against nectar robbers, both of which might affect the production and/or availability of EFN. The number and species of ants on each plant were then recorded at five times during the following 24 h (22:00, 07:00, 10:00, 13:00, 16:00). The number of ant recruitment events was also recorded for each plant. Ant recruitment was deemed to have occurred when three or more ants of the same species were observed on a single plant at the same time.

Calculating sugar content of EFN—Refractometers, used in experiments 1 and 2, were calibrated using a series of artificial nectar solutions. A sugar mix containing fructose (42%), sucrose (23%), maltose (21. 5%), and glucose (13.5%) was formulated to closely resemble a generalized EFN. An 80% nectar solution was produced by dissolving 80 g of the sugar mix in 100 mL distilled water. The resulting solution was then serially diluted to produce 70%, 60%, 50%, 40%, 30%, 20%, and 10% solutions. By taking refractometer measurements of solutions with known sugar content, the following formula was established to calculate the sugar content (mg) of EFN produced by experimental plants: mg sugar/μL nectar = (refractometer reading × 0.00001729 + 0.0000073) × 1000.

Statistical analysis—For experiment 1, nonparametric Mann–Whitney *U* tests were used because data were not normally distributed. Extrafloral nectar production in young and old seedlings was compared to determine whether plant age affects EFN production. Data were aggregated by plant, so that the eight nectar measurements from each plant were reduced to one value, the mean (N = 120).

To determine whether plants produce more nectar during the night or during the day, data were aggregated by plant and time (N = 240). The eight observations for each plant were, therefore, reduced to two data points, mean nectar collected at 7am, and 7pm. Night-time and day-time sugar production were then compared among all plants.

To determine whether EFN production increased in response to leaf damage, data were again aggregated by plant. Sugar production (mg) in damaged and control plants was compared for young plants (N = 60), old plants (N = 60), and all plants (N = 120).

For establishing the duration that EFN production was increased after damage, data could not be aggregated. Sugar production in damaged and control plants were compared at 12, 24, 36, 48, 60, 72, and 84 h post damage (N = 120).

Finally, for determining whether the time at which damage occurs affects plant response, data were aggregated by plant, and sugar production was compared between plants damaged at 07:00 and 19:00 hours. This analysis was repeated for young plants (N = 30) and older plants (N = 30). Only EFN collected 12 and 24 h post treatment was included in this analysis because this time period represented the peak in EFN response to leaf damage.

For experiment 2, Kruskal–Wallace *H* tests were used to compare EFN production among the three treatments (N = 90) because data were not normally distributed. Post hoc analyses were then conducted separately using Mann–Whitney *U* tests between pairs of treatments (N = 60). Holm's sequential Bonferroni adjustments were applied to control for type 1 errors.

For experiment 3, Mann–Whitney *U* tests were used to determine whether ant attendance and ant recruitment differed between damaged and undamaged plants (N = 100).

RESULTS

Experiment 1—Old seedlings produced significantly more sugar than young seedlings both when damaged ($z = -4.421$, df = 28, $P < 0.001$) and undamaged ($z = -3.319$, df = 28, $P = 0.001$). All plants consistently produced more sugar during the night (median = 0.2923 mg) than during the day (median = 0.0438 mg) ($z = -9.891$, df = 238, $P < 0.001$). This result was true for both damaged ($z = -8.015$, df = 118, $P < 0.001$) and undamaged plants ($z = -7.382$, df = 118, $P < 0.001$) (Fig. 2).

Both young and old seedlings subjected to 50% leaf damage produced significantly more sugar than undamaged plants (young: $z = -4.938$, df = 58, $P < 0.001$; old: $z = -5.396$, df = 58, $P < 0.001$). In young seedlings, sugar production was significantly higher in damaged plants compared with control plants at 12 h ($z = -5.042$, df = 58, $P < 0.000$), 24 h ($z = -4.337$, df = 58, $P < 0.001$), and 36 h ($z = -2.653$, df = 58, $P = 0.008$) post treatment. In older seedlings, damaged plants produced significantly more sugar at 12 h ($z = -3.722$, df = 58, $P < 0.001$), 24 h ($z = -3.187$, df = 58, $P = 0.001$) and 72 h ($z = -2.233$, df = 58, $P = 0.026$) post treatment. When young and old seedlings were analyzed together, damaged plants produced significantly more sugar than control plants at every time except 60 h ($z = -1.643$, df = 118, $P = 0.1$) post treatment (Fig. 3).

The timing of leaf damage did not affect plant response in young seedlings ($z = -0.684$, df = 58, $P = 0.512$). However, older seedlings damaged at 07:00 hours produced significantly more sugar in the 24 h after leaf damage than those damaged at 19:00 hours ($z = -1.970$, df = 58, $P = 0.049$) (Fig. 4).

Experiment 2—The Kruskal–Wallis comparison among treatments showed that the location of plant damage affected EFN production. Subsequent Mann–Whitney *U* tests, with Bonferroni adjustments, showed that plants with 50% damage to young leaves produced significantly more sugar than plants with 50% damage to old leaves ($z = -2.935$, df = 58, $P = 0.003$), and control plants ($z = -2.676$, df = 58, $P = 0.007$). Plants with 50% damage to old leaves did not produce more sugar than control plants ($z = -0.643$, df = 58, $P = 0.520$) (Fig. 5). Plants with damage to young leaves also produced a higher volume of nectar than control plants ($z = -4.391$, df = 58, $P < 0.001$) and plants with damage to old leaves ($z = -4.539$, df = 58, $P < 0.000$). The volume of nectar produced by plants with damage to old leaves was not greater than that of control plants ($z = -0.488$, df = 58, $P = 0.626$). The mean concentration of EFN produced by plants with young leaf damage was also significantly higher than that

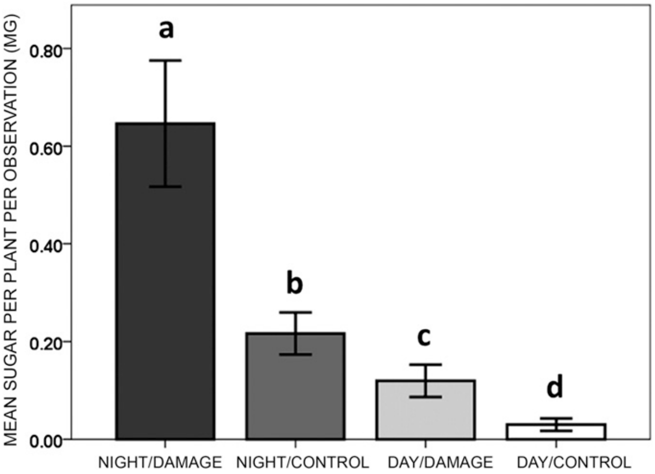

Fig. 2. Sugar production in all plants of *Senna chapmanii* in experiment 1 at night and during the day. Bars indicate mean sugar accumulated during each 12-h period. Letters indicate significant differences.

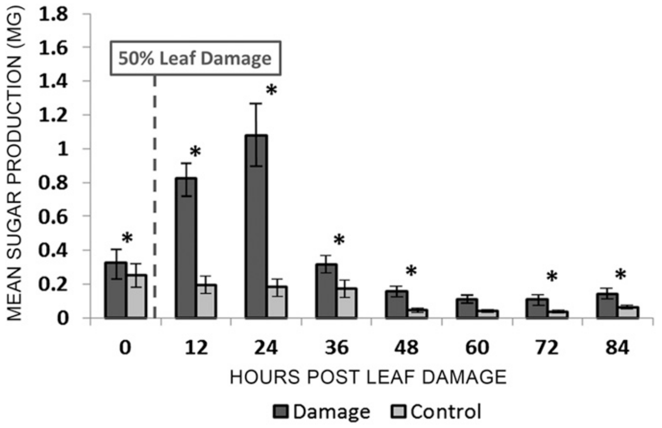

Fig. 3. Mean sugar production in damaged vs. control plants of *Senna chapmanii* at 12-h intervals post damage. Bars indicate mean sugar accumulated during each 12-h period. Results for plants damaged at 07:00 and 19:00 hours are combined, so each bar consists of measurements at both time points. Overall patterns of EFN production in response to leaf damage can therefore be observed controlling for natural diurnal variations in nectar production. The dotted line indicates the time of damage. Asterisks indicate significant differences between damaged and control plants.

produced by plants with old leaf damage ($z = -2.935$, df = 58, $P = 0.003$), and control plants ($z = -2.676$, df = 58, $P = 0.007$) (Fig. 6). Nectar concentration was not increased in plants suffering damage to older leaves in comparison with controls ($z = -0.555$, df = 58, $p = 0.579$).

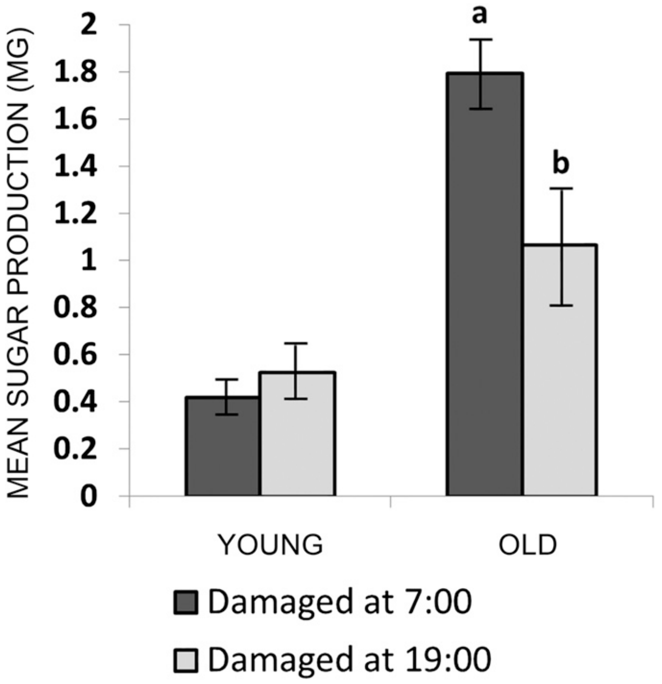

Fig. 4. Mean sugar production 12–24 h post leaf damage in young and old seedlings of *Senna chapmanii* damaged at 07:00 vs. young and old seedlings damaged at 19:00. Bars indicate mean sugar accumulated over each 12-h period and therefore represent the average between 1 daytime measurement and one nighttime measurement for each plant. Letters indicate significant differences.

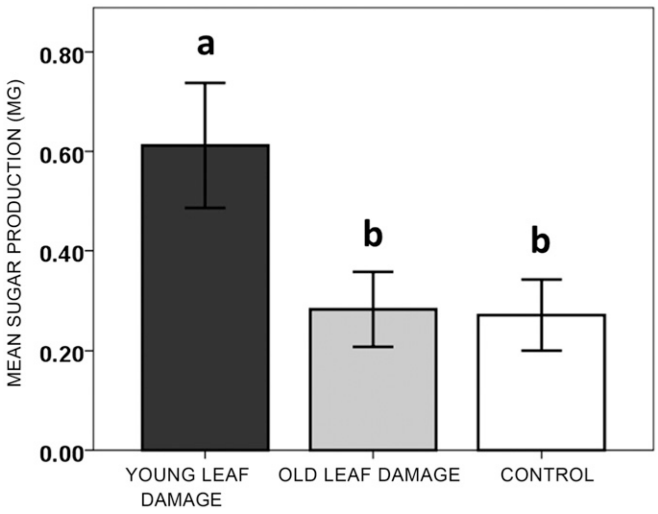

Fig. 5. Mean sugar production by plants of *Senna chapmanii* with damage to young leaves, old leaves, or no leaf damage. Bars indicate mean sugar accumulated during each 12-h period. Letters indicate significant differences.

Experiment 3—The total number of ants observed was significantly higher on damaged plants than on undamaged plants ($z = -3.468$, df = 98, $P = 0.001$) (Fig. 7). The number of ant recruitment events was also greater on damaged vs. undamaged plants ($z = -2.716$, df = 98, $P = 0.007$) (Fig. 8). Nine ant species were observed on damaged plants, while only six were observed on undamaged plants. Of the 299 ant visits observed, over 90% could be attributed to three species, *Brachymyrmex obscurior* Forel, *Wasmannia auropunctata* Roger, and *Paratrechina longicornis* (Latreille).

DISCUSSION

Extrafloral nectar is an extremely widespread, often inducible trait that mediates food for protection interactions between plants and ants. Although the ecological role of EFN as a form

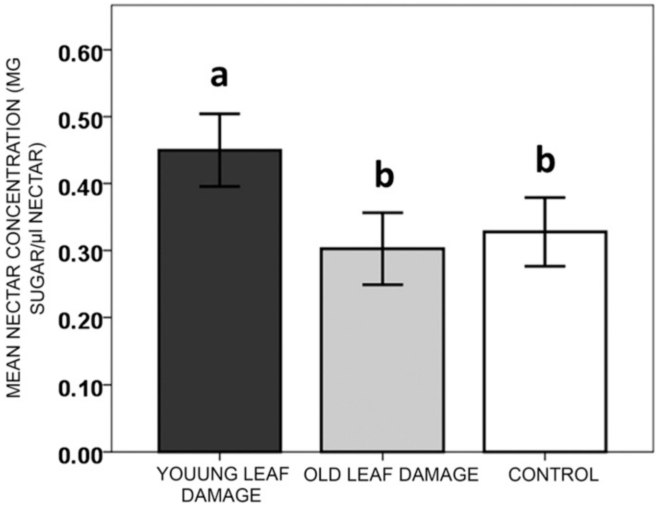

Fig. 6. Mean nectar concentration in plants of *Senna chapmanii* with damage to young leaves, old leaves, or no leaf damage. Bars indicate mean nectar concentration for each 12-h period. Letters indicate significant differences.

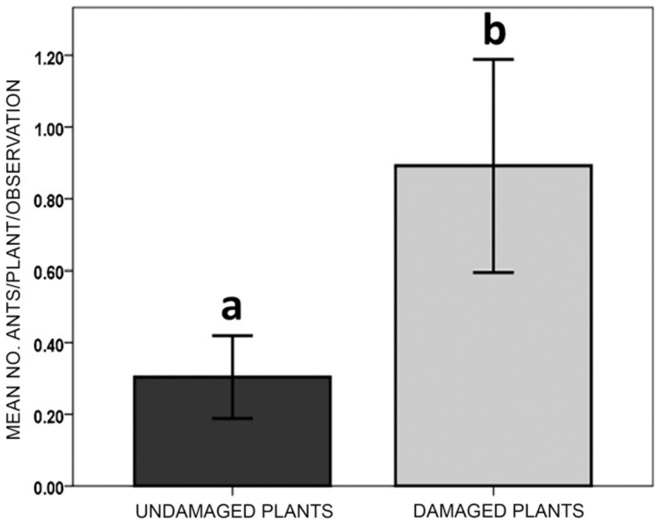

Fig. 7. Mean ant attendance on damaged and undamaged plants of *Senna chapmanii*. Bars indicate the average number of ants observed on each plant during each observation. Letters indicate significant differences.

of biotic defense against herbivores is well supported (Bentley, 1977; Koptur, 1992; Rosumek et al., 2009), far less is known about how plants control EFN secretion. Here we examine the effects of time of day, plant age, leaf damage, and the timing of leaf damage on EFN production in *S. chapmanii*.

Time of day—All plants produced significantly more nectar during the night than during the day. Overall, EFN production was over five times higher during the night (Fig. 2), and even damaged plants produced less nectar during the day than control plants produced at night. Nocturnal peaks in EFN production have been observed in several other studies (Bentley, 1977; Heil et al., 2000), but diurnal patterns of EFN production vary greatly among species (Heil et al., 2000; Raine et al., 2002). In most cases, patterns seem to correspond with consumer activity (Tilman, 1978; Corbet and Delfosse, 1984; Kuo and Pate, 1985; Heil et al., 2000). More observations of herbivore and ant activity, in natural growing conditions, are required to determine the selection pressures that drive nocturnal EFN production in *S. chapmanii*.

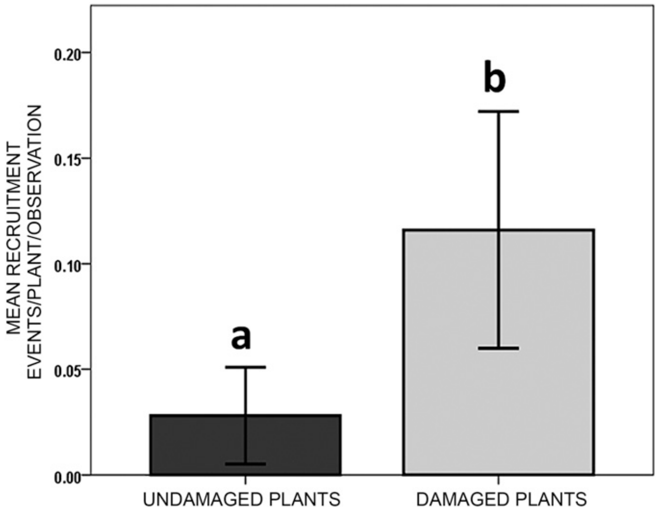

Fig. 8. Mean ant recruitment on damaged and undamaged plants of *Senna chapmanii*. Bars indicate the average number of recruitment events observed on each plant during each observation. Letters indicate significant differences.

Leaf damage—Both young and old seedlings produced significantly more EFN in response to leaf damage, providing further support for the assumption that EFN functions as an inducible defensive response. Numerous studies have reported increased EFN production in response to leaf damage (Stephenson, 1982; Koptur, 1989; Agrawal and Rutter, 1998; Engel et al., 2001; Heil et al., 2001; Mondor and Addicott, 2003; Choh and Takabayashi, 2006; Lach et al., 2009). In most cases, however, only the volume or concentration of nectar is reported, and the period of elevation is no greater than 48 h. Here we report elevated sugar production in *S. chapmanii* for up to 84 h post leaf damage. Inducible EFN production has been seen less commonly in tropical and subtropical plants (but see Heil et al., 2000), and it has been suggested that tropical plants, often subjected to greater herbivore pressures than their temperate counterparts, tend to express indirect defenses constitutively (Bixenmann et al., 2011).

The production of EFN in *S. chapmanii* in response to leaf damage was strongly dependent on the age of the damaged leaves, with damage to young leaves resulting in significantly greater sugar production. This weighting of defensive investment toward more valuable, and vulnerable tissues, provides strong support for ODT. Damage to young leaves is thought to have a greater impact on future plant fitness, as they have already caused high construction costs, but have not yet contributed significantly to the plants pool of photosynthetic products (Harper, 1989). Several authors have used ODT to predict patterns of EFN production. Holland et al. (2009) observed constitutive EFN production in the fruits of cactus, *Pachycereus schottii*, but only inducible production in less valuable flower buds. Radhika et al. (2008) showed not only that EFN production was greater in young leaves of lima bean and castor, but also that carbohydrate assimilates were transported from older to younger leaves to facilitate this investment. No authors, to our knowledge, have previously measured EFN production after controlled damage to tissues of different ages. Our results, therefore, provide new support for ODT and further evidence of the costs of inducible plant defenses.

Extrafloral nectar production in *S. chapmanii* was almost exclusive to the youngest mature leaves and, although this was not explicitly measured, the response to leaf damage did not appear to be systemic. Damage to leaves 6–10 had no impact on nectar production in leaves 1–5. This relationship requires further study before any conclusions can be drawn for *S. chapmanii*; however, a lack of systemic response has been noted in other species (Swift and Lanza, 1993; Heil et al., 2000).

Damage to young leaves of *S. chapmanii* elicited an increase not only in the overall quantity of sugar secreted, but also in the volume and concentration of EFN. Many studies have shown changes in nectar quantity in response to damage (e.g., Stephenson, 1982; Koptur, 1989; Agrawal and Rutter, 1998), but few have reported changes in nectar quality. The extent to which plants can manipulate EFN composition is an active area of research. The concentration of carbohydrates in EFN is known to vary widely (Heil et al., 2000; Bluthgen et al., 2004), and changes in concentration play a crucial role in determining the identity and behavior of its consumers (Heil, 2011). Nectar composition can even affect prey choice in some ants (Wilder and Eubanks, 2010), and plants may manipulate the nutritional value of nectar to encourage defensive mutualists to feed on their most damaging herbivores. While plants are known to manipulate nectar concentration (Heil, 2011), such manipulation has rarely been observed in response to short-term ecological cues. A few species, however, have been reported to manipulate nectar composition in response to the identity of visitors (Heil et al., 2009). Our results indicate that *S. chapmanii* can manipulate nectar concentration in response to the location of leaf damage.

Plant age—Baseline extrafloral nectar (EFN) production was higher in older vs. younger seedlings of *S. chapmanii*. Older seedlings also increased EFN production more in response to leaf damage. These findings may indicate an age-dependent shift in the balance of costs and benefits associated with EFN production in *S. chapmanii*. It should also be considered, however, that the observed increase in EFN production with plant age may be a function of the availability of sucrose in the phloem. Millán-Cañongo et al. (2014) found that spatiotemporal patterns of EFN production in *Ricinus communis* were mirrored by the activity of the plant hormone jasmonic acid (JA) and the enzyme cell-wall invertase (CWI). In experimentally shaded leaves, EFN production, but not the activity of JA or CWI, was greatly reduced. These findings highlight the importance of sucrose availability as a limiting factor for EFN production. In our experiments, older seedlings possessed a higher photosynthetic capacity, and damage treatments represented a smaller percentage loss of leaf tissue. The increased EFN production that we observed in older seedlings may, therefore, have resulted from greater availability of photosynthetic assimilates.

Our observations of the relationship between plant age and EFN production provide support for the GDBH. Older plants have greater photosynthetic potential and may satisfy nutrient requirements for growth with more to spare (Herms and Mattson, 1992; Stamp, 2003). The current study, however, observed only immature life stages, and work is ongoing to examine ontogenetic changes in EFN production throughout the life span of *S. chapmanii* in more detail.

Timing of leaf damage—Although many factors are known to affect patterns of EFN production, interactions among these factors have rarely been considered. We have clearly shown that EFN production in *S. chapmanii* increases in response to leaf damage and that it follows a predictable diurnal rhythm. We supplemented these findings by asking the question: Does the time of day at which damage occurs affect plant response? Our results clearly suggest that the answer is yes. Older seedlings damaged at 07:00 hours produced significantly more nectar in the 24 h that followed, than those damaged at 19:00 hours. Plant responses to attack are known to vary based on the severity of damage (Koptur, 1989) and even on the feeding guild of the attacker (Schmidt et al., 2009; Sotelo et al., 2014). It is not surprising, therefore, that defensive responses seem to also be sensitive to diurnal rhythms. The mechanisms behind this phenomenon deserve further study. The timing of leaf damage may provide the plant with useful cues as to the source and severity of the threat. Alternatively, or additionally, plants may face changing resource limitations over the course of 24 h. Wickers (1997), for example, suggested that diurnal patterns of EFN production in *Inga thibaudiana* result from changes in water availability. Indeed, in experiment 1, we observed a steady reduction in EFN production rates over 84 h (Fig. 3), probably due to declining water availability over the course of the experiment. Plants were watered 24 h before damage treatments, and water was replenished through light spraying after each nectar measurement. In future, plants should be watered fully after each nectar measurement to minimize any reduction in water availability during the study.

One potential limitation in all three experiments was that damaged and control plants remained in close proximity throughout the study. Exposure to herbivore-infested conspecifics has been observed to stimulate EFN production in several plants (Choh et al., 2006; Heil and Silva Bueno, 2007; Li et al., 2012). Our results, however, indicate that this is not the case in *S. chapmanii*. Indeed, future studies should explore the possibility that defensive investment in *S. chapmanii* may be downregulated in response to highly invested neighbors.

The effects of leaf damage on ant attendance—Leaf damage and the consequent increase in EFN production resulted in higher ant attendance in *S. chapmanii*. Extrafloral nectar is widely known to mediate food for protection mutualisms between ants and plants, and many plants have been shown to derive significant fitness benefits from these interactions (Koptur, 1979; Oliveira, 1997; Rudgers, 2004; Koptur et al., 2013). The majority of studies, however, have used an all or nothing exclusion of ants or of EFN (Rosumek et al., 2009), and few have demonstrated a correlation between investment in EFN production and the intensity of ant–plant interactions (but see Heil et al., 2001; Kost and Heil, 2005). Volatile organic chemicals (VOCs) produced by damaged plants may also have affected ant attendance; however, our experimental design placed damaged plants and control plants in close proximity, so it is likely that the effects of long-distance chemical cues would be seen in both treatments. Volatile chemicals within EFN itself, however, have been shown to affect ant attraction over short distances. Choice tests, conducted using the EFN of *Acacia* myrmecophytes, showed that ants preferred nectar over sugar solutions based on odor alone (González-Teuber and Heil, 2009). In addition to overall ant attendance, we observed a significantly higher number of recruitment events on damaged plants of *S. chapmanii*. While individual ants feeding at EFNs might represent chance encounters, recruitment events may better demonstrate manipulation of ant behavior by *S. chapmanii*.

Conclusions—Variations in EFN production may be ontogenetically programmed and/or occur in response to a host of abiotic and biotic factors. Observing these variations in controlled experiments can help us to understand patterns of plant investment in defense, the costs of those defenses, and how plants maintain mutualisms with ants. Ontogenetic changes in EFN production support GDBH in *S. chapmanii*. Older seedlings produce more EFN, probably as a result of increased availability of sucrose in the phloem. Changes in EFN production within individual leaves, however, provided strong support for ODT. Younger leaves produced significantly more EFN, in line with values per unit area of tissue and vulnerability to attack.

Our most important findings, however, relate to the response of *S. chapmanii* to leaf damage. Older seedlings damaged at 07:00 hours produced significantly more nectar over 24 h than did those damaged at 19:00 hours. The ability of plants to fine-tune their investment in indirect defenses in response to the timing of plant damage may have evolved in response to patterns of herbivore or mutualist activity. Alternatively, contrasting diurnal and nocturnal responses may reflect patterns of resource availability.

Damage to young leaves elicited a significant increase in EFN production, while damage to older leaves had no effect. In addition, EFN volume and concentration increased in response to young leaf damage. These results provide further support for ODT and suggest that *S. chapmanii* can manipulate EFN production with a high degree of sensitivity. This ability likely affords the plants better protection from herbivores, while optimizing their use of resources. Few studies have considered the effects of leaf damage on EFN quality, and the current study considers only carbohydrate concentration. More extensive analyses are required to determine nectar composition in *S. chapmanii* and discern how nectar constituents vary temporally and in response to leaf damage. We contribute to an improved understanding of plant resource allocation and the dynamics of defensive traits. Such an understanding is directly applicable in agricultural settings where the loss of crops to herbivores and the overuse of pesticides cause social and environmental problems worldwide.

LITERATURE CITED

Agrawal, A. A., and M. T. Rutter. 1998. Dynamic anti-herbivore defense in in ant-plants: The role of induced responses. *Oikos* 83: 227–236.

Baker, D. A., L. J. Hall, and J. R. Thorpe. 1978. A study of the extrafloral nectaries of *Ricinus communis. New Phytologist* 81: 129–137.

Becerra, J. X. I., and D. L. Venable. 1989. Extrafloral nectaries: A defence against ant–Homoptera mutualisms? *Oikos* 55: 276–280.

Bentley, B. L. 1977. The protective function of ants visiting the extrafloral nectaries of *Bixa orellana* (Bixaceae). *Journal of Ecology* 65: 27–38.

Bixenmann, R. J., P. D. Coley, and T. A. Kursar. 2011. Is extrafloral nectar production induced by herbivores or ants in a tropical facultative ant–plant mutualism? *Oecologia* 165: 417–425.

Bluthgen, N., G. Gottsberger, and K. Fiedler. 2004. Sugar and amino acid composition of ant attended nectar and honeydew sources from an Australian rainforest. *Austral Ecology* 29: 418–429.

Boege, K., and R. J. Marquis. 2005. Facing herbivory as you grow up: The ontogeny of resistance in plants. *Trends in Ecology & Evolution* 20: 441–448.

Choh, Y., S. Kugimiya, and J. Takabayashi. 2006. Induced production of extrafloral nectar in intact lima bean plants in response to volatiles from spider mite-infested conspecific plants as a possible indirect defence against spider mites. *Oecologia* 147: 455–460.

Choh, Y., and J. Takabayashi. 2006. Herbivore-induced extrafloral nectar production in lima bean plants enhanced by previous exposure to volatiles from infested conspecifics. *Journal of Chemical Ecology* 32: 2073–2077.

Clark, C. J., J. R. Poulsen, and D. J. Levey. 2012. Vertebrate herbivory impacts seedling recruitment more than niche partitioning or density-dependent mortality. *Ecology* 93: 554–564.

Coley, P. D., and J. A. Barone. 1996. Herbivory and plant defenses in tropical forests. *Annual Review of Ecology and Systematics* 27: 305–335.

Corbet, S. A., and E. S. Delfosse. 1984. Honeybees and the nectar of *Echium plantagineum* L. in southeastern Australia. *Australian Journal of Ecology* 9: 125–139.

Doak, P., D. Wagner, and A. Watson. 2007. Variable extrafloral nectary expression and its consequences in quaking aspen. *Canadian Journal of Botany* 85: 1–9.

Elger, A., D. G. Lemoine, M. Fenner, and M. E. Hanley. 2009. Plant ontogeny and chemical defence: Older seedlings are better defended. *Oikos* 118: 767–773.

Engel, V., M. K. Fischer, F. L. Wackers, and W. Volkl. 2001. Interactions between extrafloral nectaries, aphids and ants: Are there competition effects between plant and homopteran sugar sources? *Oecologia* 129: 577–584.

Falcao, P. F., G. F. D. Melo-de-Pinna, I. R. Leal, and J. S. Almeida-Cortez. 2003. Morphology and anatomy of extrafloral nectaries in *Solanum stramonifolium* (Solanaceae). *Canadian Journal of Botany* 81: 859–864.

Folgarait, P. J., and D. W. Davidson. 1995. Myrmecophytic *Cecropia*: Antiherbivore defenses under different nutrient treatments. *Oecologia* 104: 189–206.

González-Teuber, M., and M. Heil. 2009. Nectar chemistry is tailored for both attraction of mutualists and protection from exploiters. *Plant Signaling & Behavior* 4: 809–813.

Harper, J. L. 1989. The value of a leaf. *Oecologia* 80: 53–58.

Heil, M. 2004. Induction of two indirect defences benefits lima bean (*Phaseolus lunatus*, Fabaceae) in nature. *Journal of Ecology* 92: 527–536.

Heil, M. 2010. Plastic defense expression in plants. *Evolutionary Ecology* 24: 555–569.

Heil, M. 2011. Nectar: Generation, regulation and ecological functions. *Trends in Plant Science* 16: 191–200.

Heil, M., D. Feil, A. Hilpert, and K. E. Linsenmair. 2004a. Spatiotemporal patterns in indirect defense of a South-East Asian ant–plant support the optimal defense hypothesis. *Journal of Tropical Ecology* 20: 573–580.

Heil, M., B. Fiala, B. Baumann, and K. E. Linsenmair. 2000. Temporal, spatial and biotic variations in extrafloral nectar secretion by *Macaranga tanarius. Functional Ecology* 14: 749–757.

Heil, M., M. González-Teuber, L. W. Clement, S. Kautz, M. Verhaagh, and J. C. Silva Bueno. 2009. Divergent investment strategies of *Acacia* myrmecophytes and the coexistence of mutualists and exploiters. *Proceedings of the National Academy of Sciences, USA* 106: 18091–18096.

Heil, M., A. Hilpert, R. Kruger, and K. E. Linsenmair. 2004b. Competition among visitors to extrafloral nectaries as a source of ecological costs of an indirect defence. *Journal of Tropical Ecology* 20: 201–208.

Heil, M., T. Koch, A. Hilpert, B. Fiala, W. Boland, and K. E. Linsenmair. 2001. Extrafloral nectar production of the ant-associated plant, *Macaranga tanarius*, is an induced, indirect, defensive response elicited by jasmonic acid. *Proceedings of the National Academy of Sciences, USA* 98: 1083–1088.

Heil, M., and J. C. Silva Bueno. 2007. Within-plant signaling by volatiles leads to induction and priming of an indirect plant defense in nature. *Proceedings of the National Academy of Sciences, USA* 104: 5467–5472.

Herms, D. A., and W. J. Mattson. 1992. The dilemma of plants—To grow or defend. *The Quarterly Review of Biology* 67: 283–335.

Holland, J. N., A. A. Chamberlain, and K. C. Horn. 2009. Optimal defence theory predicts investment in extrafloral nectar resources in an ant–plant mutualism. *Journal of Ecology* 97: 89–96.

Inouye, D. W., and O. R. Taylor. 1979. A temperate region plant–ant–seed predator system: Consequences of extrafloral nectary secretion by *Helianthella quinquenervis. Ecology* 60: 1–7.

Itino, T., T. Itioka, A. Hatada, and A. A. Hamid. 2001. Effects of food rewards offered by ant–plant *Macaranga* on the colony size of ants. *Ecological Research* 16: 775–786.

Janzen, D. H. 1966. Coevolution of mutualism between ants and acacias in central America. *Evolution* 20: 249–275.

Jezorek, H., P. Stiling, and J. Carpenter. 2011. Ant predation on an invasive herbivore: Can an extrafloral nectar-producing plant provide associational resistance to *Opuntia* individuals? *Biological Invasions* 13: 2261–2273.

Kobayashi, S., T. Asai, Y. Fujimoto, and S. Kohshima. 2008. Anti-herbivore structures of *Paulownia tomentosa*: Morphology, distribution, chemical constituents and changes during shoot and leaf development. *Annals of Botany-London* 101: 1035–1047.

Koptur, S. 1979. Facultative mutualism between weedy vetches bearing extrafloral nectaries and weedy ants in California. *American Journal of Botany* 66: 1016–1020.

KOPTUR, S. 1984. Experimental evidence for defense of *Inga* (Mimosoideae) saplings by ants. *Ecology* 65: 1787–1793.

KOPTUR, S. 1989. Is extrafloral nectar production an inducible defence? *In* J. Bock and Y. Linhart [eds.], Evolutionary ecology of plants, 323–339. Westview Press, Boulder, Colorado, USA.

KOPTUR, S. 1992. Extrafloral nectary-mediated interactions between insects and plants. *In* E. A. Bernays [ed]. Insect–plant interactions, vol. IV, 81–129. CRC Press, Boca Raton, Florida, USA.

KOPTUR, S., AND J. H. LAWTON. 1988. Interactions among vetches bearing extrafloral nectaries, their biotic protective agents, and herbivores. *Ecology* 69: 278–283.

KOPTUR, S., M. PALACIOS-RIOS, C. DIAZ-CASTELAZO, W. P. MACKAY, AND V. RICO-GRAY. 2013. Nectar secretion on fern fronds associated with lower levels of herbivore damage: Field experiments with a widespread epiphyte of Mexican cloud forest remnants. *Annals of Botany* 111: 1277–1283.

KOST, C., AND M. HEIL. 2005. Increased availability of extrafloral nectar reduces herbivory in Lima bean plants (*Phaseolus lunatus*, Fabaceae). *Basic and Applied Ecology* 6: 237–248.

KUO, J., AND P. J. S. PATE. 1985. The extrafloral nectaries of cowpea (*Vigna unguiculata* (L.) Wapp). 1. Morphology, anatomy and fine-structure. *Planta* 166: 15–27.

KWOK, K. E., AND R. A. LAIRD. 2012. Plant age and the inducibility of extrafloral nectaries in *Vicia faba. Plant Ecology* 213: 1823–1832.

LACH, L., R. J. HOBBS, AND J. D. MAJER. 2009. Herbivory-induced extrafloral nectar increases native and invasive ant worker survival. *Population Ecology* 51: 237–243.

LAMBDON, P. W., AND M. HASSALL. 2005. How should toxic secondary metabolites be distributed between leaves of a fast-growing plant to minimize the impact of herbivory? *Functional Ecology* 19: 299–305.

LEE, D., AND S. WEST. 2011. Wayside trees of tropical Florida. Tellus Books, Mebane, North Carolina, USA.

LI, T., J. K. HOLOPAINEN, H. KOKKO, A. I. TERVAHAUTA, AND J. D. BLANDE. 2012. Herbivore induced aspen volatiles temporally regulate two different indirect defences in neighbouring plants. *Functional Ecology* 26: 1176–1185.

LUCAS-BARBOSA, D. L., J. J. A. VAN LOON, AND M. DICKE. 2011. The effects of herbivore-induced plant volatiles on interactions between plants and flower-visiting insects. *Phytochemistry* 72: 1647–1654.

MARAZZI, B., AND M. J. SANDERSON. 2010. Large-scale patterns of diversification in the widespread legume genus *Senna* and the evolutionary role of extrafloral nectaries. *Evolution* 64: 3570–3592.

MARAZZI, B., J. L. BRONSTEIN, AND S. KOPTUR. 2013. The diversity, ecology and evolution of extrafloral nectaries: Current perspectives and future challenges. *Annals of Botany* 111: 1243–1250.

MCKEY, D. 1974. Adaptive patterns in alkaloid physiology. *American Naturalist* 108: 305–320.

MILLÁN-CAÑONGO, C., D. ORONA-TAMAYO, AND M. HEIL. 2014. Phloem sugar flux and jasmonic acid-responsive cell wall invertase control extrafloral nectar secretion in *Ricinus communis*. *Journal of Chemical Ecology* 40: 760–769.

MONDOR, E. B., AND J. F. ADDICOTT. 2003. Conspicuous extra-floral nectaries are inducible in *Vicia faba. Ecology Letters* 6: 495–497.

NOSS, R. F. 2011. Between the devil and the deep blue sea: Florida's unenviable position with respect to sea level rise. *Climatic Change* 107: 1–16.

OLIVEIRA, P. S. 1997. The ecological function of extrafloral nectaries: Herbivore deterrence by visiting ants and reproductive output in *Caryocar brasiliense* (Caryocaraceae). *Functional Ecology* 11: 323–330.

QUINTERO, C., K. E. BARTON, AND K. BOEGE. 2013. The ontogeny of plant indirect defences. *Perspectives in Plant Ecology, Evolution and Systematics* 15: 245–254.

RADHIKA, V., C. KOST, S. BARTRAM, M. HEIL, AND W. BOLAND. 2008. Testing the optimal defense hypothesis for two indirect defences: Extrafloral nectar and volatile organic compounds. *Planta* 228: 449–457.

RAINE, N. E., P. WILLMER, AND G. N. STONE. 2002. Spatial structuring and floral avoidance behaviour prevent ant–pollinator conflict in a Mexican ant–acacia. *Ecology* 83: 3086–3096.

RIOS, R. S., R. J. MARQUIS, AND J. C. FLUNKER. 2008. Population variation in plant traits associated with ant attraction and herbivory in *Chamaecrista fasciculata* (Fabaceae). *Oecologia* 156: 577–588.

ROSUMEK, F. B., F. A. O. SILVEIRA, F. D. NEVES, N. P. D. BARBOSA, L. DINIZ, Y. OKI, F. PEZZINI, G. W. FERNANDES, AND T. CORNELISSEN. 2009. Ants on plants: A meta-analysis of the role of ants as plant biotic defenses. *Oecologia* 160: 537–549.

RUDGERS, J. A. 2004. Enemies of herbivores can shape plant traits: Selection in a facultative ant–plant mutualism. *Ecology* 85: 192–205.

SCHMIDT, L., U. SCHURR, AND U. S. R. ROSE. 2009. Local and systemic effects of two herbivores with different feeding mechanisms on primary metabolism of cotton leaves. *Plant, Cell & Environment* 32: 893–903.

SOTELO, P., E. PEREZ, A. NAJAR-RODRIGUEZ, A. WALTER, AND S. DORN. 2014. *Brassica* plant responses to mild herbivore stress elicited by two specialist insects from different feeding guilds. *Journal of Chemical Ecology* 40: 136–149.

SOUTHWICK, E. E. 1984. Photosynthate allocation to floral nectar: A neglected energy investment. *Ecology* 65: 1775–1779.

STAMP, N. 2003. Out of the quagmire of plant defense hypotheses. *Quarterly Review of Biology* 78: 23–55.

STEPHENSON, A. G. 1982. The role of extrafloral nectaries of *Catalpa speciosa* in limiting herbivory and increasing fruit production. *Ecology* 63: 663–669.

SWIFT, S., AND J. LANZA. 1993. How do *Passiflora* vines produce more extrafloral nectar after simulated herbivory? *Bulletin of the Ecological Society of America* 74: 451.

TILMAN, D. 1978. Cherries, ants and tent caterpillars: Timing of nectar production in relation to susceptibility of caterpillars to ant predation. *Ecology* 59: 686–692.

VAN DAM, N. M., T. J. DE JONG, Y. IWASA, AND T. KUBO. 1996. Optimal distribution of defenses: Are plants smart investors? *Functional Ecology* 10: 128–136.

VAN DAM, N. M., R. VERPOORTE, AND E. VAN DER MEIJDEN. 1994. Extreme differences in pyrrolizidine alkaloid levels between leaves of *Cynoglossum officinale. Phytochemistry* 37: 1013–1016.

WAGNER, D., AND A. KAY. 2002. Do extrafloral nectaries distract ants from visiting flowers? An experimental test of an overlooked hypothesis. *Evolutionary Ecology Research* 4: 293–305.

WICKERS, S. 1997. Study of nectariferous secretion in a pioneer plant, *Inga thibaudiana*, in relation with ants. *Acta Botanica Gallica* 144: 315–326.

WILDER, S. M., AND M. D. EUBANKS. 2010. Extrafloral nectar content alters foraging preferences of a predatory ant. *Biology Letters* 6: 177–179.

YAMAWO, A., N. SUZUKI, J. TAGAWA, AND Y. HADA. 2012. Leaf ageing promotes the shift in defence tactics in *Mallotus japonicas* from direct to indirect defence. *Journal of Ecology* 100: 802–809.

YOUNG, T. P., C. H. STUBBLEFIELD, AND L. A. ISBELL. 1997. Ants on swollen thorn acacias: Species coexistence in a simple system. *Oecologia* 109: 98–107.