



## Changing light conditions in pine rockland habitats affect the intensity and outcome of ant–plant interactions

Ian M. Jones<sup>1,3</sup>, Suzanne Koptur<sup>1</sup>, Hilma R. Gallegos<sup>1</sup>, Joseph P. Tardanico<sup>1</sup>, Patricia A. Trainer<sup>1</sup>, and Jorge Peña<sup>2</sup>

<sup>1</sup> Department of Biological Sciences, Florida International University, 11200 S.W. 8th Street, Miami, FL, 33199, USA

<sup>2</sup> Tropical Research and Education Center, University of Florida, Homestead, FL, 33031, USA

### ABSTRACT

Extrafloral nectar (EFN) mediates food-for-protection mutualisms between plants and ants. Such mutualisms exist within a complex web of biotic interactions, and in a framework provided by the abiotic environment. Both biotic and abiotic factors, therefore, affect the outcome of ant–plant interactions. We conducted an experiment to determine the effects of ant activity, and light intensity, on herbivory rates, growth, and reproductive fitness in *Senna mexicana* var. *chapmanii*, a perennial legume native to pine rockland habitats of south Florida. Forty plants were divided among four treatments in a factorial experimental design with two independent variables: ant activity and light intensity. Plants were divided equally between sunny and shady habitats, and ants were excluded from half of the plants in each habitat type. The presence of ants significantly reduced herbivory rates in *S. chapmanii*. In shaded habitats, the presence of ants had no effect on plant reproductive fitness, however, in sunny habitats plants with ants produced significantly more seeds over the duration of the 1-yr study. Ants represent an important biotic defense against herbivores in *S. chapmanii*; however, their effects on plant fitness are dependent on light conditions. Pine rockland habitats in south Florida have been widely destroyed or mismanaged. In fragments that remain, suppression of fire has led to increased canopy closure and shading of the understory. These changes will likely negatively impact plants that rely on ants for defense. We highlight the importance of conservation efforts to preserve the pine rocklands and the fire regimes on which they rely.

*Key words:* extrafloral nectar; Florida; plant defenses; *Senna mexicana* var. *chapmanii*.

EXTRAFLOREAL NECTARIES (EFNs), SUGAR-SECRETING GLANDS LOCATED OUTSIDE OF FLOWERS, HAVE BEEN REPORTED ON SPECIES BELONGING TO 93 FAMILIES AND 332 GENERA (Koptur 1992a, Marazzi *et al.* 2013). These glands are structurally diverse, and may be found on almost any vegetative or reproductive plant structure (Bentley 1977, Inouye & Taylor 1979, Koptur 1992a). A wide range of ecological functions have been suggested for EFNs (Baker *et al.* 1978, Becerra & Venable 1989, Wagner & Kay 2002, Gonzalez-Teuber & Heil 2009, Heil 2011), however, they are most noted for providing indirect defense against herbivory by attracting natural enemies (Janzen 1966, Koptur & Lawton 1988, Heil *et al.* 2001, Heil 2015). Ants represent the most common visitors to EFNs, and have regularly been observed to benefit host plant fitness (Bentley 1977, Koptur 1992a, Rosumek *et al.* 2009, Heil 2015).

A host of studies have identified food-for-protection mutualisms between ants and plants (Koptur 1992a, Rosumek *et al.* 2009, Koptur *et al.* 2015). Plants, known as myrmecophytes, may provide domatia and food bodies as well as EFN, and engage in obligate interactions with ants. Janzen (1966), for example, observed that *Acacia cornigera* plants succumbed to herbivory when resident *Pseudomyrmex ferruginea* ants were experimentally removed. A far greater number of plants, however, provide only

EFN and engage in facultative interactions with ants. These plants are described as myrmecophiles and, although their interactions with ants are more varied in their outcomes (Rosumek *et al.* 2009), significant fitness benefits have been reported in many plants (Koptur 1979, Oliveira 1997, Rudgers 2004, Kost & Heil 2005, Leal *et al.* 2006, Koptur *et al.* 2013).

In a number of myrmecophiles, the experimental exclusion of ants has resulted in reduced herbivory and an increase in plant reproductive fitness (Cuautle & Rico-Gray 2003, Heil 2004, Rutter & Rausher 2004, Leal *et al.* 2006). In the majority of these studies, however, plant fitness has been observed a relatively short time after ant exclusion, usually within one growing season (but see Torres-Hernandez *et al.* 2000, Rudgers 2004). In reality, the fitness benefits gained by plants as a result of reduced herbivory may not occur in the same growing season. Studies conducted over longer periods are required to account for potential lag-times between the deterrence of herbivores by ants and changes in plant reproductive fitness.

In one such long-term study, Torres-Hernandez *et al.* (2000) evaluated the effects of different ant species on the reproductive fitness of *Turnera ulmifolia* over a period of 2 yr. Plants associated with ants suffered lower levels of herbivory, but the level of protection provided by ants, and the effects on plant reproductive fitness, depended on the size and species of ants concerned. Furthermore, when ants were excluded, visits from other predators such as bees and wasps increased, and these insects offered

Received 6 May 2016; revision accepted 3 June 2016.

<sup>3</sup>Corresponding author; e-mail: ijone002@fiu.edu

greater protection than did some ant species. The outcomes of facultative ant–plant mutualisms, therefore, are strongly dependent on level dynamics (Heil *et al.* 2004).

Ant–plant mutualisms do not function in isolation, but within a framework imposed by abiotic factors (Kersch & Fonseca 2005). Increased nutrient availability in *Macaranga triloba*, for example, has been observed to increase EFN production and ant attendance, leading to reduced herbivory rates (Heil *et al.* 2001). Soil moisture levels have also been shown to affect EFN production and subsequent ant visitation in *Mallotus japonicus* (Yamawo *et al.* 2012). Light may be a particularly important factor influencing ant–plant mutualisms, as it not only represents a crucial part of resource availability but may also serve as an indicator of insect activity (Karban *et al.* 1999).

Extrafloral nectar production has been observed to increase in response to high light intensity (Yamawo & Hada 2010, Jones & Koptur 2015b). Furthermore, the induction of EFN production, either through treatment with jasmonic acid (Radhika *et al.* 2010) or through leaf damage (Izaguirre *et al.* 2013), has been found to be dependent on light intensity and light quality. Few studies have considered the effects of light conditions on EFN-mediated ant–plant interactions in natural growing conditions, and those that exist have yielded mixed results. In *Stryphnodendron microstachyum*, plants shaded by the forest canopy were exposed to more herbivores than plants growing in open pasture. In response, these shaded plants produced more EFN and attracted more ants (de la Fuente & Marquis 1999). Conversely, *Cecropia* trees exposed to increased sunlight, in forest gaps, more efficiently attract mutualistic ants (Davidson & Fischer 1991). In the most comprehensive study of its type, Kersch and Fonseca (2005) found that the presence of ants on *Inga vera* resulted in significant fitness benefits in sunny habitats, but not in the shade. It is clear from these studies that the outcome of insect–plant interactions may be conditional upon light conditions. It is likely that plants fare best from their interactions with ants when growing in the ecological conditions to which they are adapted.

*Senna mexicana* var. *chapmanii* (hereafter referred to as *Senna chapmanii*) (Fabaceae), is a perennial low-lying shrub native to the pine rocklands of south Florida and the Caribbean. The species bears single globe-shaped EFNs on the rachis between the first pair of leaflets, as well as on the pedicels, which are commonly patrolled by ants (Jones & Koptur 2015a,b, Koptur *et al.* 2015). We have previously shown that ants remove key herbivores from *S. chapmanii* (Koptur *et al.* 2015). We have also demonstrated that EFN production in *S. chapmanii* occurs predominantly at night (Jones & Koptur 2015a,b). Pine rockland habitats contain a high proportion (around 27%) of extrafloral nectary-bearing plants (Koptur 1992b), but ant–plant interactions have been studied in detail only in a few species (*e.g.*, Rutter & Rausher 2004). Much of the Florida pine rocklands have been destroyed in recent history and, in the remaining habitat fragments, light conditions are changing in predictable ways due to the suppression of fires that would maintain the open canopy that characterizes the habitat (Possley *et al.* 2008). We have shown that light intensity significantly affects EFN production in *S. chapmanii* (Jones & Koptur

2015b). Here we sought to investigate how changing light conditions might affect ant–plant interactions in *S. chapmanii*, as a model for the potential effects on the many other EFN producing species in the pine rocklands.

We conducted a 1-yr field study to observe EFN-mediated ant–plant interactions in *S. chapmanii*. We manipulated ant activity and light intensity to determine the effects of both factors on plant size, growth rate, herbivory, and seed set. The intensity and outcome of mutualistic interactions varies considerably in space and time (Bentley 1976, Barton 1986, Rico-Gray *et al.* 1998, Heil *et al.* 2000, Moya-Raygoza & Larsen 2001, Bronstein *et al.* 2003, Kersch & Fonseca 2005, Lange *et al.* 2013). Here we contribute to a growing literature, which aims to understand the causes of such variations.

## METHODS

A 1-yr field study was carried out at the University of Florida's Tropical Research and Education Center (TREC) in Homestead, Florida (25°30'27.52N, 8°30'13.67"W), between April 2013 and March 2014. The climate is subtropical, with average minimum and maximum temperatures of 3.2–24.8°C in January and 22.7–32.4°C in July. Elevation is roughly 2.5 m asl, and mean annual precipitation is 1496 mm (Koptur *et al.* 2015). The study site consisted of flat calcareous limestone rocklands that had been rock-plowed for agriculture. We utilized a 2-acre plot, previously overgrown with exotic pest plants, which had been mostly cleared of all vegetation except for a few stands of large native trees. Adjacent to the western edge of the plot there is a fragment of pine rockland habitat, a protected natural area.

*Senna chapmanii* plants were grown from seed in a greenhouse at Florida International University. Seeds were collected from multiple individuals in a single population on Big Pine Key (under Research Permit # FFO4RFKD-2014-0, National Wildlife Refuge System—National Key Deer Refuge). After 3 mo, 40 plants were transplanted into the experimental site, in an evenly spaced array, with each plant at least 4 m from its nearest neighbor. Sites were chosen to ensure an equal number of plants in sunny versus shaded habitats. Light conditions at each plant location were later characterized as described below.

Plants were mulched with wood chips, and watered for 2 mo until they were established. Plants were then divided into a factorial experimental design with two independent variables, ants (present/absent) and light intensity (sun vs. shade). Ten plants were allocated to each of four treatments: (i) sunny habitats with ants present; (ii) sunny habitats with ants excluded; (iii) shady habitats with ants present; (iv) shady habitats with ants excluded. Ant exclusion treatments were assigned systematically to ensure even distribution of treatments across the site.

Ants were excluded by painting a sticky gel (Tanglefoot™) around the base of the stem. Tanglefoot was reapplied on an as-needed basis, to maintain the efficacy of ant-exclusion treatments. Light intensity was measured at the apex of each plant at the beginning of the study, using a digital illuminance meter (Dr.Meter LX1330B, Union City, CA 94587). Light was measured

three times throughout the day (0900 h, 1200 h, and 1500 h), on a clear day with no cloud cover, and mean light intensity was calculated for each plant location. Mean illuminance ranged from 7800–51,366 Lux in the shade and 59,600–98,000 Lux in sunny habitats.

Insect activity was observed on each plant during weekly surveys. Plant size, growth rate, and reproductive fitness, as well as percentage herbivore damage, was measured every 2 wk. Extrafloral nectar production (volume and concentration) was measured every 2 mo.

**INSECT ACTIVITY.**—Every week, a single branch was chosen from each plant as a census locale. The chosen branch was observed for a period of 2 min, and the number and species of ants and herbivores were recorded. Voucher specimens were collected where necessary, for identification, and stored at Florida International University (FIU). Insect observations were conducted during the day (1000–1500 h), and at night (2000–0100 h) on an alternating basis, to control for differences in diurnal and nocturnal patterns of insect activity. At the conclusion of the study, daytime and nighttime insect observations were combined and analyzed together. The mean numbers of ants and herbivores per observation were calculated for each plant, and these means were compared between treatments.

**PLANT REPRODUCTIVE FITNESS.**—Every 2 wk, throughout the study, the number of open flowers and the number of mature fruits were counted on each plant. Mature fruit were collected and returned to the laboratory at FIU where the seeds of each fruit were counted. At the end of the study we calculated the mean number of seeds collected from each plant, and these means were compared between treatments. Where we refer to plant reproductive fitness in the results and discussion, we refer to seed set unless otherwise stated.

**HERBIVORY RATES.**—Every 2 wk, throughout the study, a single branch with an intact growing tip (with no herbivore damage) was chosen from each plant. A jewelry tag was fastened beneath the most recently matured leaf. After 2 wk, percentage herbivore damage was estimated for each new leaf above the jewelry tag, so that all herbivory observed was sure to have taken place during that 2-wk period. The mean percentage herbivore damage among all of the new leaves was taken as the overall bi-weekly herbivory score for that plant. On rare occasions, all of the leaves above the jewelry tag were removed by herbivores. In these cases a herbivory score of 100 percent was given. At the end of the study all of the 2-wk herbivory scores were averaged to give an overall mean herbivory score, for each plant, for the entire year. These means were compared between treatments.

**PLANT GROWTH RATES.**—Prior to the commencement of the study, 200 leaves at various developmental stages were collected from the experimental plants. Leaves were measured (length in cm) before being placed in a drying oven for 48 h, and dry biomass was recorded for each leaf. A significant regression equation was

found ( $R^2 = 0.682$ ,  $F(1, 198) = 424.4$ ,  $P < 0.001$ ). The following formula was established to estimate the dry biomass of a leaf from its length:

$$\text{Leaf dry biomass (g)} = \text{Leaf length (cm)} * 0.0188$$

During the study, every 2 wk, a single branch with an intact growing tip was chosen from each plant in order to measure vegetative growth rates. A jewelry tag was placed beneath the most recently matured leaf, and the length of that leaf was measured. After 2 wk, the number of mature leaves above the jewelry tag was counted, and the length of each leaf was measured again. The total dry biomass of the leaves above the jewelry tag was calculated at the beginning and end of each 2-wk period, and the increase in biomass was taken as a relative measure of plant growth during those 2 wk. Plant growth rates were measured in this way every 2 wk throughout the study. At the end of the study, the mean growth rate was calculated for each plant, and these means were compared between treatments.

**PLANT SIZE.**—*Senna chapmanii* is a perennial sprawling subshrub, which branches close to the base and often spreads broader than tall. As such, the number of branches on a plant is a useful proxy for plant size. Every 2 wk, throughout the study, the size of each plant was estimated by counting the number of branches. Branches were classified as any growing stem with 10 or more leaves. At the conclusion of the study, the mean number of branches was calculated for each plant, and these means were compared between treatments.

**EXTRAFLOREAL NECTAR PRODUCTION.**—At 2-mo intervals throughout the study, extrafloral nectar (EFN) production was measured in each plant. A single branch was selected from each plant, and any insects present were removed by hand. Nectaries were then washed by lightly spraying with water. Leaves were then dried with paper towel, and branches were sealed within fine mesh bags to exclude insects. Bags were placed on branches at 1900 h, and removed 12 h later for nectar measurements at 0700 h.

For each plant, combined nectar volume from the five most recently matured leaves on the chosen branch was measured using 1-, 2-, and 10- $\mu$ l micropipettes. Nectar concentration was measured using a handheld refractometer, and total sugar production was estimated from the combination of these measurements (see Jones & Koptur 2015a,b). In the results, EFN is expressed as total sugar production. At the end of the study, mean sugar production over the course of the entire study was calculated for each plant, and these means were compared between treatments.

**STATISTICAL ANALYSIS.**—Due to the high number of zeros in the data, insect observation data and seed set data could not meet parametric assumptions even after transformation. The effects of Tanglefoot and light conditions on ant and herbivore abundance as well as seed production were, therefore, tested independently using non-parametric Mann-Whitney U tests.

Repeated-measures factorial ANOVAs were used to investigate the effects of the independent factors, ants (presence/absence) and light conditions (sun vs. shade), on the dependent variables, herbivory level, plant growth rate, plant size, and production of EFN. Dependent variables were  $\log_{10}(x + 1)$  transformed to meet parametric assumptions. All statistical analyses were performed using SPSS23.

## RESULTS

**ANT ACTIVITY.**—Overall ant abundance was significantly higher on plants without Tanglefoot than on Tanglefoot-treated plants ( $N = 40$ ,  $df = 1$ ,  $U = 11$ ,  $P < 0.001$ ), indicating that Tanglefoot successfully excluded ants from treatment plants. On plants with Tanglefoot, a mean of 0.17 ants was observed per observation (1080 observations), and ants were present in only 185 observations (17.1%). On these occasions, ant numbers on a single branch ranged from 1 to 11. On plants without Tanglefoot, a mean of 1.34 ants was observed per observation (1080 observations), and ants were present in 606 observations (56.1%). On occasions where ants were present, numbers ranged from 1 to 18.

Ants were significantly more abundant on plants in sunny habitats than those in the shade ( $N = 40$ ,  $df = 1$ ,  $U = 117.5$ ,  $P = 0.024$ ). On plants in sunny habitats, a mean of 1.07 ants was observed per observation (1080 observations). Ants were present in 468 observations (43.3%). On these occasions, ant numbers ranged from 1 to 18. On plants in the shade, a mean of 0.53 ants were observed per observation (1080 observations). Ants were present in 323 observations (29.9%). On these occasions, ant numbers ranged from 1 to 14.

Six ant species made up the vast majority of observations: *Brachymyrmex obscurior* (38.04%), *Camponotus floridanus* (19.37%), *Solenopsis invicta* (19.02%), *Camponotus sexguttatus* (10.23%), *Cardiocondyla emeryi* (9.2%), and *Pheidole moerens* (2.7%). The remaining 1.44 percent of observations included four species, of which sightings were rare enough to be deemed unimportant for plant fitness.

**HERBIVORE ACTIVITY.**—Overall herbivore numbers were not affected by the presence or absence of Tanglefoot ( $N = 40$ ,  $df = 1$ ,  $U = 193$ ,  $P = 0.862$ ). In the presence of Tanglefoot, a mean of 0.32 herbivores was observed per observation (1080 observations). Herbivores were present in 251 observations (23.2%). On these occasions, herbivore numbers ranged from 1 to 7. In the absence of Tanglefoot, a mean of 0.35 herbivores was observed per observation (1080 observations). Herbivores were present in 213 observations (19.7%). On these occasions, herbivore numbers ranged from 1 to 11.

There were no differences in the abundance of herbivores between plants in sunny and shady habitats ( $N = 40$ ,  $df = 1$ ,  $U = 161$ ,  $P = 0.301$ ). In sunny habitats, a mean of 0.28 herbivores was observed per observation (1080 observations). Herbivores were present in 212 observations (19.6%). On these occasions, herbivore numbers ranged from 1 to 7. In the shade,

a mean of 0.39 herbivores was observed per observation (1080 observations). Herbivores were present in 252 observations (23.2%), in which herbivore numbers ranged from 1 to 11.

A total of 725 herbivores were observed over 2160 observations (a mean of 0.34 herbivores per observation). The most common herbivores were psyllids (412, 56.83%), leafhoppers (114, 15.72%), sulfur caterpillars (57, 7.86%), and scale insects (45, 6.21%). The remaining 13.35 percent of observations were made up of a wide range of taxa, including other Hemiptera (60, 8.28%), weevils and other Coleoptera (31, 4.28%), and other Lepidopteran larvae (6, 0.83%).

**THE EFFECTS OF SUNLIGHT AND ANTS ON PLANT REPRODUCTIVE FITNESS.**—Light intensity did not affect seed set overall, although the difference was close to significant ( $N = 40$ ,  $df = 1$ ,  $U = 134$ ,  $P = 0.051$ ), with plants in sunny habitats producing more seeds (Fig. 1). The presence of ants did not affect seed set overall ( $N = 40$ ,  $df = 1$ ,  $U = 146.5$ ,  $P = 0.113$ ); however, in sunny habitats, plants with ants produced significantly more seeds than plants from which ants were excluded ( $N = 20$ ,  $df = 1$ ,  $U = 16.0$ ,  $P = 0.009$ ). In shady habitats, ants had no effect on seed production ( $N = 20$ ,  $df = 1$ ,  $U = 46.5$ ,  $P = 0.796$ ). In the presence of ants, plants in sunny habitats produced significantly more seeds than plants in shady habitats ( $N = 20$ ,  $df = 1$ ,  $U = 15.0$ ,  $P = 0.007$ ). In the absence of ants, sunlight had no impact on seed set ( $N = 20$ ,  $df = 1$ ,  $U = 50.0$ ,  $P = 1.000$ ).

**THE EFFECTS OF SUNLIGHT AND ANTS ON HERBIVORY.**—A repeated-measures factorial ANOVA revealed no interaction between light and ants on herbivory rates ( $N = 40$ ,  $F(1, 36) = 2.290$ ,  $P = 0.139$ ). Light conditions did not significantly affect herbivory rates

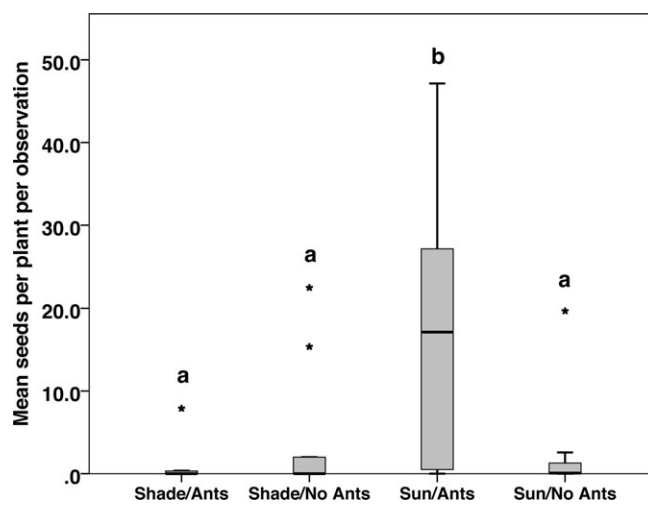


FIGURE 1. Seed set in *Senna chapmanii* plants from all four treatments. Bars represent mean seed set values for plants within each treatment over the course of the 1-yr study. Bold lines represent the median values, and bars and error bars represent quartiles. Outliers are represented by asterisks. Different letters indicate significant differences.



( $N = 40$ ,  $F(1, 36) = 0.804$ ,  $P = 0.376$ ); however, plants with ants present suffered significantly less herbivory than plants from which ants were excluded ( $N = 40$ ,  $F(1, 36) = 6.620$ ,  $P = 0.014$ ) (Fig. 2A; Table 1).

Within individual plants a significant interaction was observed between time and light conditions ( $N = 40$ ,  $F(20, 720) = 3.284$ ,  $P < 0.001$ ). Plants in the shade suffered greater levels of

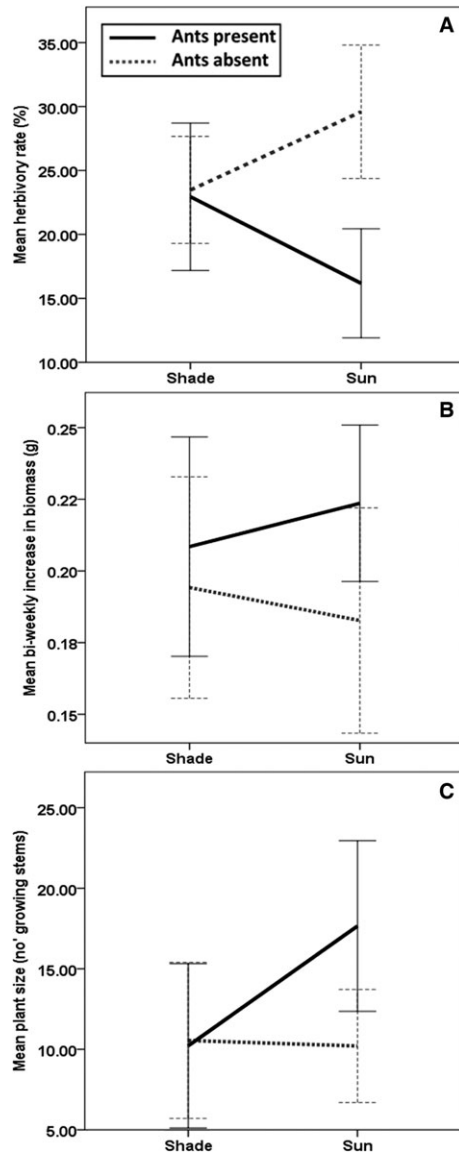


FIGURE 2. The effects of ants (presence/absence) and light (sun vs. shade) on (A) mean percentage herbivory rates. No significant interaction was observed between light and the presence of ants. Plants with no ants suffered significantly more herbivore damage than plants with ants present; (B) mean plant growth rates. No interaction was observed between light and the presence of ants. No single treatment had a significant effect on plant growth rates; (C) mean plant size. No interaction was observed between light and the presence of ants. No single treatment had a significant effect on overall plant size. Error bars represent standard error.

TABLE 1. Summary of  $F$  values generated by repeated-measures ANOVA performed on herbivory rate, plant growth rate, and plant size. Treatments are ants (present/absent) and light conditions (sun/shade).

Source of variation	df	Herbivory level	Plant growth	Plant size
Between subjects				
Light	1	0.804	0.051	0.866
Ants	1	6.620*	3.646	1.598
Light*Ants	1	2.290	0.308	2.480
Error between subjects	36			
Within subjects				
Time	20	3.311***	13.742***	2.564
Time*Light	20	3.284***	1.473	1.707
Time*Ants	20	0.546	0.932	1.382
Time*Light*Ants	20	1.200	1.286	1.619
Error within subjects	720			

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

herbivory during the summer months (May–July), while plants in sunny habitats suffered more herbivory during the winter months (September–January). Herbivory rates, overall, varied significantly over time ( $N = 40$ ,  $F(20, 720) = 3.311$ ,  $P < 0.001$ ; Table 1).

THE EFFECTS OF SUNLIGHT AND ANTS ON PLANT GROWTH RATES.—A repeated-measures factorial ANOVA revealed no significant interaction between light and ants on plant growth rates ( $N = 40$ ,  $F(1, 36) = 0.308$ ,  $P = 0.583$ ). Neither light ( $N = 40$ ,  $F(1, 36) = 0.051$ ,  $P = 0.823$ ) nor the presence of ants ( $N = 40$ ,  $F(1, 36) = 3.646$ ,  $P = 0.064$ ) significantly affected plant vegetative growth rates (Fig. 2B; Table 1).

Plant growth rates varied significantly over time ( $N = 40$ ,  $F(20, 720) = 13.742$ ,  $P < 0.001$ ), but no significant interactions were observed between time and ants ( $N = 40$ ,  $F(20, 720) = 0.932$ ,  $P = 0.496$ ), or time and light conditions ( $N = 40$ ,  $F(20, 720) = 1.473$ ,  $P = 0.158$ ) (Table 1).

EFFECTS OF SUNLIGHT AND ANTS ON PLANT SIZE.—A repeated-measures factorial ANOVA revealed no interaction effect between sunlight and the presence of ants on plant size ( $N = 40$ ,  $F(1, 36) = 2.480$ ,  $P = 0.124$ ). Plants in sunny habitats were not significantly larger than those in shady habitats ( $N = 40$ ,  $F(1, 36) = 0.866$ ,  $P = 0.358$ ), and the presence of ants did not have a significant impact on plant size overall ( $N = 40$ ,  $F(1, 36) = 1.598$ ,  $P = 0.214$ ). Plant size did not vary significantly with time throughout the study ( $N = 40$ ,  $F(20, 720) = 2.564$ ,  $P = 0.082$ ) (Fig. 2C; Table 1). When plants in sunny habitats were analyzed in isolation, however, the effect of ants on plant size was almost significant ( $N = 20$ ,  $F(1, 18) = 4.392$ ,  $P = 0.051$ ), with plants growing larger in the presence of ants (Fig. 3).

EXTRAFLOREAL NECTAR PRODUCTION.—No interaction was observed between ants and light conditions ( $N = 40$ ,  $F(1, 36) = 0.075$ ,  $P = 0.786$ ), and neither the presence of ants ( $N = 40$ ,

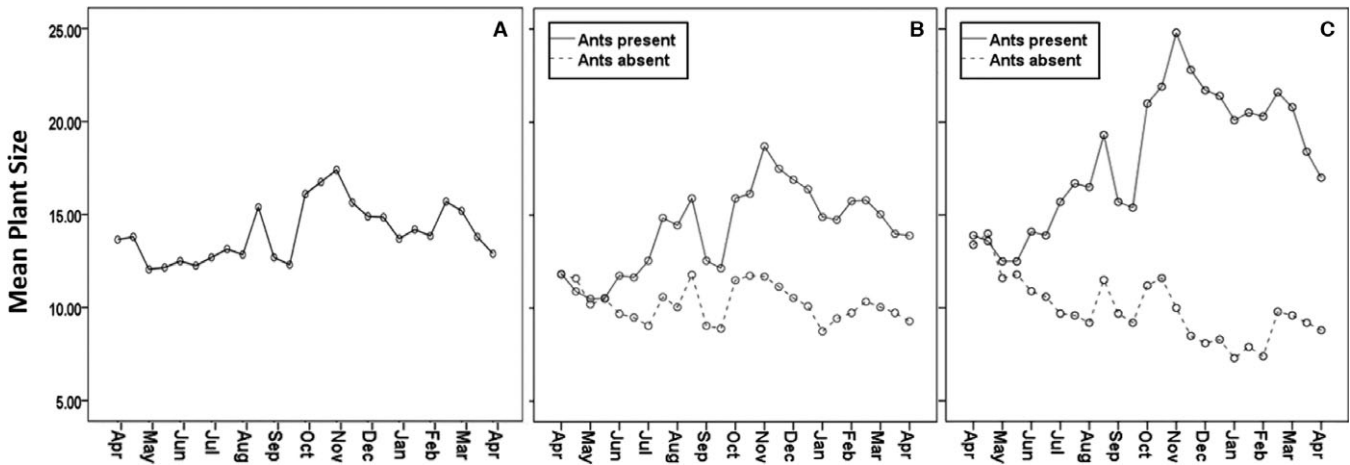


FIGURE 3. Temporal changes in plant size (number of branches); (A) in all plants ( $N = 40$ ); (B) in all plants ( $N = 40$ ), with plants with and without ants represented by separate lines; (C) in plants in sunny habitats ( $N = 20$ ), with plants with and without ants represented by separate lines.

$F(1, 36) = 0.051$ ,  $P = 0.823$ ) nor light intensity ( $N = 40$ ,  $F(1, 36)$ ,  $P = 0.492$ ) had a significant effect on EFN production. The per leaf production of EFN, however, decreased significantly with time over the duration of the study ( $N = 40$ ,  $F(5, 180) = 8.575$ ,  $P < 0.001$ ) (Fig. 4).

## DISCUSSION

Our results show that ants can act as a highly effective biotic defense against herbivores in *Senna chapmanii*, and that the

outcome of these ant–plant interactions is dependent upon light conditions. Plants with ants suffered less herbivore damage irrespective of light conditions. In sunny habitats, where reproductive fitness was highest, plants with ants produced significantly more seeds than plants without ants. In shaded habitats, however, no such differences were seen. That plants received greater benefits from their ant partners in sunny habitats was not surprising, as ant activity was significantly higher in the sun than in the shade.

Light conditions may affect the outcome of ant–plant interactions in a number of ways. Firstly, light conditions are known to affect ant activity, with many species occurring in higher abundance in sunny microhabitats (Varon *et al.* 2007). The tropical fire ant, *Solenopsis geminata*, for example, is particularly abundant in unshaded areas in Costa Rican coffee plantations (Perfecto & Vandermeer 1996). In addition to ant activity, light conditions have been found to affect EFN production in several species (Radhika *et al.* 2010, Yamawo & Hada 2010, Izaguirre *et al.* 2013), including *S. chapmanii* (Jones & Koptur 2015b). Yamawo and Hada (2010), for example, found that EFN production in *Mallotus japonicus* was increased at high light intensities. Furthermore, Izaguirre *et al.* (2013) observed that EFN production in passion fruit (*Passiflora edulis*) is down-regulated in response to changes in light spectral quality that signal the proximity of other plants. Although we did not observe increased EFN production in plants in sunny habitats, our results suggest that light conditions significantly impacted the outcome of ant–plant interactions, predominantly due to localized variations in ant activity.

Most studies of the effects of ants on plant fitness have been limited by their duration. Heil *et al.* (2001) compared the effects of ant exclusion on herbivory rates in three *Macaranga* species. After 2 mo, herbivory rates had increased by an average of <2 percent compared to control (ants present) plants. Within 1 yr, however, plants with ants excluded lost between 70–80 percent of their leaf area. These findings suggest that longer term studies (of at least 1 yr) are required to accurately determine the

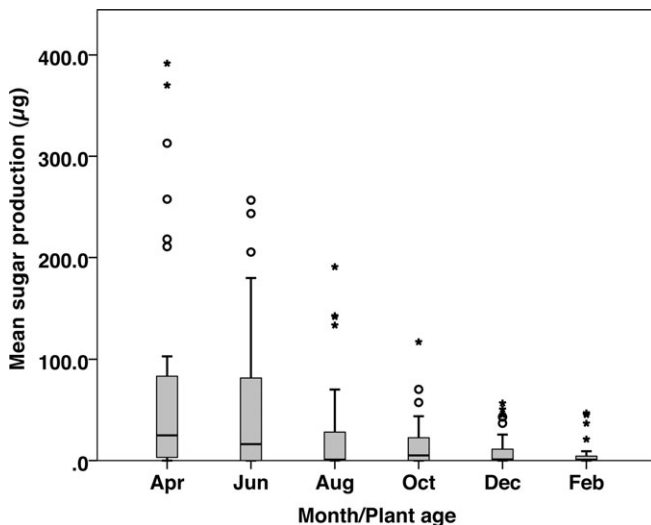


FIGURE 4. Temporal/ontogenetic variations in EFN production. The x axis not only shows months of the year but also represents age of study plants. Bold lines indicate median sugar production ( $\mu\text{g}$ ) across all 40 plants. Bars and error bars indicate quartiles. Circles indicate outliers and asterisks indicate extreme outliers.

effects of ants on herbivory rates. In many existing studies, plant fitness is estimated only a short time after ant exclusion, often within the same growth season. The true effects of herbivory on plant fitness may be cumulative, as damage can affect not only short-term reproductive investment but also plant size, thereby reducing future reproductive potential (Rosumek *et al.* 2009). Here, we addressed these shortcomings in two ways. Firstly, an experiment duration of one calendar year surpasses that of most studies, and represents more than a snapshot in a relatively short-lived plant. Secondly, we recorded changes in plant size over the course of the study, as a measure of future reproductive potential. Plants with ants grew steadily larger over the course of the year, while plants without ants gradually decreased in size (Fig. 3). In sunny habitats this trend was more obvious, and the effect of ants on plant size was close to significant. These observations provide further evidence that ants are more effective plant bodyguards for *S. chapmanii* in sunny habitats, and suggest that the gap in reproductive fitness between plants with and without ants would only widen over time.

It should be noted that we have focused only on foliar herbivory, which may be misleading as ants can be equally important in defending reproductive structures (Falcão *et al.* 2014, Heil 2015). Indeed, EFN production has been observed to increase during fruit production in several species (Holland *et al.* 2009, Falcão *et al.* 2014). During our study we regularly saw ants patrolling developing fruit, an activity that may account for much of the difference in seed set between plants with and without ants. Future work should focus on the effects of ants on the activity of pre-dispersal seed predators.

Bi-monthly measurements revealed a gradual reduction in EFN production over the course of the study. These measurements were taken between April and February, and we have observed a similar pattern in a subsequent field study, during which measurements were taken between October and May (Jones *et al.* unpublished data). These results suggest that the decline in EFN production with age in *S. chapmanii* represents an ontogenetic rather than a seasonal or phenological pattern. Previous studies of the ontogeny of EFN secretion, particularly among myrmecophytic plants, have yielded mixed results. 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). Our results suggest that ant-plant interactions may be most important for establishment and early growth in *S. chapmanii*. Future studies could observe establishment rates of *S. chapmanii* saplings in natural growing conditions, in the presence and absence of ants.

Understanding the effects of small-scale changes in light conditions is particularly important for plants in pine rockland habitats, which are threatened in south Florida. Over the last century, more than 98 percent of pine rockland habitat in south Florida (with the exception of Everglades National Park) has been destroyed for agriculture and urban development (Barrios *et al.* 2011). Due to their close proximity to dense human

populations, the remaining pine rockland fragments are frequently mismanaged. In particular, the fires that are necessary to maintain healthy pine rocklands are often suppressed (Possley *et al.* 2008). Pine rockland habitats are characterized by an open canopy, with high levels of light reaching the diverse, endemic-rich herb layer. In the absence of fire, trees and shrubs quickly grow up in the site, and many understory species are shaded out, decreasing plant diversity. While the transition to a closed canopy hammock environment will inevitably threaten many pine rockland species, our results suggest that even minor changes in habitat structure could impact the fitness of low-lying herbaceous species, particularly those that rely on mutualistic ants for defense.

## ACKNOWLEDGMENTS

The authors thank S. Zona for his generosity with greenhouse facilities. Help with insect specimen identification came from M. Deyrup and J. Clayborn. Constructive input on the manuscript was provided by J. Theobald, J. Richards, J. Clayborn, B. Harris, A. Salas, C. Pimienta, in addition to four anonymous reviewers. Financial support was provided in the form of three research grants from the Lloyd Kelly Foundation for Tropical Botany. Statistical consultation was provided by A. Matamala. This is contribution number 320 to the Florida International University Program in Tropical Biology.

## DATA AVAILABILITY

Data deposited in the Dryad Repository: <http://dx.doi.org/10.5061/dryad.p52kg> (Jones *et al.* 2016).

## LITERATURE CITED

- BAKER, D. A., L. J. HALL, AND J. R. THORPE. 1978. A study of the extrafloral nectaries of *Ricinus communis*. *New Phytol.* 81: 129–137.
- BARRIOS, B., G. ARELLANO, AND S. KOPTUR. 2011. The effects of fire and fragmentation on occurrence and flowering of a rare perennial plant. *Plant Ecol.* 212: 1057–1067.
- BARTON, A. M. 1986. Spatial variation in the effect of ants on an extrafloral nectary plant. *Ecology* 67: 495–504.
- BECERRA, J. X. I., AND D. L. VENABLE. 1989. Extrafloral nectaries: a defence against ant-homoptera mutualisms? *Oikos* 55: 276–280.
- BENTLEY, B. L. 1976. Plants bearing extrafloral nectaries and associated ant community—interhabitat differences in reduction of herbivore-damage. *Ecology* 57: 815–820.
- BENTLEY, B. L. 1977. The protective function of ants visiting the extrafloral nectaries of *Bixa orellana* (Bixaceae). *J. Ecol.* 65: 27.
- BRONSTEIN, J. L., W. G. WILSON, AND W. E. MORRIS. 2003. Ecological dynamics of mutualistic/antagonist communities. *Am. Nat.* 162: 24–39.
- CUAUTLE, M., AND V. RICO-GRAY. 2003. The effect of wasps and ants on the reproductive success of the extrafloral nectaried plant *Turnera ulmifolia* (Turneraceae). *Funct. Ecol.* 17: 417–423.
- DAVIDSON, D. W., AND B. L. FISCHER. 1991. Symbiosis of ants with *Cecropia* as function of light regime. In C. R. Huxley, and D. F. Cutler (Eds.). *Ant-plant interactions*, pp. 289–309. Oxford University Press, New York, NY.
- DOAK, P., D. WAGNER, AND A. WATSON. 2007. Variable extrafloral nectary expression and its consequences in quaking aspen. *Can. J. Bot.* 85: 1–9.

- FALCÃO, J. C. F., W. DÁTILLO, AND T. J. IZZO. 2014. Temporal variation in extrafloral nectar secretion in different ontogenic stages of the fruits of *Alibertia verrucosa* S. Moore (Rubiaceae) in a Neotropical savanna. *J. Plant Interact.* 9: 137–142.
- 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). *Can. J. Bot.* 81: 859–864.
- de la FUENTE, M. A. S., AND R. J. MARQUIS. 1999. The role of ant-tended extrafloral nectaries in the protection and benefit of a neotropical rain-forest tree. *Oecologia* 118: 192–202.
- GONZALEZ-TEUBER, M., AND M. HEIL. 2009. Nectar chemistry is tailored for both attraction of mutualists and protection from exploiters. *Plant Signal. Behav.* 4: 809–813.
- HEIL, M. 2004. Induction of two indirect defences benefits lima bean (*Phaseolus lunatus*, Fabaceae) in nature. *J. Ecol.* 92: 527–536.
- HEIL, M. 2011. Nectar: generation, regulation and ecological functions. *Trends Plant Sci.* 16: 191–200.
- HEIL, M. 2015. Extrafloral nectar at the plant-insect interface: a spotlight on chemical ecology, phenotypic plasticity and food webs. *Annu. Rev. Entomol.* 60: 213–232.
- HEIL, M., B. FIALA, B. BAUMANN, AND K. E. LINSENMAIR. 2000. Temporal, spatial and biotic variations in extrafloral nectar secretion by *Macaranga tanarius*. *Funct. Ecol.* 14: 749–757.
- HEIL, M., A. HILPERT, B. FIALA, AND K. E. LINSENMAIR. 2001. Nutrient availability and indirect (biotic) defence in a Malaysian ant-plant. *Oecologia* 126: 404–408.
- HEIL, M., A. HILPERT, R. KRUGER, AND K. E. LINSENMAIR. 2004. Competition among visitors to extrafloral nectaries as a source of ecological costs of an indirect defence. *J. Trop. Ecol.* 20: 201–208.
- 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. *J. Ecol.* 97: 89–96.
- INOUE, D. W., AND O. R. TAYLOR. 1979. A temperate region plant-ant-seed predator system: consequences of extrafloral nectar secretion by *Heliantibella quinquenervis*. *Ecology* 65: 618.
- IZAGUIRRE, M. M., C. A. MAZZA, M. S. ASTIGUETA, A. M. CIARLA, AND C. L. BALLARE. 2013. No time for candy: passionfruit (*Passiflora edulis*) plants down-regulate damage-induced extra floral nectar production in response to light signals of competition. *Oecologia* 173: 213–221.
- JANZEN, D. H. 1966. Coevolution of mutualism between ants and acacias in central America. *Evolution* 20: 249–275.
- JONES, I. M., AND S. KOPTUR. 2015a. Dynamic extrafloral nectar production: The timing of leaf damage affects the defensive response in *Senna mexicana* var. *chapmanii* (Fabaceae). *Am. J. Bot.* 102: 58–66.
- JONES, I. M., AND S. KOPTUR. 2015b. Quantity over quality: light intensity, but not red/far-red ratio, affects extrafloral nectar production in *Senna mexicana* var. *chapmanii*. *Ecology and Evolution*. 5(18): 4108–4114.
- JONES, I. M., S. KOPTUR, H. R. GALLEGOS, J. P. TARDANICO, P. A. TRAINER, AND J. PEÑA. 2016. Data from: Changing light conditions in pine rockland habitats affect the intensity and outcome of ant-plant interactions. doi:10.5061/dryad.p52 kg
- KARBAN, R., A. A. AGRAWAL, J. S. THALER, AND L. S. ADLER. 1999. Induced plant responses and information content about risk of herbivory. *Trends Ecol. Evol.* 14: 443–447.
- KERSCH, M. F., AND C. R. FONSECA. 2005. Abiotic factors and the conditional outcome of an ant-plant mutualism. *Ecology* 86: 2117–2126.
- 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. *Ann. Bot.* 101: 1035–1047.
- KOPTUR, S. 1979. Facultative mutualism between weedy vetches bearing extrafloral nectaries and weedy ants in California. *Am. J. Bot.* 66: 1016–1020.
- KOPTUR, S. 1992a. Interactions between insects and plants mediated by extrafloral nectaries. In E. Bernays (Ed.). CRC series on insect/plant interactions, Vol. 4, pp. 85–132. CRC Press, Boca Raton, Florida.
- KOPTUR, S. 1992b. Plants with extrafloral nectaries and ants in everglades habitats. *Fla. Entomol.* 75: 38–50.
- KOPTUR, S., I. M. JONES, AND J. E. PEÑA. 2015. The influence of host plant extrafloral nectaries on multitrophic interactions: an experimental investigation. *PLoS ONE*. e0138157.
- 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. *Ann. Bot.* 111: 1277–1283.
- KOST, C., AND M. HEIL. 2005. Increased availability of extrafloral nectar reduces herbivory in lima bean plants (*Phaseolus lunatus*, Fabaceae). *Basic Appl. Ecol.* 6: 237–248.
- LANGE, D., W. DÁTILLO, AND K. DEL-CLARO. 2013. Influence of extrafloral nectary phenology on ant-plant mutualistic networks in a neotropical savanna. *Ecol. Entomol.* 38: 463–469.
- LEAL, I. R., E. FISCHER, C. KOST, M. TABARELLI, AND R. WIRTH. 2006. Ant protection against herbivores and nectar thieves in *Passiflora coccinea* flowers. *Ecoscience* 13: 431–438.
- MARAZZI, B., J. L. BRONSTEIN, AND S. KOPTUR. 2013. The diversity, ecology and evolution of extrafloral nectaries: current perspectives and future challenges. *Ann. Bot.* 111: 1243–1250.
- MOYA-RAYGOZA, G., AND K. J. LARSEN. 2001. Temporal resource switching by ants between honeydew produced by the five spotted gamma grass leafhopper (*Dalbulus quinquenotatus*) and nectar produced by plants with extrafloral nectaries. *Am. Midl. Nat.* 146: 311–320.
- OLIVEIRA, P. S. 1997. The ecological function of extrafloral nectaries: herbivore deterrence by visiting ants and reproductive output in *Caryocar brasiliense* (Caryocaraceae). *Funct. Ecol.* 11: 323–330.
- PERFECTO, I., AND J. VANDERMEER. 1996. Microclimatic changes and the indirect loss of ant diversity in a tropical agroecosystem. *Oecologia* 108: 577–582.
- POSSLEY, J., S. W. WOODMANSEE, AND J. MASCHINSKI. 2008. Patterns of plant composition in fragments of globally imperiled pine rockland forest: effects of soil type, recent fire frequency, and fragment size. *Nat. Areas J.* 28: 379–394.
- RADHIKA, V., C. KOST, A. MITHOFER, AND W. BOLAND. 2010. Regulation of extrafloral nectar secretion by jasmonates in lima bean is light dependent. *Proc. Natl Acad. Sci. USA* 107: 17228–17233.
- RICO-GRAY, V., J. G. GARCIA-FRANCO, M. PALACIOS-RIOS, C. DIAZ-CASTELAZO, V. PARRA-TABLA, AND J. A. NAVARRO. 1998. Geographical and seasonal variation in the richness of ant-plant interactions in Mexico. *Biotropica* 30: 190–200.
- 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.
- RUTTER, M. T., AND M. D. RAUSHER. 2004. Natural selection on extrafloral nectar production in *Chamaecrista fasciculata*: the costs and benefits of a mutualism trait. *Evolution* 58: 2657–2668.
- TORRES-HERNANDEZ, L., V. RICO-GRAY, C. CASTILLO-GUEVARA, AND J. A. VERGARA. 2000. Effect of nectar-foraging ants and wasps on the reproductive fitness of *Turnera ulmifolia* (Turneraceae) in a coastal sand dune in Mexico. *Acta Zool. Mex.* 81: 13–21.
- VARON, E. H., P. HANSON, J. T. LONGINO, O. BORBON, M. CARBALLO, AND L. HILJE. 2007. Temporal and spatial distribution of ants in a light



- gradient in a coffee agroforestry system, Turrialba, Costa Rica. *Rev. Biol. Trop.* 55: 943–956.
- WAGNER, D., AND A. KAY. 2002. Do extrafloral nectaries distract ants from visiting flowers? An experimental test of an overlooked hypothesis. *Evol. Ecol. Res.* 4: 293–305.
- YAMAWO, A., AND Y. HADA. 2010. Effects of light on direct and indirect defences against herbivores of young plants of *Mallotus japonicus* demonstrate a trade off between two indirect defence traits. *Ann. Bot.* 106: 143–148.
- YAMAWO, A., Y. HADA, AND N. SUZUKI. 2012. Variations in direct and indirect defenses against herbivores on young plants of *Mallotus japonicus* in relation to soil moisture conditions. *J. Plant. Res.* 125: 71–76.