



## **Exploring Whether and How Ants (Hymenoptera: Formicidae) Affect Reproductive Fitness in *Senna mexicana* var. *chapmanii* (Fabaceae)**

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# Exploring whether and how ants (Hymenoptera: Formicidae) affect reproductive fitness in *Senna mexicana* var. *chapmanii* (Fabaceae)

Ian M. Jones<sup>1,\*</sup>, Suzanne Koptur<sup>2</sup>, and Jorge E. Peña<sup>3</sup>

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## Abstract

Extrafloral nectar mediates food-for-protection mutualisms between plants and ants. Ant-plant mutualisms are keystone associations, occurring within a complex web of biotic interactions. As such, these interactions may affect plant fitness in a number of ways, both positive and negative. In *Senna mexicana* var. *chapmanii* (Isely) H. S. Irwin & Barneby (Fabaceae), the presence of ants has been shown to reduce herbivory and increase fruit and seed set. These effects, however, are not the result of only one interaction but the balance of many. We conducted a field study to determine the processes by which ants affect reproductive fitness in *S. chapmanii*. Thirty plants were established in a semi-natural area adjacent to native pine rockland habitat in southern Florida. Ants were excluded from half of the plants by painting a sticky resin (Tanglefoot™) around the base of each stem. Over the course of a single flowering season (Oct–May), we observed the effects of ants on the activity of herbivores, predators, pollinators, and pre-dispersal seed predators. We also observed the overall effects of ants on plant size and reproductive output. Plants with ants were quicker to establish, grew larger, and produced floral displays that attracted more pollinators. Contrary to our expectations, relative pollinator efficiency and rates of pre-dispersal seed predation were unaffected by ants. In *S. chapmanii*, ants did not appear to affect the outcome of other plant–insect associations, but they affected the scale at which they occurred. Ants facilitated plant growth and establishment in *S. chapmanii*, with subsequent effects on reproductive investment.

Key Words: ant–plant interactions; extrafloral nectar; herbivory; multi-trophic interactions; plant defense; pollination

## Resumen

El néctar extrafloral interviene el mutualismo de alimento-para-protección entre plantas y hormigas. Los mutualismos de la hormiga-planta son asociaciones claves, que ocurren dentro de una red compleja de interacciones bióticas. Como tales, estas interacciones pueden afectar la aptitud de la planta de varias maneras, tanto positivas como negativas. En *Senna mexicana* var. *chapmanii* (Isely) H. S. Irwin & Barneby (Fabaceae), se ha demostrado que la presencia de hormigas reduce la herbivoría y aumenta el número de frutos y semillas. Estos efectos, sin embargo, no son el resultado de una sola interacción sino el equilibrio de muchos. Realizamos un estudio de campo para determinar los procesos por los cuales las hormigas afectan la aptitud reproductiva en *S. chapmanii*. Se establecieron treinta plantas en un área semi-natural adyacente al hábitat nativo rocoso de pino en el sur de la Florida. Se excluyeron las hormigas de la mitad de las plantas al pintar una resina pegajosa (Tanglefoot™) alrededor de la base de cada tallo. A lo largo de una sola temporada de floración (octubre–mayo), observamos los efectos de las hormigas sobre la actividad de herbívoros, depredadores, polinizadores y predadores de semillas pre-dispersantes. También observamos los efectos generales de las hormigas sobre el tamaño de la planta y la producción reproductiva. Las plantas con hormigas fueron más rápidas de establecer, crecieron más y produjeron muestras florales que atrajeron a más polinizadores. Contrariamente a nuestras expectativas, la eficacia relativa de los polinizadores y las tasas de predación de las semillas antes de la dispersión no fueron afectadas por las hormigas. En *S. chapmanii*, aparentemente las hormigas afectaron el resultado de otras asociaciones de plantas e insectos, pero afectaron la escala en la que ocurrieron. Las hormigas facilitaron el crecimiento y establecimiento de las plantas en *S. chapmanii*, con efectos subsecuentes sobre la inversión reproductiva.

Palabras Clave: interacciones hormiga–planta; néctar extrafloral; herbivoría; interacciones multi-tróficas; defensa de plantas; polinización

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Extrafloral nectaries (EFN) are glands that secrete nectar (solutions of sugars, amino acids, lipids, and other organic compounds) located outside of flowers and have been reported on species belonging to 110 families and 806 genera (Koptur 1992; Marazzi et al. 2013; Weber et al. 2015). These nectaries may serve diverse ecological roles (Baker et al. 1978; Becerra & Venable 1989; Wagner & Kay 2002; Gonzalez-Teuber & Heil 2009; Heil 2011). However, their primary function is the attraction of predatory insects, predominantly ants, which provide plants

with a form of indirect defense against herbivores (Bentley 1977; Koptur 1992; Rosumek et al. 2009).

Many studies have identified food-for-protection mutualisms between ants and plants (Koptur 1992; Rosumek et al. 2009). In some cases, plants provide domatia and food bodies, as well as EFN, and the resulting interactions may be highly specialized. Janzen (1966) observed that *Acacia cornigera* (L.) Willd. (Fabaceae) plants succumbed to herbivory when resident *Pseudomyrmex ferruginea* Smith (Hyme-

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noptera: Formicidae) ants were experimentally removed. Plants that provide only EFN are normally involved only in facultative interactions with ants (Rosumek et al. 2009). Despite the less specialized nature of these interactions, significant fitness benefits for plants have been reported (Koptur 1979; Koptur 1984; Oliveira 1997; Rudgers 2004; Koptur et al. 2013).

The presence of ants may benefit plants in a number of ways. Aggressive ants have been shown to reduce herbivore numbers on many plant species (Letourneau & Barbosa 1999; Dejean et al. 2010), and numerous studies have observed reduced rates of herbivore damage in the presence of ants (Janzen 1966; Bruna et al. 2004; Del-Claro et al. 2006; Jones et al. 2017). Extrafloral nectar is found on the fruits of many species. Indeed, production of EFN has been shown to increase during fruit production, in line with optimal defense theory (Wackers & Bonifay 2004; Holland et al. 2009). These observations suggest a role for ants in the protection of developing fruit. In addition to the provision of defense, the proximity of ant nests may benefit plants by enhancing soil nutrient concentrations (Wagner 1997; Wagner & Nicklent 2010). Wagner (1997) found that nests of *Formica perpilosa* Wheeler (Hymenoptera: Formicidae) at the base of *Acacia constricta* Benth. (Fabaceae) shrubs enhanced nitrate, ammonium, and phosphorus availability in the surrounding soil. Plants with nests at their base produced significantly more seeds than plants without nests (Wagner 1997).

Despite these well-documented benefits, plants may also suffer a range of costs associated with their ant partners. Some less aggressive ant species consume nectar, but provide no defensive benefits for plants (Freitas et al. 2000; Ruhren 2003). Indeed, in a few cases ants have even been seen to affect plant defense negatively (Fredrickson & Gordon 2007; Mooney 2007; Rosumek et al. 2009). Conversely, overly aggressive ants may deter beneficial insects such as predators (Torres-Hernandez et al. 2000; Nahas et al. 2012), parasitoids (Styrsky & Eubanks 2007; Rosumek et al. 2009), and pollinators (Ness 2006; Hernández-Cumplido et al. 2010; Assunção et al. 2014). Several studies have shown that pollinators recognize the dangers posed by aggressive ants (Hernández-Cumplido et al. 2010; Assunção et al. 2014). Assunção et al. (2014) placed plastic ants on the petals of *Heteropterys pteropetala* A. Juss. (Malpighiaceae) and found that flowers with plastic ants produced significantly less fruit than control flowers.

Ant-plant associations occur not in isolation, but within a complex web of biotic interactions, and in a framework provided by the abiotic environment. In the cactus *Ferocactus wislizeni* Engelm. (Cactaceae), plants defended by *Solenopsis xyloni* McCook (Hymenoptera: Formicidae) ants exhibit reduced herbivory and increased flowering. These plants, however, receive fewer and shorter visits from pollinators, which are deterred by the same ferocious ants (Ness 2006). Understanding the outcome of ant-plant interactions, therefore, requires that we focus on the broader multi-guild, multi-trophic interactions of which they are a part. While the presence of ants has been shown to boost reproductive fitness in a number of EFN producing species (Oliveira 1997; Freitas et al. 2000; Rudgers 2004; Rosumek et al. 2009; Heil et al. 2015), the specific mechanisms by which ants facilitate increased plant fitness are not fully understood for many species. In the present study, we aimed to determine not just whether, but how ants increase plant reproductive fitness in the known myrmecophile, *Senna mexicana* var. *chapmanii* (Isely) H. S. Irwin & Barneby.

*Senna mexicana* var. *chapmanii* (hereafter referred to as *S. chapmanii*) is native to south Florida and the Caribbean. This species grows in pine rockland habitat and rockland hammock edges as an upright or sprawling subshrub up to 1.2 m in height, spreading broader than tall. Flowers offer no nectar to floral visitors and are visited by bees collecting pollen by ‘buzzing’ the anthers (Koptur, personal observa-

tion). Extrafloral nectaries occur on the pedicels of flowers in the inflorescences, as well as throughout the foliage between basal leaflets. Previous work with *S. chapmanii* has shown that EFN production is responsible for the recruitment of ants to plants (Jones & Koptur 2015). Ants also remove key herbivores (Koptur et al. 2015), and plants with ants excluded suffer increased herbivore damage and reduced seed set (Jones et al. 2016).

We conducted an 8 mo field study (Oct–May), encompassing 1 entire flowering season for *S. chapmanii*. The presence of ants on test plants was manipulated to address the question: how does the presence of ants increase reproductive fitness in *S. chapmanii*? We determined the effects of ants on rates of flowering, fruit set, and seed production. We observed changes in plant size over the course of the study. Finally, we observed the effects of ants on the activity and effectiveness of pollinators and on pre-dispersal seed predators. We predicted that the presence of ants would lead to increased plant size and increased flower production. We also predicted that ants would have a neutral or negative effect on pollination rates, but that those flowers that did set fruit would be more likely to reach maturity in the presence of ants. During previous studies, we have regularly seen ants patrolling developing fruit, and therefore we hypothesized that a reduction in pre-dispersal seed predation would account for much of the difference in seed set between plants with and without ants.

## Materials and Methods

This field study was conducted at the University of Florida, Tropical Research and Education Center (TREC) in Homestead, Florida, USA. The climate is subtropical, with average minimum and maximum temperatures of 3.2 to 24.8 °C in Jan and 22.7 to 32.4 °C in Jul (Koptur et al. 2015). The study site consisted of flat calcareous limestone rocklands that have been rock-ploughed for agriculture. We used a 2 acre plot, previously overgrown with exotic pest plants, which had been cleared of vegetation except for a few large native trees. Adjacent to the western edge of the plot is a fragment of pine rockland habitat, a protected natural area.

*Senna chapmanii* plants were grown from seeds in a greenhouse at Florida International University (FIU), Miami, Florida. Seeds were collected from multiple individuals in a single population on Big Pine Key, in the lower Florida Keys (under Research Permit # FFO4RFKD-2014-0, National Wildlife Refuge System, National Key Deer Refuge). Three mo after germination, 30 plants were transplanted into the experimental site. Plants were mulched with wood chips and watered for 2 mo, beginning in Aug 2014, until they were established. Plants were installed in an evenly spaced array, at least 4 m from their nearest neighbor, and divided into 2 treatments: (1) ants present (control) and (2) ants excluded. In the ants excluded treatment, a sticky resin (Tanglefoot™) was painted around the base of the stem 1 mo prior to the start of data collection. Tanglefoot™ was re-applied every 2 wk for the duration of the experiment. Treatments were assigned systematically to ensure an even distribution across the site. Data were collected over a period of 8 mo from Oct 2014 to May 2015.

## INSECT SURVEYS

Each week, 1 active inflorescence was chosen from all plants that were in flower, and used as a census locale. Each chosen branch was studied over a period of 2 min, and the numbers and species of ants, herbivores, and predators were recorded. Any new species were collected and brought back to the lab at FIU for identification.

In addition to surveys of insects on the inflorescences, monthly ant surveys were conducted throughout the experiment, both to verify that ant exclusion treatments were working and to determine any temporal changes in ant activity at the study site. A single non-flowering branch was chosen from each plant and observed for 2 min. The number and species of ants observed on the branch were recorded.

Pollinator observations were conducted on a weekly basis. Plants with active inflorescences were observed for periods of 15 min, and the number of flower visitors was recorded. The identity of each visitor and the length of the visit were recorded. For each plant observed, the number of inflorescences at the time of the observation was recorded, so that the effects of inflorescence number on pollinator attraction could be determined. Pollinator observations were carried out between 8 am and 5 pm, and over 15 h of observation time was accumulated, equally distributed between treatment and control plants.

### PLANT SIZE

*Senna chapmanii* is a sprawling subshrub, growing broader than tall and commonly branching from near the base. As such, the number of branches is an effective proxy for plant size. Plant size was estimated monthly throughout the study by counting the number of branches. A branch was classified as any growing stem with at least 10 leaves.

### PLANT REPRODUCTIVE FITNESS

During the course of the experiment, 3 open flowers were collected from each study plant to determine the average number of ovules per flower. Although flowers were collected from each plant on an opportunistic basis, each week, an equal number of flowers were collected from treatment and control plants. Where possible, flowers from an individual plant were taken from different inflorescences. Flowers were returned to the laboratory, and their ovaries were dissected under a light microscope to determine number of ovules. The mean number of ovules per flower was calculated for each plant.

Measures of gross plant reproductive output (numbers of inflorescences, flowers, mature fruit, and mature seeds) were measured on a weekly basis throughout the experiment. Each week, any new inflorescences were labelled with a numbered jewelry tag. The number of open flowers, new fruit set, and the number of mature fruit were then recorded for both new and previously marked inflorescences. All mature fruit were collected from each plant and returned to the lab. Fruit were dissected to determine the number of fertilized ovules, the number of intact seeds, and the number of herbivore-damaged seeds. The number of fertilized ovules could be determined by counting the seed chambers in the mature fruit. Because *Senna* species are buzz-pollinated, and require an insect visitor to set seeds (Marazzi et al. 2015), the proportion of ovules fertilized in each plant can be used as a measure of pollinator effectiveness. Herbivore-damaged seeds were counted as any seeds with obvious herbivore damage, along with empty seed chambers that contained seed debris. Empty seed chambers that contained no evidence of herbivore activity were assumed to be aborted seeds.

The overall rate of pre-dispersal seed predation was calculated for each plant as the number of herbivorized seeds divided by the total number of developing seeds (fertilized ovules minus aborted seeds). Effective fecundity was calculated for each plant as the number of non-predated mature seeds divided by the total number of ovules. Herbivores found within seed pods were collected and identified. Larval herbivores were reared in the lab and identified as adults. Voucher specimens of these, and all other insects, were preserved and stored at FIU.

### EXTRAFLOREAL NECTAR

Extrafloral nectar production was measured in each plant, every 2 mo throughout the study. 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 tissue paper, and branches were sealed within fine mesh bags to exclude insects. Bags were placed on branches at 7 PM, and removed 12 h later for nectar measurements at 7 AM.

Combined nectar volume from the 5 most apical leaves was measured with 1, 2, and 10  $\mu$ L micropipettes. Nectar concentration was measured with a handheld refractometer, and total sugar production ( $\mu$ g) was estimated from the combination of these measurements (Jones & Koptur, 2014). In the results, EFN is expressed as total sugar production.

### STATISTICAL ANALYSES

Due to the high number of zeros in the data, insect survey data, pollinator watch data, seed herbivory data, and plant reproductive fitness data could not meet parametric assumptions even after transformation. As such, median insect numbers from each guild were compared between treatments by Mann–Whitney *U* tests. The number and duration of pollinator visits, the rate of pre-dispersal seed predation, and median measures of plant reproductive fitness (numbers of inflorescences, flowers, fruits, and seeds) were compared between treatments by Mann–Whitney *U* tests. The relationship between pollinator visits and the number of active inflorescences was analyzed by 2-tailed Spearman correlation.

Repeated-measures ANOVAs were used to investigate the effects of ant presence or absence on the dependent variables of plant size and production of EFN. Plant size data were  $\log_{10}$  transformed to meet parametric assumptions. Extrafloral nectar data were  $\log_{10}(x+1)$  transformed as the data set contained some zeros. All statistical analyses were performed with SPSS Version 23 and, in all cases, results were considered statistically significant when *P* values were  $\leq 0.05$ .

## Results

### ANTS

During our censuses, a total of 96 ants from 9 species were observed in the inflorescences of *Senna chapmanii*. The majority of ant activity within the inflorescences (84%) could be attributed to 4 species: *Camponotus floridanus* Buckley (Hymenoptera: Formicidae) (25%), *Camponotus sexguttatus* Fabricius (Hymenoptera: Formicidae) (23.9%), *Camponotus planatus* Roger (Hymenoptera: Formicidae) (19.8%), and *Brachymyrmex obscurior* Forel (Hymenoptera: Formicidae) (14.6%).

A total of 144 ants were observed on the foliage of *S. chapmanii* during monthly ant surveys. The same 4 species found on inflorescences accounted for the majority of ant activity on leaves, representing 15.5%, 9.7%, 9%, and 45% of ant activity, respectively. *Brachymyrmex obscurior* was the dominant species on the foliage, while *Camponotus* species were seen most frequently in the inflorescences.

Ant numbers were significantly higher on control plants than on Tanglefoot™-treated plants, both in the inflorescences ( $N = 30$ ;  $df = 29$ ;  $U = 12.5$ ;  $P < 0.001$ ), and during monthly foliar ant surveys ( $N = 30$ ;  $df = 29$ ;  $U = 27.5$ ;  $P < 0.001$ ).

### HERBIVORES AND PREDATORS

A total of 34 herbivores were observed in the inflorescences of *S. chapmanii*, of which the most abundant were pierid caterpillars (Lepi-

doptera: Pieridae) belonging to 3 species, *Abaeis nicippe* (Cramer) (the sleepy orange), *Phoebis philea* (L.) (the orange-barred sulfur), and *Phoebis sennae* (L.) (the cloudless sulfur). Thirteen pierid caterpillars were recorded, accounting for 38.2% of herbivore observations. The 2nd most abundant herbivore was the leucaena psyllid, *Heteropsylla cubana* Crawford (Hemiptera: Psyllidae). Eight groups of psyllid nymphs were observed in inflorescences, accounting for 23.5% of herbivore observations. Numbers of pierid caterpillars ( $N = 30$ ;  $df = 29$ ;  $U = 97$ ;  $P = 0.539$ ) and psyllid nymphs ( $N = 30$ ;  $df = 29$ ;  $U = 107$ ;  $P = 0.758$ ) did not differ between treatments.

A total of 21 predators were observed in the inflorescences of *S. chapmanii*. Predators included spiders (14), coccinellid beetles (4), and predatory wasps (3). The total number of predators observed did not differ significantly between treatments ( $N = 30$ ;  $df = 29$ ;  $U = 89.5$ ;  $P = 0.3$ ).

### PRE-DISPERSAL SEED PREDATORS

Although evidence of pod-boring seed predators (i.e., frass and residue from predated seeds) was often observed, the herbivores themselves were only recovered on 4 occasions, 3 times from control plants, and once from plants with ants excluded. The pod borers observed belonged to 2 species: 1 coleopteran and 1 lepidopteran. The rate of seed predation did not differ between treatments ( $N = 30$ ;  $df = 29$ ;  $U = 112.5$ ;  $P = 1.0$ ) (Fig. 1A).

### POLLINATORS

A total of 14 pollinator visits were observed during 63 observations (15 h and 45 min). Ten of those visits (71%) were by the sweat bee, *Augochlora pura* Say (Hymenoptera: Halictidae). The remaining visits involved the honeybee, *Apis mellifera* L. (Hymenoptera: Apidae) (2), a metallic hoverfly, *Ornidia obesa* F. (Diptera: Syrphidae) (1), and a skipper butterfly, *Euphyes arpa* Boisduval & Le Conte (Lepidoptera: Hesperidae) (1). *Augochlora pura* was the only visitor observed that is able to remove pollen from the poricidal anthers of *S. chapmanii* flowers. As such, analyses of pollinator visits took into account only this visitor.

Plants with ants present were visited by pollinators significantly more frequently than plants from which ants were excluded (Control  $N = 33$ , Tanglefoot™  $N = 30$ ;  $df = 62$ ;  $U = 348$ ;  $P = 0.003$ ). The mean duration of pollinator visits was also significantly longer on plants with ants present (Control  $N = 33$ , Tanglefoot™  $N = 30$ ;  $df = 62$ ;  $U = 346$ ;  $P = 0.003$ ) (Fig. 2). Pollination efficiency (number of fertilized ovules per total number of ovules produced), however, did not differ between plants with and

without ants ( $N = 30$ ;  $df = 29$ ;  $U = 108$ ;  $P = 0.818$ ) (Fig. 1B). No correlation was seen between the number of active inflorescences on a given plant and the number of pollinator visits ( $N = 63$ ;  $r = 0.253$ ;  $P = 0.516$ ) (Fig. 3).

### PLANT SIZE

A repeated measures ANOVA revealed that, within individual plants, the number of branches increased significantly over time ( $N = 30$ ;  $F_{7,196} = 3.66$ ;  $P = 0.013$ ). No significant interaction was observed between time and the presence or absence of ants ( $N = 30$ ;  $F_{7,196} = 0.91$ ;  $P = 0.444$ ). The presence of ants, however, significantly affected plant size ( $N = 30$ ;  $F_{1,28} = 4.99$ ;  $P = 0.034$ ). The mean number of branches on plants in the presence of ants (6.84) was higher than when ants were absent (3.12) (Fig. 4).

### PLANT REPRODUCTIVE FITNESS

The number of ovules counted in dissected flowers ranged from 25 to 39, and the mean number of ovules per flower was 30 (SD = 2.962). Measures of gross reproductive fitness, such as number of inflorescences ( $N = 30$ ;  $df = 29$ ;  $U = 81$ ;  $P = 0.187$ ), flowers ( $N = 30$ ;  $df = 29$ ;  $U = 84.5$ ;  $P = 0.244$ ), mature fruit ( $N = 30$ ;  $df = 29$ ;  $U = 109.5$ ;  $P = 0.878$ ), and mature seeds ( $N = 30$ ;  $df = 29$ ;  $U = 106$ ;  $P = 0.739$ ) did not differ significantly between treatments (Fig. 5). Effective fecundity was not significantly different between treatment and control plants ( $N = 30$ ;  $df = 29$ ;  $U = 103$ ;  $P = 0.627$ ) (Fig. 1C).

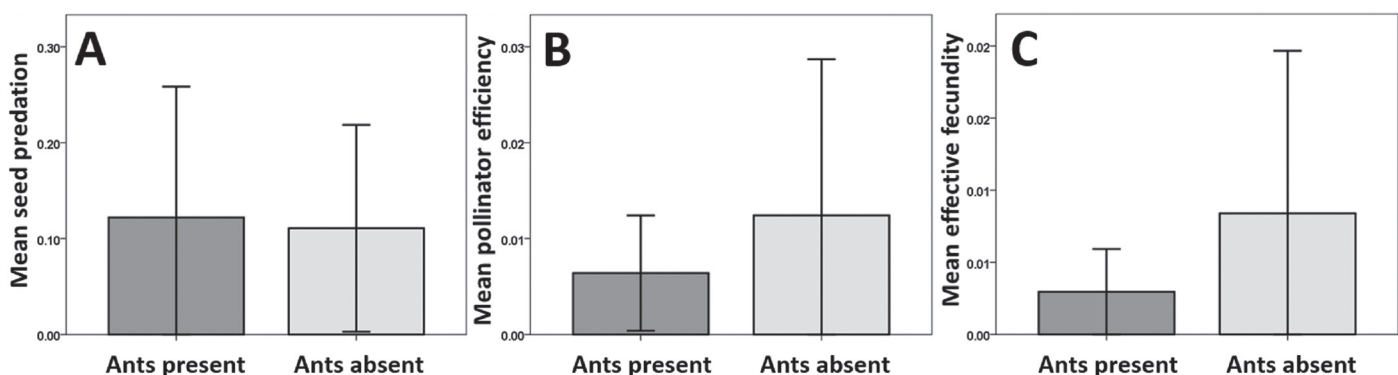
### EXTRAFLOREAL NECTAR PRODUCTION

A repeated measures ANOVA revealed that, within individual plants, the production of EFN decreased significantly over time ( $N = 30$ ;  $F_{3,84} = 3.58$ ;  $P = 0.028$ ). No significant interaction was observed between time and the presence/absence of ants ( $N = 30$ ;  $F_{3,84} = 0.401$ ;  $P = 0.700$ ). The presence of ants had no effect on EFN production ( $N = 30$ ;  $F_{1,28} = 0.45$ ;  $P = 0.509$ ).

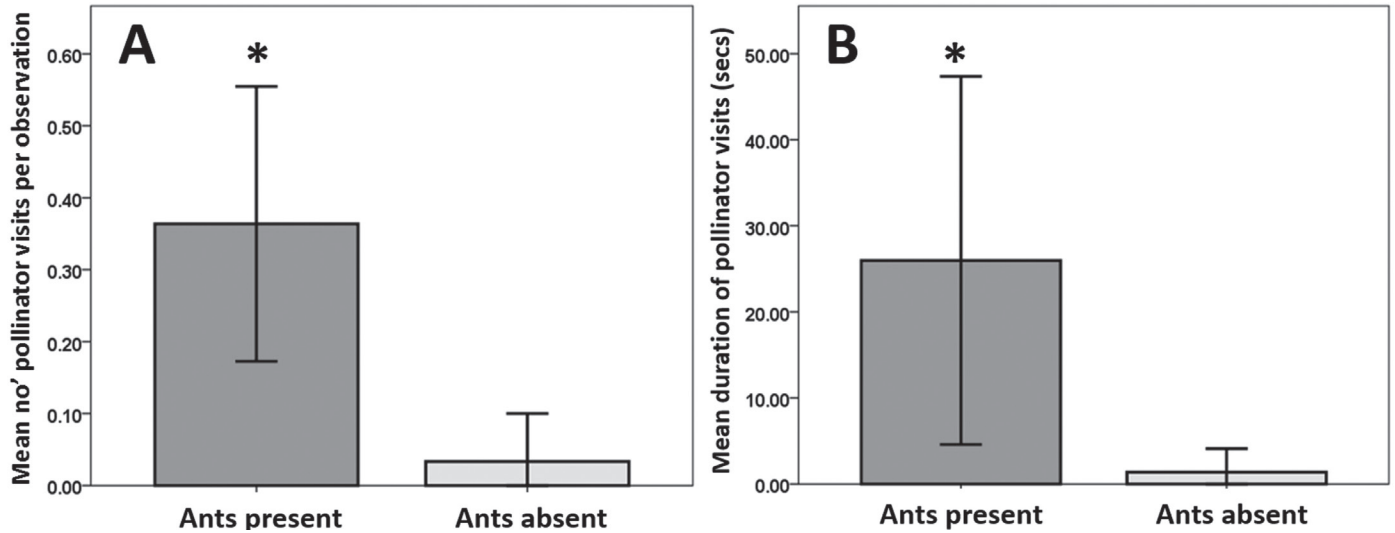
## Discussion

### GENERAL DISCUSSION

Ants have long been known to defend plants through the removal of herbivores, and this phenomenon has been seen on many plant spe-



**Fig. 1.** A. Mean rates of pre-dispersal seed predation on *Senna chapmanii* plants with (Control) and without (Tanglefoot™) ants. Seed predation was calculated for each plant as the number of herbivore damaged seeds divided by the total number of developing seeds (fertilized ovules minus aborted seeds); B. Mean pollinator efficiency on *S. chapmanii* plants with and without ants. Pollinator efficiency was measured as the proportion of ovules fertilized for each plant; C. Mean effective fecundity of *S. chapmanii* plants with and without ants. Effective fecundity was calculated for each plant as the number of non-predated mature seeds divided by the total number of ovules. In all 3 cases, comparisons were made between treatments using Mann–Whitney  $U$  tests, and results were deemed statistically significant if  $P$  values were  $\leq 0.05$ . Error bars represent standard error in all cases. No significant differences were observed between treatments.

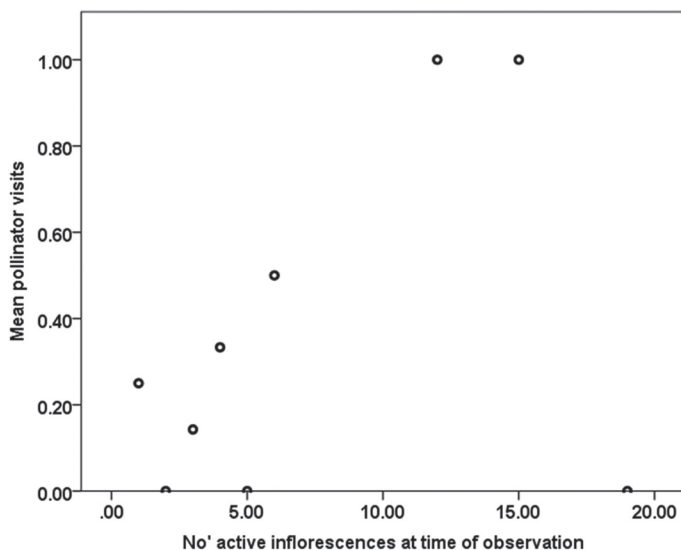


**Fig. 2.** Mean number (A) and duration (B) of pollinator visits on *Senna chapmanii* plants with and without ants. In both cases, comparisons were made between treatments using Mann–Whitney *U* tests, and results were deemed statistically significant if *P* values were  $\leq 0.05$ . Error bars represent standard error. Asterisks indicate significant differences. In the axis label, no' refers to number.

cies, including *S. chapmanii* (Bentley 1977; Rosumek et al. 2009; Heil 2015; Koptur et al. 2015; Jones et al. 2016). The application of Tanglefoot™ was effective in excluding ants from treatment plants for the duration of the experiment.

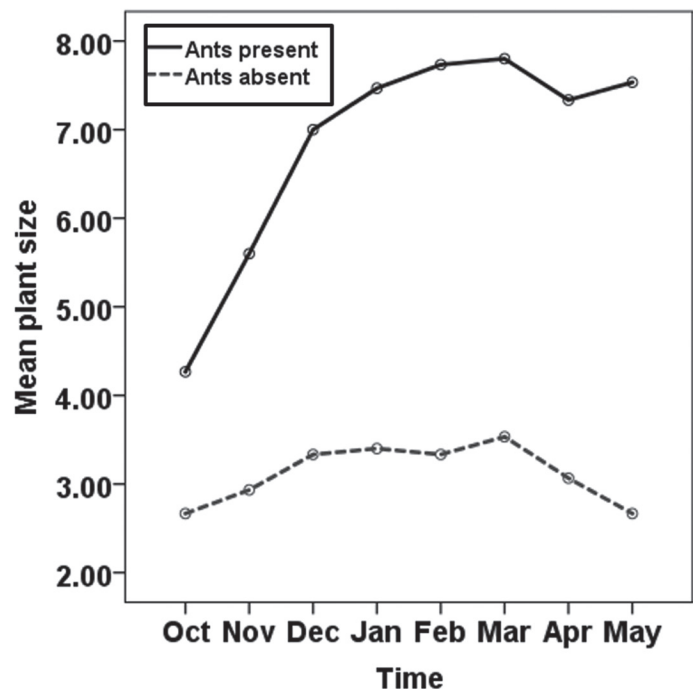
In the present study, we did not observe significant differences in measures of gross reproductive fitness (numbers of inflorescences, flowers, mature fruit, and seeds) between treatments, perhaps due to relatively low sample sizes. Previous studies have, however, shown increased seed production in *S. chapmanii* plants with ants present, when compared with plants from which ants were excluded (Jones et al. 2016). Here, we focus greater attention on the effects of ants on interactions between *S. chapmanii* and other insects that may affect its reproductive fitness.

On control plants, ants were regularly seen patrolling flowers and developing fruit. Despite this, no difference was observed in the rate



**Fig. 3.** Correlation between the number of active inflorescences on *Senna chapmanii* plants at the time of observation and the number of pollinator visits. Circles represent the mean number of pollinator visits per observation on plants with different numbers of inflorescences. In the axis label, No' refers to number.

of seed predation between treatments. Although surprising, this result was not wholly unexpected. While several authors have observed increased EFN production on and around developing fruit, in line with optimal defense theory (Holland et al. 2009; Falcão et al. 2014), few studies have shown ants to reduce pre-dispersal seed predation (but see Inouye & Taylor 1979; Schemske 1980). Lenoir and Pihlgren (2006) observed 10 species of ants attracted to EFNs of the Bush vetch, *Vicia sepium* L. (Fabaceae). Despite their numbers, ants had no effect on seed predation, which was predominantly mediated by the leaf beetle, *Bruchus atomarius* L. (Coleoptera: Atellabidae). Ruhren (2003) also observed no effects of ants on the specialist seed predator, *Sennius cru-*



**Fig. 4.** Mean size of *Senna chapmanii* plants in the presence and absence of ants over the course of the 8 mo experiment. Plant size was estimated as the number of growing stems with 10 or more leaves.

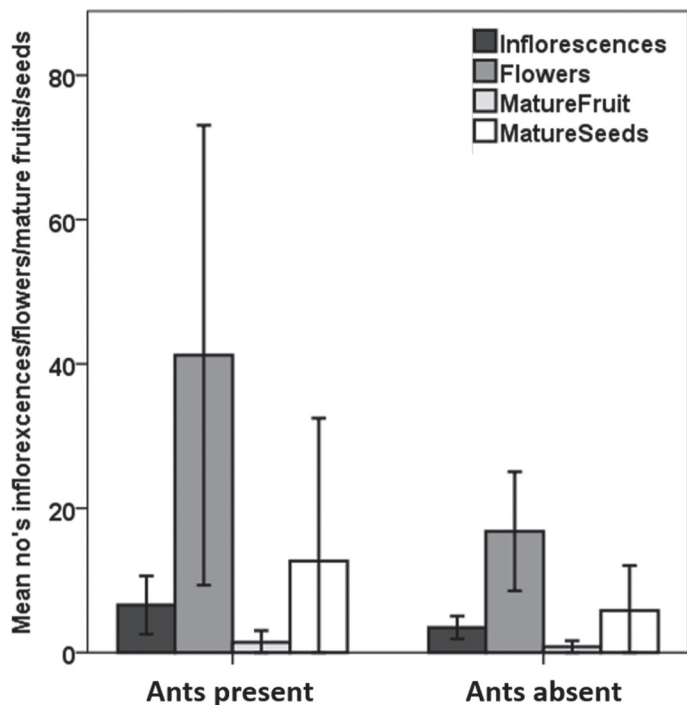


Fig. 5. Reproductive fitness of *Senna chapmanii* plants with (Control) and without (Tanglefoot™) ants. Bars represent the means of weekly measurements taken over the course of the 8 mo experiment. Error bars represent standard error. In the axis label, “no’s.” refers to numbers.

*entatus* Horn (Coleoptera: Chrysomelidae), on *Chamaecrista nictitans* (L.) Moench (Fabaceae). In this case, the beetles may have evaded detection by living inside developing seed pods. Pod-boring seed predators may pose a particular problem for ant defended plants. In the common vetch, *Vicia sativa* L. (Fabaceae), seed damage by pod-boring tortricid moths has been shown to be greater in the presence of ants (Koptur & Lawton 1988). It was suggested that ants may even facilitate pod-boring herbivores by patrolling the fruit and deterring predators and parasitoids (Koptur & Lawton 1988).

In the present study, effective fecundity, defined as the proportion of ovules that survived to become mature seeds, did not differ between treatments. These results, along with our seed predation data and insect surveys, indicate that in the case of *S. chapmanii*, ants do not benefit plant fitness by increasing the survivorship of flowers or developing fruit.

The flowers of *Senna* species are buzz-pollinated by pollen collecting bees (Marazzi et al. 2015). Only 1 insect, the sweat bee (*A. pura*), was observed to effectively remove pollen from the poricidal anthers of *S. chapmanii* flowers. Flower visits by this insect were significantly more frequent, and their duration significantly longer, on plants with ants present. This result was surprising, as aggressive ants more often have been seen to deter pollinators (Ness 2006; Assunção et al. 2014; Ohm & Miller 2014), and pollinators have been observed to recognize the danger posed by ants (Hernández-Cumplido et al. 2010; Assunção et al. 2014). However, similar to our findings, another study (Holland et al. 2011) observed increased pollination rates in the presence of ants. Holland et al. (2011) excluded ants from senita cacti in the Sonoran Desert and observed a reduction in pollination rates in the absence of ants.

Although the number and duration of pollinator visits was higher in the presence of ants, pollination efficiency (defined as the proportion of ovules fertilized for each plant) did not differ between

treatments. So the question remains, what drives increased pollinator visitation in the presence of ants, and how might this affect plant reproductive fitness? Throughout the course of the study, plant size increased rapidly in the presence of ants, and remained fairly constant in their absence. After 4 mo, plants with ants had significantly more growing stems than plants with ants excluded. These results suggest that by removing herbivores (Koptur et al. 2015) and reducing rates of leaf damage (Jones et al. 2016), ants on *S. chapmanii* facilitate increased plant growth. It is likely, therefore, that the observed increase in pollinator visits in the presence of ants simply reflected the larger size and increased floral displays of plants with ants. Oliveira (1997) observed higher pollination rates in *Caryocar brasiliense* A. St. -Hil. (Caryocaraceae) in the presence of ants and drew similar conclusions. Ants reduced herbivore damage to vegetative tissues, resulting in healthier plants that supported larger, more attractive floral displays (Oliveira 1997).

Extrafloral nectar measurements revealed a sharp decline in per leaf sugar production over the course of the study. These measurements were taken between Oct and May, and the same pattern was seen in plants of a similar age during previous field season, in which measurements were taken between Apr and Feb (Jones et al. 2016). These results suggest that the decline in EFN production represents an ontogenetic rather than a seasonal or phenological pattern, and that EFN may be most important for plant establishment and early growth in *S. chapmanii*.

## CONCLUSIONS

Ants may benefit plant reproductive fitness in a number of ways. For example, by reducing herbivory rates (Janzen 1966; Bruna et al. 2004; Del-Claro et al. 2006) or herbivore numbers (Letourneau & Barbosa 1999), facilitating effective pollination (Oliveira 1997; Holland et al. 2011), protecting flowers or developing fruits (Wackers & Bonifay 2004; Holland et al. 2009), or even providing nutrient subsidies by nesting among plant roots (Wagner 1997; Wagner & Nicklent 2010). Conversely, plants that host ants may incur certain ecological costs. Aggressive ants have been known to deter beneficial insects such a pollinators (Ness 2006; Hernández-Cumplido et al. 2010; Assunção et al. 2014), predators (Torres-Hernandez et al. 2000; Nahas et al. 2012; Koptur et al. 2015), and parasitoids (Styrsky & Eubanks 2007; Rosumek et al. 2009). Some defensive ants are also known to cheat their mutualistic partners by removing reproductive structures to promote vegetative growth (Yu & Pierce 1998). In order for plants to benefit from their interactions with ants, the combined effects of these interconnected processes must be weighed in their favor.

The effects of ants on reproductive fitness in *S. chapmanii* appear inconsistent and are strongly influenced by ecological context (Jones et al. 2016), as is likely the case in many facultative ant-plant associations. The mechanisms by which ants effect plant reproductive fitness, however, appear relatively simple in this case. The presence of ants on plants, particularly during the months of establishment, contribute to enhanced plant size. The relative effects of ants on rates of pollination, fruit development, and seed predation appear minimal. Any differences in reproductive fitness in the presence of ants likely occur because plants are faster to establish, reach larger sizes, and can allocate more resources to reproductive traits.

Food-for-protection mutualisms between plants and ants have often been shown to enhance plant reproductive fitness (Rosumek et al. 2009). Understanding the intraguild and multitrophic interactions that underline these benefits may allow us to harness ant-plant interactions in agricultural settings where herbivore damage and the overuse of pesticides are worldwide concerns.

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## References Cited

- Assunção MA, Torezan-Silingardi HM, Del-Claro K. 2014. Do ant visitors to extrafloral nectaries of plants repel pollinators and cause an indirect cost of mutualism? *Flora—Morphology, Distribution, Functional Ecology of Plants* 209: 244–249.
- Baker DA, Hall LJ, Thorpe JR. 1978. A study of the extrafloral nectaries of *Ricinus communis*. *New Phytologist* 81: 129–137.
- Becerra JXI, Venable DL. 1989. Extrafloral nectaries: a defence against ant–Hymenoptera mutualisms? *Oikos* 55: 276–280.
- Bentley BL. 1977. The protective function of ants visiting the extrafloral nectaries of *Bixa orellana* (Bixaceae). *Journal of Ecology* 65: 27–38.
- Bruna EM, Lapola DM, Vasconcelos HL. 2004. Interspecific variation in the defensive responses of obligate plant–ants: experimental tests and consequences for herbivory. *Oecologia* 138: 558–565.
- Dejean A, Leroy C, Corbara B, Céréghino R, Roux O, Hérault B, Rossi V, Guerrero RJ, Delabie JHC, Orivel J, Boulay R. 2010. A temporary social parasite of tropical plant–ants improves the fitness of a myrmecophyte. *Naturwissenschaften* 97: 925–934.
- Del-Claro K, Byke J, Yague GM, Morato MG. 2006. Conservative benefits in an ant–hemipteran association in the Brazilian tropical savanna. *Sociobiology* 47: 415–421.
- Falcão JCF, Dáttilo W, Izzo TJ. 2014. Temporal variation in extrafloral nectar secretion in different ontogenic stages of the fruits of *Alibertia verrucosa* S. Moore (Rubiaceae) in a Neotropical savanna. *Journal of Plant Interactions* 9: 137–142.
- Frederickson ME, Gordon DM. 2007. The devil to pay: a cost of mutualism with *Myrmelachista schumanni* ants in ‘devil’s gardens’ is increased herbivory on *Duroia hirsuta* trees. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 274: 1117–1123.
- Freitas L, Galetto L, Bernardello G, Paoli AAS. 2000. Ant exclusion and reproduction of *Croton sarcopetalus* (Euphorbiaceae). *Flora* 195: 398–402.
- Gonzalez-Teuber M, Heil M. 2009. Nectar chemistry is tailored for both attraction of mutualists and protection from exploiters. *Plant Signaling and Behaviour* 4: 809–813.
- Heil M. 2011. Nectar: generation, regulation and ecological functions. *Trends in Plant Science* 16: 191–200.
- Heil M. 2015. Extrafloral nectar at the plant–insect interface: A spotlight on chemical ecology, phenotypic plasticity, and food webs. *Annual Review of Entomology* 60: 213–232.
- Hernández-Cumplido J, Benrey B, Heil M. 2010. Attraction of flower visitors to plants that express indirect defence can minimize ecological costs of ant–pollinator conflicts. *Journal of Tropical Ecology* 26: 555–577.
- Holland JN, Chamberlain SA, Horm KC. 2009. Optimal defence theory predicts investment in extrafloral nectar resources in an ant–plant mutualism. *Journal of Ecology* 97: 89–96.
- Holland JN, Chamberlain SA, Miller TEX. 2011. Consequences of ants and extrafloral nectar for pollinating seed-consuming mutualism: ant satiation, floral distraction, or plant defense? *Oikos* 120: 381–388.
- IBM Corp. 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, New York.
- Inouye DW, Taylor OR. 1979. A temperate region plant–ant–seed predator system: consequences of extra floral nectar secretion by *Helianthella quinque-nervis*. *Ecology* 60: 1–7.
- Janzen DH. 1966. Coevolution of mutualism between ants and acacias in Central America. *Evolution* 20: 249–275.
- Jones IM, Koptur S. 2015. Dynamic extrafloral nectar production: the timing of leaf damage affects the defensive response in *Senna mexicana* var. *chapmanii* (Fabaceae). *American Journal of Botany* 102: 58–66.
- Jones IM, Koptur S, Gallegos HR, Tardanico JP, Trainer PA, Pena JE. 2017. Changing light conditions in pine rockland habitats may affect the intensity and outcome of ant–plant interactions. *Biotropica* 49: 83–91.
- 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. 1992. Extrafloral nectary-mediated interactions between insects and plants, pp 81–129 In Bernays EA [ed]. *Insect-Plant Interactions Vol IV*. CRC Press, Boca Raton, USA.
- Koptur S, Lawton JH. 1988. Interactions among vetches bearing extrafloral nectaries, their biotic protective agents, and herbivores. *Ecology* 69: 278–283.
- Koptur S, Palacios-Rios M, Diaz-Castelazo C, Mackay WP, Rico-Gray V. 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.
- Koptur S, Jones IM, Pena JE. 2015. The influence of host plant extrafloral nectaries on multitrophic interactions: an experimental investigation. *PLOS One* 10: e0138157.
- Lenoir L, Pihlgren A. 2006. Effects of grazing and ant/beetle interaction on seed production in the legume *Vicia sepium* in a seminatural grassland. *Ecological Entomology* 31: 601–607.
- Letourneau DK, Barbosa P. 1999. Ants, stem borers, and pubescence in *Endospermum* in Papua New Guinea. *Biotropica* 31: 295–302.
- Marazzi BJ, Bronstein L, Koptur S. 2013. The diversity, ecology and evolution of extrafloral nectaries: current perspectives and future challenges. *Annals of Botany* 111: 1243–1250.
- Marazzi B, Conti E, Endress PK. 2015. Diversity in anthers and stigmas in the buzz-pollinated genus *Senna* (Leguminosae, Cassiinae). *International Journal of Plant Sciences* 168: 371–391.
- Mooney KA. 2007. Tritrophic effects of birds and ants on a canopy food web, tree growth, and phytochemistry. *Ecology* 88: 2005–2014.
- Nahas L, Gonzaga MO, Del-Claro K. 2012. Emergent impacts of ant and spider interactions: herbivory reduction in a tropical savanna tree. *Biotropica* 44: 498–505.
- Ness JH. 2006. A mutualism’s indirect costs: the most aggressive plant bodyguards also deter pollinators. *Oikos* 113: 506–514.
- Ohm JR, Miller TEX. 2014. Balancing anti-herbivore benefits and anti-pollinator costs of defensive mutualists. *Ecology* 95: 2924–2935.
- Oliveira PS. 1997. The ecological function of extrafloral nectaries: herbivore deterrence by visiting ants and reproductive output in *Caryocar brasiliense* (Caryocaraceae). *Functional Ecology* 11: 323–330.
- Rosumek FB, Silveira FAO, Neves FDS, Barbosa NPDU, Diniz L, Oki Y, Pezzini F, Fernandes GW, Cornelissen T. 2009. Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia* 160: 537–549.
- Rudgers A. 2004. Enemies of herbivores can shape plant traits: selection in a facultative ant–plant mutualism. *Ecology* 85: 192–205.
- Ruhren S. 2003. Seed predators are undeterred by nectar-feeding ants on *Chamaecrista nictitans* (Caesalpinaceae). *Plant Ecology* 166: 189–198.
- Schemske DW. 1980. The evolutionary significance of extrafloral nectar production by *Costus woodsonii* (Zingiberaceae), an experimental analysis of ant protection. *Journal of Ecology* 68: 959–967.
- Styrsky JD, Eubanks MD. 2007. Ecological consequences of interactions between ants and honeydew-producing insects. *Proceedings of the Royal Society of London, Series B, Biological Sciences* 274: 151–164.
- Torres-Hernandez L, Rico-Gray V, Castillo-Guevara C, Vergara JA. 2000. Effect of nectar-foraging ants and wasps on the reproductive fitness of *Turnera ulmifolia* (Turneraceae) in a coastal sand dune in Mexico. *Acta Zoologica Mexicana* 81: 13–21.
- Wackers FL, Bonifay C. 2004. How to be sweet? Extrafloral nectar allocation by *Gossypium hirsutum* fits optimal defense theory predictions. *Ecology* 85: 1512–18.
- Wagner D. 1997. The influence of ant nests on *Acacia* seed production, herbivory, and soil nutrients. *Journal of Ecology* 85: 83–93.
- Wagner D, Kay A. 2002. Do extrafloral nectaries distract ants from visiting flowers? An experimental test of an overlooked hypothesis. *Evolutionary Ecology Research* 4: 293–305.
- Wagner D, Nicklent EF. 2010. Ant nest location, soil nutrients, and nutrient uptake by ant-associated plants: does extrafloral nectar attract ant nests and thereby enhance plant nutrition? *Journal of Ecology* 98: 614–624.
- Weber MG, Porturas LD, Keeler KH. 2015. World list of plants with extrafloral nectaries. [www.extrafloralnectaries.org](http://www.extrafloralnectaries.org) (last accessed 21 Feb 2017).
- Yu DW, Pierce NE. 1998. A castration parasite of an ant–plant mutualism. *Proceedings of the Royal Society of London, Series B, Biological Sciences* 265: 375–382.