

# On the inability of ants to protect their plant partners and the effect of herbivores on different stages of plant reproduction

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**Abstract** Ants co-occur with herbivorous thrips in several tropical plants, but their interactions are largely unexplored. Should thrips be deterred by ants, a positive effect of ants on plant fitness might be expected. Here, by using an experimental study design with ant-present and ant-excluded treatments, we investigated the influence of *Camponotus blandus* on *Pseudophilothrips obscuricornis* abundance and herbivory in three extrafloral nectaried species: *Banisteriopsis malifolia*, *B. laevifolia* and *B. stellaris*. In addition, we examined the effect of thrips herbivory on flower set and fruit development and dispersion. Thrips abundance and herbivory were higher on ant-present stems of *B. malifolia* and *B. laevifolia*, where thrips managed to escape from ants by hiding in between clusters of flower buds (thygmataxis behaviour). In *B. stellaris* the results were the opposite, as flower bud clusters did not offer hiding places, so thrips were unable to hide from ants; thus both thrips abundance and herbivory were lower on ant-present stems. Thrips herbivory had no significant effect on flower and fruit set, but samaras (V-shaped winged fruits of Malpighiaceae) attacked by thrips presented severe distortions and asymmetries. This caused damaged fruits to be dispersed closer to the mother plant, whereas uninjured fruits were dispersed further away. This study is evidence that ant–plant–herbivore systems have variable outcomes depending on the species involved, their behaviour and the plant structure under consideration. Unlike other herbivores, thrips negatively influence the very last stage of plant reproduction. The minute and furtive herbivorous thrips have long been ignored in natural systems, but because of their wide host range, they may be important herbivores even in extrafloral nectaried plants, which are usually fiercely protected by ants.

**Key words:** *Banisteriopsis*, *Camponotus*, Malpighiaceae, *Pseudophilothrips*, samara.

## INTRODUCTION

Thrips are widespread insect herbivores in the Neotropics (Mound 2002) where their co-occurrence with patrolling ants on extrafloral nectaried plants is commonplace (Del-Claro *et al.* 1997). Such co-occurrence might indicate that thrips populations could be maintained at lower levels because extrafloral nectar feeding ants are aggressive towards insect herbivores in general (González-Teuber *et al.* 2012). Several studies have clearly demonstrated that this guild of ants can significantly deter a wide range of herbivores (e.g. lepidopterans, beetles, orthopterans and hemipterans), thus benefiting the plant (Vilela *et al.* 2014; Del-Claro & Marquis 2015). Nonetheless, there are few detailed studies of thrips–ant interactions (Alves-Silva & Del-Claro 2014) and unfortunately most studies are observational, thus limiting knowledge of the actual effect of ants on thrips populations (Del-Claro & Mound 1996; Sakai 2001; Peng &

Christian 2004). Because of their ubiquity and high abundance in plants, herbivorous thrips may kill growing tips and shoots, thus reducing plant performance, vigour, architectural complexity and fitness (Mound & Zapater 2003; Cuda *et al.* 2008, 2009).

In the case of plants bearing extrafloral nectaries (EFNs), the tending ants are assumed to protect the plant against herbivores in general (Heil & McKey 2003), including thrips (Kersch & Fonseca 2005). Nonetheless, in some cases ants fail to protect the plants, as insect herbivores may have morphological and behavioural adaptations to escape from ant contact and attack (Mody & Linsenmair 2004; Nogueira *et al.* 2012; Endo & Itino 2013; Alves-Silva *et al.* 2014). Concerning thrips, ants can affect their behaviour and reduce their populations (Peng & Christian 2004), but some thrips species use chemical repellents to discourage ant contact or hide in places inaccessible to ants (Crespi & Mound 1997; Suzuki *et al.* 2004). In such cases, once free from their possible natural enemies (i.e. ants), thrips can feed on and cause severe damage to their host plants (Alves-Silva & Del-Claro 2014; Vilela *et al.* 2014).

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In ant–plant systems, authors usually consider the fruit set as a good estimator of plant fitness (Rosumek *et al.* 2009; Assunção *et al.* 2014). Herbivorous thrips have a wide feeding flexibility and consume mostly leaves, flower buds and flowers (Mound & Marullo 1996), but a few species can also feed on fruits, often causing serious aesthetic damage with distortions and necrosis over the fruit surface (Pinent *et al.* 2008). Therefore, it is important to take into account the natural history of fruit/seed consumer insects in order to verify their influence on plant reproductive success (Ruhren 2003). If ants are able to deter these herbivores, the plant should gain an actual benefit in terms of fitness (Guimarães *et al.* 2006).

In the Brazilian savanna, several Malpighiaceae shrubs possess EFNs (Araújo *et al.* 2010), and support a rich ant fauna together with herbivorous thrips (Vilela *et al.* 2014). Therefore, Malpighiaceae might be considered an ideal model to investigate the effects of thrips on plant reproduction, as well as the influence of ant guards on deterring these small herbivores. In the present study, thrips–ant–plant interactions were studied in *Banisteriopsis malifolia* (Nees et Mart.) B. Gates, *B. laevifolia* (A. Juss.) B. Gates and *B. stellaris* (Griseb.) B. Gates, all of which possess EFNs. These plants are patrolled by *Camponotus blandus* (Smith 1858) Formicinae, and the flower buds and fruits are severely attacked by *Pseudophilothrips* aff. *obscuricornis* (Priesner 1921) (Phlaeothripidae) (pers. obs. 2013). We addressed two main questions: (i) Can ants deter (e.g. prey on, chase and/or expel) thrips from these plants? (ii) To what extent is thrips herbivory detrimental to plant reproduction?

Thrips damage to reproductive structures was compared between ant-present and ant-excluded plants. According to our hypotheses, (i) patrolling ants might reduce thrips populations (Reimer 1988) and thus herbivory rates (e.g. Peng & Christian 2004); or (ii) thrips might not be affected by ants (Souza *et al.* 1998; Mody & Linsenmair 2004) and thus be able to inflict damage on flower buds. We also investigated the influence of thrips herbivory on fruit performance and dispersion. During the fruiting season, all *Banisteriopsis* (*malifolia*, *laevifolia*, *stellaris*) have no active EFNs and ants are absent, so thrips are free of any potential disturbance from ants. We hypothesized that thrips herbivory would affect fruit quality, as measured by fruit size and shape, and that herbivory on fruits (samaras) would influence their dispersive capabilities by wind.

## METHODS

### Study area

The fieldwork was conducted in a Brazilian tropical savanna area (230 ha, 18°59'S–48°18'W) in Uberlândia City, Brazil.

The area is dominated by herbaceous vegetation and shrubs, such as Malpighiaceae, Myrtaceae and Fabaceae. Trees of the Caryocaraceae and Ochnaceae, among other families, occur scattered throughout the area, whereas grasses are common. The wettest season (October to April) contributes more than 90% of the annual rainfall (1500 mm per year). The mean monthly temperature ranges from 24.8°C in February to 19.9°C in June, with an annual mean of 23°C.

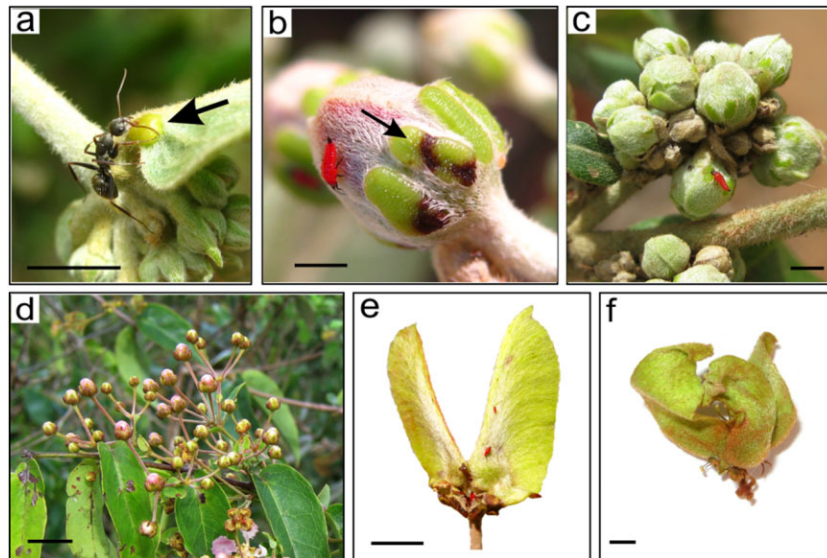
### Study organisms

*Banisteriopsis malifolia* and *B. laevifolia* are shrubs (<2 m high), whereas *B. stellaris* is a vine. Fully expanded leaves may reach up to 15 cm long and 10 cm wide in *B. malifolia*; 10 cm and 5 cm in *B. laevifolia*; and 7 cm and 4 cm in *B. stellaris*. Leaves of all species have a pair of EFNs at the base near the petiole at each side of the midrib (Fig. 1a). Flower bud production in *B. malifolia* peaks in March–April; in *B. laevifolia* in August–September; and in *B. stellaris* in January–February (Appendix S1). In all these species, flower buds grow on inflorescences located at the apex of branches and stems (Fig. 1b–d). Mature flower buds are round and on average 8, 5 and 6 mm in diameter in *B. malifolia*, *B. laevifolia* and *B. stellaris*, respectively, and are surrounded by eight oil glands. Fruiting peaks in May, November and May in *B. malifolia*, *B. laevifolia* and *B. stellaris*, respectively (Appendix S1). All plants produce wind-dispersed V-shaped samaras with crispy wings and tiny trichomes distributed over the surface (Fig. 1e,f).

*Camponotus blandus* is frequently found on Malpighiaceae and it is very aggressive towards insects in general, including other predatory arthropods (Guimarães *et al.* 2006; Alves-Silva *et al.* 2013). *Pseudophilothrips obscuricornis* are dominant herbivores in *Banisteriopsis*, feeding on leaves, flower buds and fruits, but not on flowers. Females lay eggs on shoots, flower buds and fruits, and hatched larvae remain on these structures until pupation, which takes place in the soil. Eggs are kidney-shaped and yellowish to brownish in colour. Larvae can remain immobile on a given plant structure throughout the day, and both adults and larvae are found in aggregations of up to 18 individuals, sucking up plant sap from leaves, buds and fruits. Larvae (about 1 mm in length) are reddish, wingless and very reticent to migrate between plant structures, whereas winged adults (approximately 2 mm long and black in colour) are more mobile, walking rapidly and migrating between plant parts (pers. obs. 2013).

### Flower bud herbivory rates

Flower bud herbivory was assessed in different periods according to the phenology of each plant (Appendix S1). *Banisteriopsis malifolia* ( $n = 40$  individuals) was studied in March–April; *B. laevifolia* ( $n = 32$  individuals) in July–August; and *B. stellaris* ( $n = 25$  individuals) in January–February 2011. Plants were distributed evenly over 30 ha within the study area and fieldwork was restricted to plants that supported *C. blandus* only. On each plant, one control and one treatment stem in the same phenological condition were randomly chosen for the experimental design. The bases



**Fig. 1.** (a) *Camponotus blandus* feeding from the extrafloral nectaries (arrow) of *Banisteriopsis malifolia*. (b) Flower bud with necrosis marks on oil glands (arrow). (c) Conformation of *B. malifolia* and (d) *B. stellaris* flower buds. (e, f) Uninjured and damaged samaras of *B. malifolia*. Scale bars: (a, d, e, f) = 5 mm; (b) = 1 mm, (c) = 2 mm.

of treatment stems were banded with a layer of atoxic sticky resin (Tanglefoot) to prevent access by ants. To control for the effect of the resin, Tanglefoot was also applied on one side of the control stems, allowing free access of ants to the plant parts (following Nahas *et al.* 2012). Leaves and other branches from surrounding plants that could be used by ants as bridges to climb onto experimental stems were removed or clipped back. Special care was taken concerning *B. stellaris*, as this plant is a vine. Whenever necessary, plants in the vicinity received resin to prevent access by ants.

After resin application, we conducted an initial assessment of flower bud herbivory (%), which was calculated as the number of damaged buds divided by the total number of buds produced, and expressed as a percentage. Thrips abundance on each stem was estimated by visually examining flower buds, shoots and both sides of leaves of the experimental and control stems. In this first sampling, there was no statistical difference in bud herbivory (%) and thrips abundance between experimental and control stems ( $P > 0.05$  in all cases) (Table 1, see Results section). Thus, study bias could be discounted.

After the beginning of the experiment (resin application), plants were visited twice a week (with a minimum interval of 2 days) to ensure that ants were indeed excluded from the experimental stems. During these visits, other herbivores that might influence our study (e.g. beetles, butterfly larvae and grasshoppers) were removed and placed on distant non-experimental plants. Three weeks after resin application (approximately 1 week before flowering), flower bud herbivory (%) and thrips abundance were estimated again on experimental and control stems.

### Thrips herbivory and flower set

To investigate whether *P. obscuricornis* herbivory in buds influenced the flower set, 20 mature flower buds were tagged

**Table 1.** Flower bud herbivory (%) and *Pseudophilothrips obscuricornis* abundance on ant-present and ant-excluded stems at the beginning of the study

Malpighiaceae species	Experimental stems comparison	
	Thrips abundance	Bud herbivory (%)
<i>Banisteriopsis malifolia</i>	$t_{78} = 1.0737^{\text{NS}}$	$t_{78} = 0.7045^{\text{n.s.}}$
<i>Banisteriopsis laevifolia</i>	$t_{62} = 0.9431^{\text{NS}}$	$t_{62} = 0.0977^{\text{n.s.}}$
<i>Banisteriopsis stellaris</i>	$t_{48} = 1.6720^{\text{NS}}$	$t_{48} = 0.5764^{\text{n.s.}}$

n.s., non-significant.

on each individual of each plant species ( $n = 25$  *B. malifolia* individuals (500 buds);  $n = 20$  *B. laevifolia* (400 buds);  $n = 18$  *B. stellaris* (360 buds)). Tagged buds were all present on the same stem and were divided into two groups, 10 flower buds with necrosis (see Fig. 1b – hereafter referred to as damaged buds) and 10 uninjured buds with no sign of thrips presence or herbivory. Each flower bud was tagged with a fine sewing thread (2 cm long) tied at its base. Thrips were manually removed from these stems to prevent them feeding on tagged uninjured buds. Plants were also visited twice a week to remove possible florivores. Flower buds were monitored until anthesis. This procedure was performed on stems other than those used for ant-exclusion/ant-present experiments. Therefore, this experiment had no connection with the previous investigation on the influence of ants on thrips abundance and bud herbivory.

### Effect of thrips on fruit development

The influence of thrips herbivory on fruit (samara) development was investigated at different times according to the



fruiting phenology of each plant. During the fruiting season, the EFNs of the studied *Banisteriopsis* are inactive, so no experimentation concerning ant-presence/absence could be performed. *Banisteriopsis malifolia* ( $n = 20$  individuals) was studied in May; *B. laevifolia* ( $n = 20$  individuals) in November and *B. stellaris* ( $n = 15$  individuals) in April 2011. From each individual plant we collected 20 samaras, which were always sampled in pairs, assessing one damaged samara and an adjacent samara without thrips damage. *Pseudophilothrips obscuricornis* females lay eggs on fruit surfaces and hatched larvae rarely migrate from fruits, so larvae can spend the whole of their immature stage feeding on a single fruit. As fruits within infructescences mature at the same time, neighbouring fruits were assumed to be the same age. Samara development was examined via measures of length and width (mm) in the laboratory. We expected that uninjured samaras would be larger and wider than damaged ones. This experiment also had no relationship with the investigation on the influence of ants on thrips abundance and bud herbivory.

### Thrips herbivory and fruit dispersion

The influence of thrips herbivory on the dispersion of samaras was examined in 15 *B. malifolia* individuals. All individuals were at least 5 m from each other and located in an area containing mostly herbaceous vegetation (<1 m in height). On each shrub, we tagged 20 samaras and divided them equally into 2 groups of 10 fruits per plant: one containing samaras damaged by thrips and the other group with uninjured samaras, totalling 150 samaras in each case. Both groups of fruits belonged to the same stems, which were located, on average, 1.62 ( $\pm 0.10$ ) cm above the soil and facing east.

Samaras were marked with a red-inked pen, and the symbols 'D' and 'H' were written in the wings of damaged and healthy (uninjured) samaras, respectively, together with the number of the individual plant (1–15). Wind speed during the experimental design was measured with a hand anemometer, placed 1.5 m above the soil and set to measure the mean wind speed in a 10-min interval during a sunny morning from 09:00 to 09:10 h. Each plant was visited on 3 days within 1 month, and a search for dispersed fruits was made on the soil and grasses in the vicinity (approximately 10 m radius) of the plants.

### Interactions between ants and thrips

In the field, we conducted 90 h of observation (*ad libitum* sense, Altmann 1974) of the behaviour of *C. blandus* towards *P. obscuricornis*. Observations were made on 10 individuals of each *Banisteriopsis* species (*laevifolia*, *malifolia* and *stellaris*) for 3 h (0800–1100 h) on consecutive sunny days. Whenever ants antennated thrips we registered whether the latter presented any change in behaviour and whether ants successfully deterred them on plants, either by preying upon or expelling them from flower buds. Due to the constant movement/activity of ants on different parts of the plants, it was difficult to determine their precise abundance on stems where observations were being conducted. Therefore, we

estimated the range of ants per stem during the observations and the maximum value was used. This value was then used to investigate the relationship between the abundance of ants and the molested thrips.

### Statistical analyses

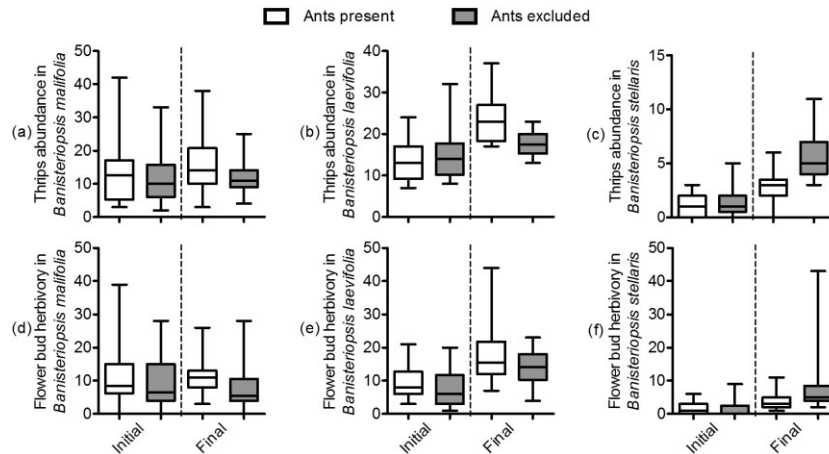
Quantitative data are presented whenever possible as mean  $\pm$  standard deviation. The comparison of the abundance of thrips and flower bud herbivory (%) at the beginning of the study was made with Student's *t*-tests for ant-present and ant-excluded stems. The original data on bud herbivory were arcsine transformed; and thrips abundance was log+1 transformed to fit variance homoscedasticity. The effect of ant presence/absence on the abundance of thrips at the end of the study was examined using analyses of covariance (ANCOVA). The abundance of thrips at the end of the study was employed as the dependent variable, the experimental stems (ants-present and ants-excluded) were regarded as factors and the abundance of thrips at the beginning of the study was considered as the covariate. The effect of ant-presence/absence on flower bud herbivory (%) was also examined with analyses of covariance. The herbivory (%) at the end of the study was employed as the dependent variable and the experimental stems were used as factors. The herbivory at the beginning of the study, together with thrips abundance on experimental stems, were used as covariates. Two models of ANCOVA, one with and one without interaction effects, were performed in order to see whether their slopes overlapped. A further comparison of the models was made to examine whether removing the interaction effects did not significantly influence the fit of the model.

The flower set was compared between buds attacked and non-attacked by thrips on the three plants with Student's *t*-tests. Measurements of fruit length and width were compared between healthy and damaged fruits using Student's *t*-tests. This test was also used to compare the dispersion distance between uninjured and damaged samaras. The abundance of thrips molested by ants on each plant species during the fieldwork was compared with a Kruskal–Wallis test, as original and transformed data did not fit the assumptions of normality or variance homoscedasticity. The relationship between the abundance of ants per stem/plant and the abundance of molested thrips was examined with a linear regression. Data from the three plants were pooled and the original data were log+1 transformed. Statistical tests were performed in GraphPad Prism and R statistical software.

## RESULTS

### Preliminary assessment

Both thrips abundance and flower bud herbivory (%) were not statistically different on experimental stems of the three *Banisteriopsis* species at the beginning of the study, thus discarding study bias (Table 1, Fig. 2). Small differences, however, were noticed at the beginning of the fieldwork. Thrips were more abundant (but



**Fig. 2.** (a,b,c) Abundance of *Pseudophyllothrips obscuricornis* and (d,e,f) bud herbivory (%) on three *Banisteriopsis* species, according to ant-presence/absence and timing of sampling (beginning or end of the study). See Tables 1–3 for statistical results. Figures show original/untransformed data. ‘Whiskers’ indicate the maximum and minimum values; boxes indicate the 25th and 75th percentiles; and lines across boxes indicate the median.

not to a statistically significant extent) on ant-present stems of *B. malifolia*, but in the other two plants these small insects were slightly more numerous on ant-excluded stems. Bud herbivory rates were all higher (roughly 15%) on ant-present stems, but these differences were also not statistically significant.

### Thrips abundance according to ant presence

The population of *P. obscuricornis* was significantly influenced by the presence/absence of ants on all three plant species at the end of the study (Table 2, Fig. 2a–c). In *B. malifolia*, the interaction effect was also significant, showing that both the experimental procedure (ant-presence/absence) and the initial population of thrips played a role in the abundance of these insects at the end of the study. In *B. malifolia*, the comparison of both models, with and without the interaction effect, revealed a significant difference ( $F_{76,77} = 6.3348$ ,  $P < 0.05$ ), so the model with interaction was given preference, as it provided a better explanation of this system. In the other two plant species, the interaction effect was not significant, as well as the comparison of models (*B. laevifolia*:  $F_{60,61} = 0.3643$ ,  $P > 0.05$ ; *B. stellaris*:  $F_{46,47} = 2.6738$ ,  $P > 0.05$ ), so we adopted the model without interaction.

At the end of the study, *P. obscuricornis* were significantly more abundant on control stems of the shrubs *B. malifolia* and *B. laevifolia*, but on the vine *B. stellaris*, thrips were more abundant on stems without *C. blandus*. At the beginning of the study, the difference in thrips abundance between ant-present and ant-excluded treatments was, on average, 11.6% and 7.9% in *B. malifolia* and *B. laevifolia*, respectively; in *B. stellaris* this difference was on average 37.5%, as

**Table 2.** Analyses of covariance in three extrafloral nectaried Malpighiaceae showing which effects were responsible for significant changes in thrips abundance at the end of the study

	Sum sq.	d.f.	F-value	P-value
<i>Banisteriopsis malifolia</i>				
Experiment	0.1384	1	4.201	0.0439
Thrips initial	0.0078	1	0.236	0.6287
Experiment × thrips initial	0.2087	1	6.335	0.0139
Residuals	2.5038	76		
<i>Banisteriopsis laevifolia</i>				
Experiment	0.2112	1	32.150	0.0001
Thrips initial	0.0155	1	2.357	0.1300
Residuals	0.4006	61		
<i>Banisteriopsis stellaris</i>				
Experiment	0.9084	1	29.204	0.0001
Thrips initial	0.0534	1	1.716	0.197
Residuals	1.4619	47		

‘Experiment’, stems with ants present and excluded; ‘Thrips initial’, abundance of thrips at the beginning of the study.

the number of thrips per stem varied greatly and their abundance was low (see Fig. 2c). At the end of the study, stems with ant access had on average 33.3% and 33.6% more thrips than ant-excluded stems in *B. malifolia* and *B. laevifolia*, respectively. The opposite scenario was observed in *B. stellaris*, where ant-excluded stems had almost twice the number of thrips (98.6% more), compared with ant-present stems.

### Thrips herbivory in flower buds

At the beginning of the study, flower bud herbivory differences between ant-present and ant-excluded

**Table 3.** Analyses of covariance in three Malpighiaceae showing which effects were responsible for significant changes in the flower bud herbivory (dependent variable) at the end of the study

	Sum sq.	d.f.	F-value	P-value
<i>Banisteriopsis malifolia</i>				
Experiment	0.1150	1	0.424	<b>0.0010</b>
Initial herbivory	0.0042	1	0.546	0.5169
Thrips initial	0.0001	1	0.014	0.9060
Thrips final	0.0001	1	0.012	0.9134
Residuals	0.7397	75		
<i>Banisteriopsis laevifolia</i>				
Experiment	0.0578	1	5.995	<b>0.0173</b>
Initial herbivory	0.0001	1	0.011	0.9163
Thrips initial	0.0007	1	0.076	0.7840
Thrips final	0.0251	1	2.605	0.1119
Residuals	0.5687	59		
<i>Banisteriopsis stellaris</i>				
Experiment	0.0011	1	8.027	<b>0.0068</b>
Initial herbivory	0.0835	1	0.105	0.7478
Thrips initial	0.0001	1	0.001	0.9750
Thrips final	0.0076	1	0.735	0.3958
Residuals	0.4679	45		

'Experiment', stems with ants present and excluded; 'Initial herbivory', flower buds (%) attacked by thrips at the beginning of the study; 'Thrips initial and final', abundance of thrips at the beginning and end of the study. The interaction effects are not shown in the table, as the models were not significant all cases.

stems were, on average, 11.7% and 13.9% in *B. malifolia* and *B. laevifolia*, respectively, and these differences were not statistically significant (Table 1). However, these differences were 33.6% and 25.1% at the end of the study, evidence that the experimental procedure (ant-presence/absence) significantly affected the levels of flower bud herbivory in these plant species (Table 3). In both *B. malifolia* and *B. laevifolia*, ant-present stems had significantly higher levels of bud herbivory (Fig. 2d,e). The most remarkable effect of ant-presence/absence on bud herbivory rates occurred in *B. stellaris*, where herbivory was two-fold greater on ant-excluded stems at the end of the study. When the fieldwork commenced the difference in herbivory between experimental stems was only 15% (Fig. 2f).

According to the ANCOVA test, there was no significant influence of initial herbivory levels (%) and thrips abundance on the herbivory of flower buds assessed at the end of the study (Table 3). In fact, the only variable to significantly affect bud herbivory was the presence or absence of ants. Two models of ANCOVA were run, one with and one without the interaction effects, but in both models, the only variable to significantly affect bud herbivory was the experimental stems. The comparison of the two models revealed no statistical difference between them (*B. malifolia*  $F_{64,75} = 1.0099$ ,  $P > 0.05$ ;

*B. laevifolia*  $F_{48,59} = 0.6993$ ,  $P > 0.05$ ; *B. stellaris*  $F_{34,45} = 0.5661$ ,  $P > 0.05$ ). Therefore, we adopted the most parsimonious model, that is, the one without interaction effects (Table 3).

### Thrips influence on flower set

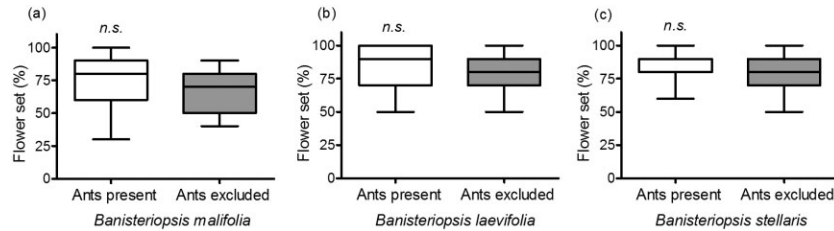
Flower buds damaged by thrips presented several black necrosis marks, especially on oil glands (Fig. 1b). Nonetheless, thrips herbivory had no effect on mean flower formation per plant and no difference was found in the flower set between healthy and damaged buds (*B. malifolia*  $t_{48} = 1.3604$ ,  $P > 0.05$ ; *B. laevifolia*  $t_{38} = 1.2302$ ,  $P > 0.05$ ; *B. stellaris*  $t_{34} = 1.1435$ ,  $P > 0.05$ ) (Fig. 3).

### Thrips effect on fruit development and dispersion

We found a marked significant difference in the size of fruits with and without thrips in all *Banisteriopsis* species. Damaged fruits were on average 8.9%, 7.5% and 5.5% shorter than healthy fruits in *B. malifolia*, *B. laevifolia* and *B. stellaris*, respectively. The difference in fruit width was 10.6%, 12.4% and 24.4%, respectively (Table 4). Among the samaras tagged to investigate dispersion ( $n = 300$ ), only 31.3% ( $n = 94$ ;  $6.71 \pm 2.46$  fruits per plant) were effectively dispersed or recovered. Some samaras were not abscised at all by the plant and eventually became senescent; others were abscised but not found on the ground during the search. We were able to collect 53 healthy and 41 damaged samaras from *B. malifolia*. The average wind speed for the days of collection (May) was 3.02 m/s. Damaged samaras were found, on average,  $85.11 \pm 5.8$  cm away from the mother plant, whereas healthy samaras were collected  $107.67 \pm 7.33$  cm away from the mother plant ( $t_{92} = 16.3591$ ,  $P < 0.0001$ ).

### Ant-thrips interactions

During observations in the field, 58 thrips were molested by ants, occurring most commonly (but not significantly) in *B. stellaris* ( $H_2 = 4.6979$ ,  $P > 0.05$ ) (Fig. 4a). Most thrips (50% of observations) did not show any conspicuous change in behaviour and remained impassively feeding on flower buds. However, on some occasions (27.6% of all observations,  $n = 16$ ) we noticed that when ants antennated thrips, the latter would raise up their abdomen and a small drop was noticed at the end of their anus. The ants then backed off rapidly and did not return to the thrips. The abdomen raising behaviour was noticed in both adults and larvae of *P. obscuricornis*. In contact

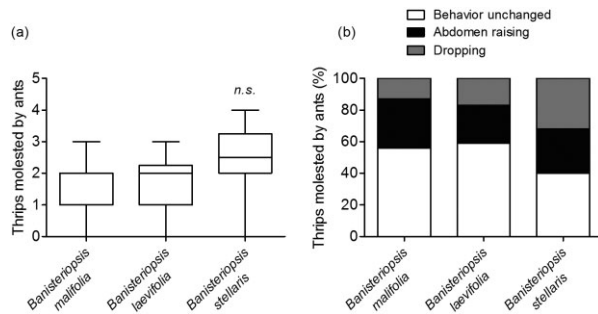


**Fig. 3.** Flower set in three *Banisteriopsis* species according to the presence or absence of patrolling ants. 'Whiskers' indicate the maximum and minimum values; boxes indicate the 25th and 75th percentiles; and lines across boxes indicate the median. 'n.s.' means non-significant.

**Table 4.** Difference in fruit size according to *Pseudophlothrips obscuricornis* herbivory in three *Banisteriopsis* species

Malpighiaceae species	Fruit length – mean $\pm$ SD ( <i>n</i> )		Statistics
	Uninjured	Damaged	
<i>Banisteriopsis malifolia</i>	23.49 $\pm$ 3.42 (200)	21.40 $\pm$ 4.22 (200)	$t_{198} = 5.4047^{***}$
<i>Banisteriopsis laevifolia</i>	20.00 $\pm$ 3.42 (200)	18.50 $\pm$ 3.25 (200)	$t_{198} = 4.4985^{***}$
<i>Banisteriopsis stellaris</i>	18.17 $\pm$ 4.01 (150)	17.09 $\pm$ 4.87 (150)	$t_{148} = 2.0923^*$
	Fruit width – mean $\pm$ SD ( <i>n</i> )		
	Uninjured	Damaged	
<i>Banisteriopsis malifolia</i>	15.00 $\pm$ 3.44 (200)	13.41 $\pm$ 3.30 (200)	$t_{198} = 4.7410^{***}$
<i>Banisteriopsis laevifolia</i>	17.58 $\pm$ 3.40 (200)	15.33 $\pm$ 3.32 (200)	$t_{198} = 6.6668^{***}$
<i>Banisteriopsis stellaris</i>	18.62 $\pm$ 3.93 (150)	14.08 $\pm$ 5.81 (150)	$t_{148} = 7.9298^{***}$

Damaged fruits were smaller and narrower than uninjured fruits. The symbols '\*' and '\*\*\*' mean  $P < 0.05$  and  $P < 0.0001$ , respectively, according to Student's *t*-tests.



**Fig. 4.** (a) Abundance of thrips molested by ants in three extrafloral nectaried Malpighiaceae. (b) Frequency (%) of thrips that changed their behaviour or not in the presence of ants. 'Whiskers' indicate the maximum and minimum values; boxes indicate the 25th and 75th percentile; and lines across boxes indicate the median. 'n.s.' means non-significant.

with ants, the abdomen-raising behaviour of one larva was sometimes repeated by the nearest larva. This synergic behaviour was enough to cause the ants to move away from the thrips aggregation. In other instances (22.4% of observations,  $n = 13$ ) thrips dropped from the plants when they were molested by ants; this behaviour was observed only in adult thrips. The dropping behaviour occurred most frequently in *B. stellaris* (Fig. 4b). We found no relationship between the abundance of ants on plants and the abundance of molested thrips (adjusted non-linear regression,

pooled data from the three plants  $R^2 = 1.7\%$ ,  $df = 28$ ,  $P > 0.60$ ). Adult individuals of *P. obscuricornis* were frequently found together with larvae, hidden in small cracks provided by the clusters of flower buds of *B. malifolia* (Fig. 1c) and *B. laevifolia*. In *B. stellaris*, thrips were very conspicuous on the plant, as the flower buds are separate from each other and do not offer hiding places (Fig. 1d).

## DISCUSSION

### Ant-thrips interactions

Ant-plant interactions mediated by the presence of EFNs have been shown to benefit both species, as ants feed on the sugar sources provided by the plant and, in turn, defend the plant against insect herbivores (Heil & McKey 2003; González-Teuber *et al.* 2012). Nevertheless, in the present study we found evidence that thrips may negatively influence the stability of *C. blandus*–*Banisteriopsis* interactions, because both the abundance of thrips and their herbivory rates (in *B. malifolia* and *B. laevifolia*) were higher on ant-present stems. In *B. stellaris*, however, the opposite results were found and this can be attributed to the different architectural characteristics of the plants. Unlike *B. malifolia* and *B. laevifolia*, buds from *B. stellaris* are fairly separate



from each other and do not provide hiding places for *P. obscuricornis* (Fig. 1c,d). Because of their small size, thrips have the ability to enter small cracks (Mound & Terry 2001; Sakai 2001) where they are inaccessible to natural enemies (Del-Claro *et al.* 1997). In the present study, thrips were common in the cracks formed by the clusters of flower buds in *B. malifolia* and *B. laevifolia*, but in *B. stellaris* they were exposed. This fact might have accounted for the high frequency of thrips molested by ants in *B. stellaris*, where they frequently dropped from the plant. Therefore, the presence of hiding places on *B. malifolia* and *B. laevifolia* might have conferred protection to thrips, which ultimately increased their population.

Other than dropping from plants, thrips were observed to raise their abdomens and release a small drop from their anus, which usually dispelled ants from the vicinity of the thrips. Ant repellents are commonplace in thrips and several compounds have been shown to act as a thrips defence mechanism against ants (Blum 1991; Blum *et al.* 1992; Suzuki *et al.* 2004). Both the abdomen-raising and the dropping behaviour were most frequently observed in *B. stellaris*, where the thrips were more exposed to ants. In the other *Banisteriopsis* (*malifolia* and *laevifolia*), thrips were also subjected to molestation by ants, but it was less common.

The mere presence of hiding places, however, is not enough to account for the striking differences in thrips abundance between experimental stems of plants. We have reason to believe that thrips gain a possible indirect benefit from living with ants, at least in *B. malifolia* and *B. laevifolia*. Individuals of *C. blandus* are very active and attack insects in general (see Guimarães *et al.* 2006). Such behaviour may be beneficial to *P. obscuricornis*, as ants may deter the thrips' own predators. In this case, the pressure exerted by ant molestation towards the thrips is counterbalanced by the net benefits of living in an enemy-free environment (e.g. Kaminski *et al.* 2010). In Malpighiaceae, *P. obscuricornis* is preyed upon by cursorial spiders, neuropterans and hemipterans, all of which are subjected to molestation by ants (Alves-Silva, unpubl. data, 2015). Therefore, the presence of hiding places and ants might have been beneficial to thrips in *B. malifolia* and *B. laevifolia*, which explains their increased abundance on ant-present stems. However, in *B. stellaris* thrips were frequently molested by ants, which can be related to their low abundance on ant-present stems.

### Thrips herbivory and plant fitness

Thrips caused necrosis on flower buds of all *Banisteriopsis* species (*laevifolia*, *malifolia* and *stellaris*). However, there was no significant relationship between

thrips abundance and bud herbivory (% of buds attacked per stem). This was expected because individuals of *P. obscuricornis* may occur alone or in aggregations of several individuals in flower buds, so relationships between both variables are unexpected. In this case, herbivory depends on the thrips presence rather than their abundance.

Flower buds attacked by thrips reached the flowering stage, indicating that herbivory did not significantly influence flower development. Both adults and larvae of *P. obscuricornis* fed on the external surface of bud tissues and the damage was mostly restricted to the oil glands. Such damage might affect pollinator visits because oil is a resource provided to *Banisteriopsis* pollinators (e.g. Centridini bees – Assunção *et al.* 2014). Nonetheless, cross-pollination by bees is performed before oil consumption (Gaglianone 2001), so damaged oil glands are assumed not to affect flower visits. The mere presence of herbivores, like *P. obscuricornis* in flowers, might also affect flower visits (e.g. Kessler & Halitschke 2009; Botto-Mahan *et al.* 2011). Nonetheless, *P. obscuricornis* do not occur in flowers at anthesis; therefore, a negative effect of thrips on flower performance, development, pollination and seed set can also be ruled out in *Banisteriopsis*.

### Fruit quality and dispersion

Despite the lack of influence on *Banisteriopsis* reproductive outputs, thrips damage on samaras was severe and fruits presented necrosis, distortions and deformations. Sucking herbivores may reduce fruit filling and seed set/size (Crawley 1989), but *P. obscuricornis* feed on the surface of samaras; thus, their influence is more aesthetic (distortion) and functional (dispersion). Samaras are dispersed by wind (Mirle & Burnham 1999); therefore, changes in their aerodynamic structure may affect how far from the mother plant samaras are taken (see Augspurger & Franson 1987). The influence of *P. obscuricornis* on fruit dispersion may negatively affect plant reproductive efforts, as according to Janzen–Connell's hypothesis, progeny near adults may suffer high mortality rates (see Janzen 1970; Connell 1971; Hyatt *et al.* 2003; Nathan & Casagrandi 2004; Hansen *et al.* 2008). In such a case, even if thrips do not affect the *Banisteriopsis* flowers or fruit set, their effects on fruit dispersion may restrict plant re-establishment. In *B. malifolia*, damaged fruits were found closer to the mother plant in comparison with uninjured fruits, but in general the dispersive capability of these samaras was low (approximately 100 cm radius away from the parents). Presumably, the wind speed at the time of the study (about 3 m/s) did not allow the flight of samaras over longer distances (see Greene & Johnson 1992; Nathan *et al.* 2001). Further studies taking into



account seedling establishment and survival may help to understand the influence of *P. obscuricornis* on plant reproduction efforts.

Our study showed that *C. blandus* failed to protect two of its extrafloral nectary plant partners (*B. malifolia* and *B. laevifolia*) against *P. obscuricornis*, as both the abundance of these insects and the herbivory on flower buds were higher on ant-present stems. In *B. stellaris*, the results were the opposite, and thrips were frequently molested and often dropped from ant-present stems, which also reveals the importance of considering plant architecture in these studies. Our study shows that ant-plant-herbivore interactions are highly conditional, and the costs and benefits to plants might depend on the herbivore's behaviour, plant characteristics and timing of herbivore damage. Because thrips in general are ubiquitous in vegetation it would be wise to examine in detail their temporal effect on plants, as they affect several stages of plant reproduction (DeBarr 1969; Kirk 1984; Childers *et al.* 1990; Tamo *et al.* 1993; Milne & Walter 2000).

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Appendix S1.** Phenology of three *Banisteriopsis* species in a Brazilian savanna.