

The effect of a flower-dwelling predator on a specialized pollination system

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Plant species vary greatly in the number and diversity of floral visitors with which they interact. Even so, pollination ecologists have focused mainly on direct pairwise interactions (mutualists), overlooking indirect effects produced by different agents, such as floral larcenists and flower-dwelling predators. In our study area in Brazil, the pollen-flowers of *Chamaecrista ramosa* (Fabaceae) harbour *Misumenops* sp. (Thomisidae) spiders, a flower-dwelling predator with unknown signalling strategy. We measured the effect of *Misumenops* on the foraging behaviour of three bee species, one behaving as a pollen robber (*Trigona spinipes*) and two as pollinators (*Xylocopa ordinaria* and *X. hirsutissima*), and the consequences for plant fitness. The presence of *Misumenops* reduced the frequency of *Trigona* bee visits and increased the proportion of undamaged anthers, and thus pollen available to *Xylocopa* bees, which seemed unaffected by spider presence. However, spider presence (detectable by both *Trigona* and *Xylocopa* bees through achromatic and chromatic contrasts) had no effect on fruit and seed set when compared to flowers without spiders. Thus, the apparently antagonistic interaction between *Trigona* and flowers can represent a commensalism, modulated by differences between pollinators and larcenists regarding the window of foraging activity, as well as regarding foraging efficiency. The results also indicate that the quantitative impact of predators of floral visitor on plant fitness will be highly dependent on context in species with specialized pollination systems.

ADDITIONAL KEYWORDS: female fitness – flower-dwelling predator – indirect effects – male fitness – pollen-flower – robber.

INTRODUCTION

In specialized pollination systems, key floral traits modulate the reproductive success of plants and their mutualists and, at the same time, hinder (mechanically and/or sensorially) the access of illegitimate pollinators to resources (Johnson & Steiner, 1997; Rodríguez-Gironés & Santamaría, 2007; Muchhala & Thomson, 2009; Bergamo *et al.*, 2016; Córdoba & Cocucci, 2017). Yet, these floral traits are not always sufficient to deter the action of floral larcenists (*sensu* Inouye, 1980). For instance, almost all flowers having tubular corollas or nectar

spurs, which prevent short-tongued pollinators from legitimately accessing the nectar, experience some sort of robbing (Irwin *et al.*, 2010). However, plants might be able to mitigate this negative effect by continuously producing nectar to maintain pollinator attractiveness to some degree, compensating for the nectar lost to robbing (Ye *et al.*, 2017). In contrast to nectar, pollen cannot be replenished. Pollen larceny in pollen-flowers (*sensu* Endress, 1996) has considerable potential to negatively affect the average reproductive success of plants, probably reducing male function, affecting pollinator attraction and visitation rates (Lau & Galloway, 2004; Hargreaves *et al.*, 2010; Solís-Montero *et al.*, 2015). While several studies have explored the causes and consequences of nectar-robbers on animal-pollinated plants (Irwin *et al.*, 2010), relatively little

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is known about the outcomes of such interactions in pollen-flowers.

Although floral larceny may have important ecological and evolutionary effects on host plants, it is not the only biotic factor that potentially affects plant fitness. Flowers are predictable places for finding relatively high concentrations of prey, because they have evolved to attract visitors (Higginson *et al.*, 2010). Predators of floral visitors can have negative effects on plant reproduction by deterring mutualists (Suttle, 2003; Knight *et al.*, 2006; Antiqueira & Romero, 2016), but their net effect will be context-dependent. If predators and larcenists are scarce, few plant individuals will be affected by their presence, resulting in a weak selection force at the population level (Rodríguez-Gironés *et al.*, 2013). If both mutualists and larcenists are at high density, flower-dwelling predators can have a strong positive effect on plant fitness, provided that larcenists avoid predator-dwelling plants (Knight *et al.*, 2006; Higginson *et al.*, 2010; Romero & Koricheva, 2011; González *et al.*, 2013). If predators deter pollinators, the cost will be low for the plant if pollinator numbers (or diversity) are high, but high if mutualists are relatively scarce (Dukas, 2005; Higginson *et al.*, 2010).

When using flowers as ambushing substrate, predatory spiders adopt at least two different strategies: crypsis, through background matching, and lure, through deceptive signals (Heiling *et al.*, 2003; Defrize *et al.*, 2010; Gawryszewski *et al.*, 2017). In the former, spiders usually match the colour of their floral background, while in the latter, spiders produce visual signals to increase their attractiveness to floral visitors (Heiling & Herberstein, 2004). In both cases, the sensory ability of receivers is involved, and the effects of predation risk vary among floral visitor species (Dukas & Morse, 2003; Heiling & Herberstein, 2004; Llandres *et al.*, 2012; González *et al.*, 2013).

Mutualists and larcenists are taxonomically and behaviourally diverse and usually vary in their impacts on plant fitness along a continuum, from positive to negative, direct to indirect (Strauss & Irwin, 2004). Considering pollen-flower species with poricidal anthers as a specialized pollination system, a mismatch between visitors and floral morphology would increase the relevance of predators in removing, at least, small larcenists from the plant population, with consequences for plant fitness (Dukas & Morse, 2005; Rodríguez-Gironés *et al.*, 2013). In this study, we attempt to measure the effect of a flower-dwelling predator (*Misumenops* sp.) using *Chamaecrista ramosa* (Vogel) H.S. Irwin & Barneby var. *ramosa* (Fabaceae) flowers as hunting platforms, on the floral visitation behaviour of three species of Apidae bees: one behaving as pollen robber and two as pollinators.

We assess the consequence for plant fitness. We specifically investigated (1) how the mutualistic and the possible antagonistic interactions with a plant species, holding a specialized pollination system, can be affected by the presence of a flower-dwelling predator, and (2) how the product of both interactions influences plant fitness, measured as male and female success. Because predators can exploit sensory signals mediated between plants and their pollinators to attract prey (Heiling *et al.*, 2006), we also observed the position spiders occupied in flowers and whether it was related to the exploitation of visual floral signal and to the ability of bees to perceive spiders against different floral parts.

MATERIAL AND METHODS

STUDY AREA

We conducted the study at the Environmental Protection Area of Maricá (Brazilian acronym: APA Maricá). APA Maricá is a managed-resource protected area of *c.* 8.3 km², located in coastal south-eastern Brazil (22°52′–22°54′S and 42°48′–42°54′W; Pereira *et al.*, 2001; Loureiro *et al.*, 2010). The vegetation is dominated by shrubs and small trees, organized in island-like patches of different sizes encircled by open sand areas (Silva *et al.*, 2001). The region has a tropical climate (type Aw of the Köppen system), with average annual temperature varying between 22 and 24 °C and average annual precipitation between 1000 and 1350 mm (Franco *et al.*, 1984).

STUDY SYSTEM

Chamaecrista ramosa is a monomorphic, enantiostylous, self-compatible (although it cannot spontaneously self-pollinate) subshrub species, with a peak of flowering around September (Oliveira-Rebouças & Gimenes, 2011; Almeida *et al.*, 2013). Flowers are pentamerous (with one falcate petal directed towards the anthers), heterantherous (two sets of stamens: one set with three large anthers and one with seven small anthers), with poricidal anthers (Pinheiro *et al.*, 1988; Almeida *et al.*, 2013). In our study area, anthesis started around 06:30 h and finished around 11:30 h. Individuals were found in clumps, in vegetation clearings, forming small islands. Due to the difficulty in separating individuals within a clump, we selected 42 different clumps, of similar size, between September and October 2014 and 2016. The distance between selected clumps ranged from *c.* 50 cm to 200 m. To estimate the abundance of *Misumenops* sp. (Thomisidae) spiders on the different clumps, we haphazardly selected particular clumps

and checked their flowers, recording the presence/absence of spiders.

EXPERIMENTAL APPROACH

We used a pairwise design to examine the effect of the presence of spiders on floral visitor behaviour, and on male (using anther damage as a proxy; $N = 42$ flowers) and female (fruit and seed set) fitness components ($N = 141$ flowers). From 07:00 to 12:00 h we visited clumps, selecting in each one two newly opened flowers, one with and the other without a spider. We thus ensured that spiders had the chance to select the most profitable flower. We observed both flowers for 20 min, and then moved to the next clump where a spider had been detected. During the observation period, we recorded every floral visitor approaching and landing on a flower, its identity, time spent on the flower and behaviour (positioning and movements on and in flowers). Visitors were classified either as pollinators, when they were of the correct size and contacted the stigma, or larcenists, when they collected pollen from flowers but did not contact the stigma. To measure body size and identify visitors and spider species, we collected individuals from flowers in the field. Using calipers, we measured body size in freshly dead spiders based on head–abdomen length, while for bees this was based on intertegular distance (Cane, 1987). Individuals were subsequently deposited in the entomological collection of the Laboratory Network at the Rio de Janeiro Botanical Garden. To determine the female and male components of fitness, we selected flowers naturally harbouring a spider at the beginning of floral aperture (others than those of focal observations) and glued the spiders to the flower (maintaining their initial hunting position). We used a small amount of commercial cyanoacrylate on their ventral abdomen to later account for the effect of the presence ($N = 59$ flowers) and absence ($N = 82$ flowers) of spiders in deterring larcenists (male component), as well as on pollination success (female component). Daily, at the end of anthesis, we counted the number of damaged/undamaged anthers resulting from treatments. We considered as damaged anthers showing clear signs of destruction. We did not account for damage variation between the different sets of anthers. We estimated fruit and seed set 2 months after treatments.

To test which ambush strategy *Misumenops* sp. spiders were using when on *C. ramosa* flowers, we measured the spectral reflectance of spiders and floral structures, using a USB2000+UV-VIS spectrometer, with a balanced deuterium tungsten source (DH-2000-BAL, Ocean Optics Inc., Dunedin, FL, USA), positioning probe and light source at an angle of 45°. The spectrometer was calibrated

with a standard white (BaSO_4) and blocking light input as a black standard. We limited the readouts to wavelengths from 300 to 700 nm, a range that encompasses the visible spectrum of most Hymenoptera (Peitsch *et al.*, 1992).

From each sampled spider ($N = 8$, four females and four males) we took three spectral measurements of the abdomen (dorsal area); for each flower ($N = 5$) we took three measurements of anthers, and falcate and normal petals. We then averaged measurements to calculate the photoreceptor excitation (E) values using the hexagon colour vision model (Chittka, 1992), considering standard daylight illumination (D65), and green (Chittka & Kevan, 2005) and grey (homogeneous reflectance of 20%) backgrounds, to check for data robustness. The E -values reflect the relative excitation (physiological receptor voltage signals) of each photoreceptor in the visual system of floral visitors when looking at the stimulus, allowing us to calculate the contrast produced by a spider, at close (chromatic) and long (achromatic) distances, when against the different floral structures (for details of calculations see, Telles & Rodríguez-Gironés, 2015). We used the photoreceptor peaks of *Trigona spinipes* and *Xylocopa brasiliatorum* (Linnaeus, 1767) as surrogates for our bee species (Peitsch *et al.*, 1992), and constructed spectral sensitivity curves (Supporting Information, Online Resource 1), used for calculation of E -values, with the *sensmodel* function, from the *pavo* package (Maia *et al.*, 2013).

STATISTICAL ANALYSES

To analyse the effect of the presence of spiders on the behaviour of approaching bees, we fitted a generalized linear mixed model (GLMM) considering the proportion of landings after approaching a flower as the response variable, and treatment (spider presence/absence) as the explanatory variable. To test whether bees that landed on a flower in the presence of a spider spent the same amount of time as when in the absence of spiders, we fitted a linear mixed model (LMM) considering the duration of flower handling (in seconds) as the response variable and treatment as the explanatory variable. We \log_e -transformed the duration of flower handling to better fit the assumption of normality on the LMM test.

To analyse the effect of the presence of spiders on plant fitness, measured based on estimated male and female components, we fitted the data to both a GLMM and an LMM, according to the response variable. We fitted a GLMM using the occurrence of damaged/undamaged anthers as a proxy of male success (response variable), and treatment (presence/absence of spider) as the explanatory variable. We analysed female success by fitting a GLMM for fruit set and an

LMM for seed set (response variables) resulting from treatments (absence/presence of spiders; explanatory variable).

We used clump as the random term for all mixed models. For GLMMs, a binomial distribution and logit link function were assumed. We used the functions *glmer* and *lmer* for running the GLMMs and LMMs, respectively, both from the *lme4* package (Bates et al., 2015). We applied type II sum of squares for all analyses, by means of the *Anova* function from the *car* package (Fox & Weisberg, 2011). Following Zuur et al. (2009) and Harrison et al. (2018), we checked for heteroscedasticity for both fixed and random terms on models by means of residual plots. To analyse the assumption of normality of deviations of the conditional means of the random effects from the global intercept, the *ranef* function from the *lme4* package was used (Bates et al., 2015). Model coefficients are presented in Online Resource 2. All analyses were performed using the 3.4.0 version of the R software (R Core Team, 2016).

RESULTS

SPIDER INCIDENCE AND BEHAVIOUR ON *C. RAMOSA* FLOWERS

Spider incidence on flowers was high, with 87% of the haphazardly sampled flowers ($N = 120$) being used as a hunting platform. We found spiders on different floral parts, from sepals to anthers, moving often when on flowers during focal observations (Fig. 1). Sometimes, but not often, when a bee approached a flower, spiders moved to a less exposed site between overlapping petals or under the calyx. After a period on a flower that did not receive any visit, spiders usually moved between floral structures or even abandoned the flower, picking another one from the same clump. We never observed movements between clumps.

FLOWER VISITORS AND THEIR BEHAVIOUR IN THE PRESENCE AND ABSENCE OF SPIDERS

During focal observations (70 h), *C. ramosa* received visits of three species: *Xylocopa ordinaria* and

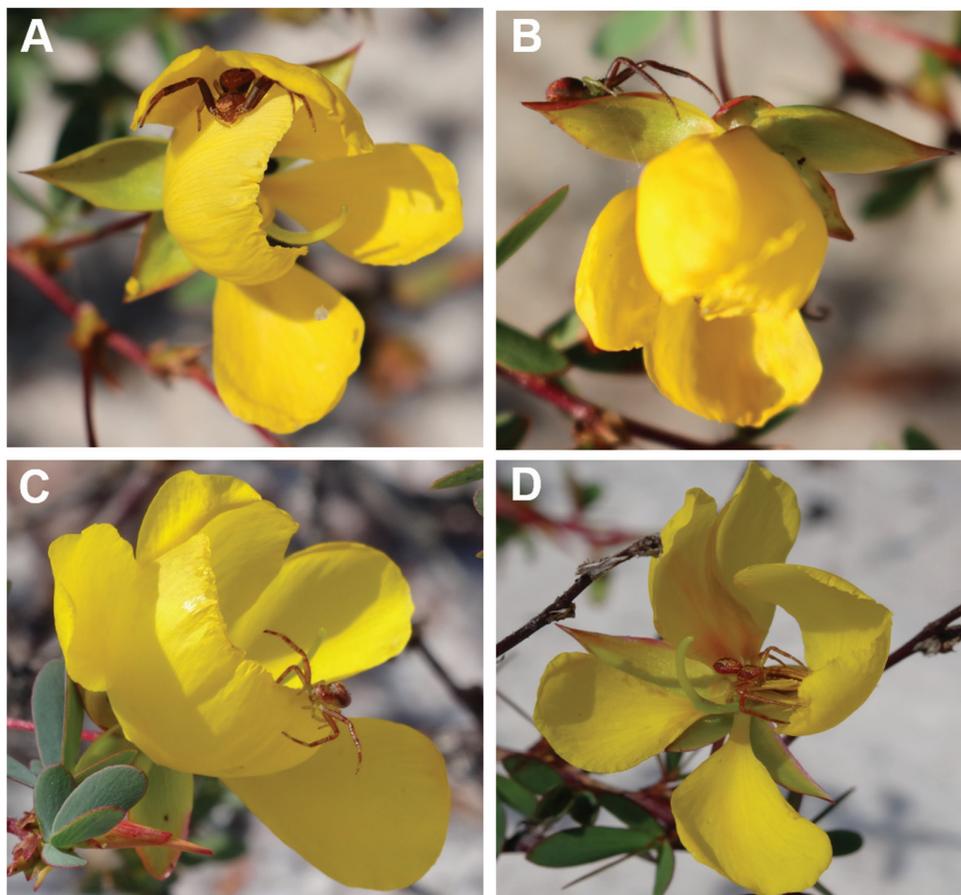


Figure 1. *Misumenops* sp. hunting spots on *Chamaecrista ramosa* flowers. Spiders did not present a specific hunting position, occupying different floral structures along the period of focal observations.

X. hirsutissima, and *Trigona spinipes* (hereafter *Trigona*). The *Xylocopa* bees show similar flower visiting behaviour and morphology (size and colour), so we could not discriminate between them in the field. They are thus termed hereafter as *Xylocopa* bees. *Xylocopa* bees behaved as legitimate pollinators. Their body size fitted flower morphology, and they positioned themselves correctly in the flowers, vibrating the anthers for a short period of time (± 3 s). *Trigona*, by contrast, acted as pollen robbers, accessing the pollen grains by cutting off the anthers (Online Resource 3). The frequency of visits varied between pollinators and robbers, with *Trigona* bees representing more than 94% of visits during focal observations (total visits = 188). *Xylocopa* bees were usually seen in the early morning (07:30–9:30 h), while *Trigona* were seen over the entire lifespan of flowers, but with an increased frequency from 08:00 to 10:00 h.

Despite the few encounters observed and thus small sample size ($N = 4$), our observations suggest that *Xylocopa* bees are unaffected by the presence of spiders on flowers, and vice versa. Nonetheless, when approaching a flower, *Xylocopa* bees hovered in front of the centre of the flower, where anthers are presented. This scanning behaviour was followed by a landing decision. Flowers with damaged anthers were avoided by *Xylocopa* bees ($N = 3$). Because visits of *Xylocopa* bees to focal flowers were few and erratic (less than 6% of total visits), we focused on the floral robber, *Trigona* bees, when visiting flowers in the presence and absence of spiders.

Trigona bees also scanned flowers. Unlike *Xylocopa*, they did not position themselves in front of the flower, but flew around it in different directions, exploring the floral area before deciding on whether to land (Online Resource 4). The presence of a spider on a flower influenced the landing decision of *Trigona* bees. The proportion of landings after approaching and scanning a flower with (38 out of 104) and without (134 out of 158) spider differed significantly ($\chi^2 = 30.5$, d.f. = 1, $P < 0.0001$, Fig. 2). In most cases, after inspection, *Trigona* bees avoided flowers with spiders. However, whenever *Trigona* bees landed on spider-harboured flowers, flower handling was shorter than when the spider was absent ($\chi^2 = 5.2$, d.f. = 1, $P = 0.02$; Fig. 3).

BODY SIZE AND PREDATION

Body size varied between *Xylocopa* (intertegular distance: *X. ordinaria* = 0.71 cm; *X. hirsutissima* = 0.85 cm; $N = 1$) and *Trigona* bees (intertegular size = 0.18 ± 0.003 cm; $N = 5$), as well as between male (head–abdomen = 0.38 ± 0.03 ; $N = 4$) and female (head–abdomen = 0.58 ± 0.10 ; $N = 6$) spiders. During focal observations we saw

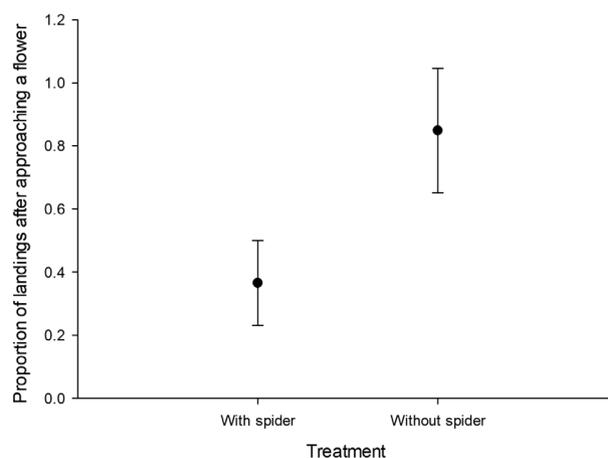


Figure 2. Proportion of *Trigona* bees landing on flowers, after approaching, in the presence ($N = 36$) or absence ($N = 32$) of spiders. Bars denote standard errors.

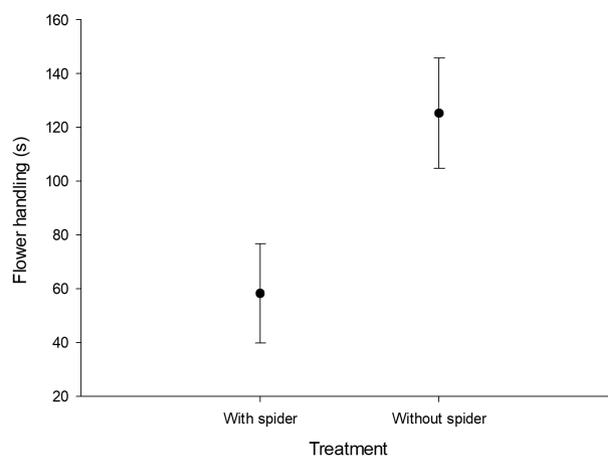


Figure 3. Flower handling (seconds) of *Trigona* bees in the presence ($N = 23$) and absence ($N = 32$) of spiders. Bars denote standard errors.

nine predation attempts and only one capture, all of *Trigona* bees. During 12 days of active search, we counted a total of 37 *Trigona* bees captured by spiders. After a successful capture, spiders would leave the flower with the prey, hiding between the sepals, stems and leaves (Fig. 4). Given the low visitation rate, we never observed any attempt or effective capture of *Xylocopa* bees in the field in both focal and active search.

EFFECT OF SPIDERS ON ANTHOR DAMAGE, FRUIT AND SEED SET

Anther damage by *Trigona* bees was negatively affected by the presence of spiders ($\chi^2 = 4.8$, d.f. = 1, $P = 0.03$; Fig. 5). The fruits formed (with spider = 25

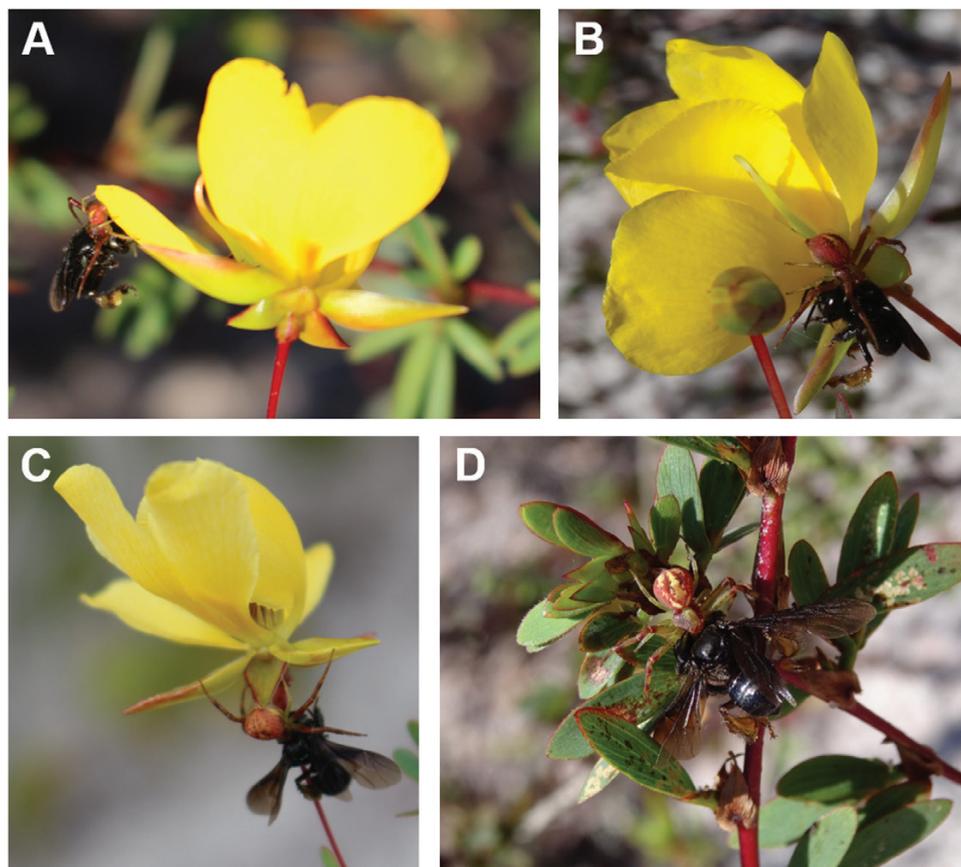


Figure 4. *Misumenops* with their prey, *Trigona* bees. After a successful capture, spiders moved to a less exposed site (B–D).

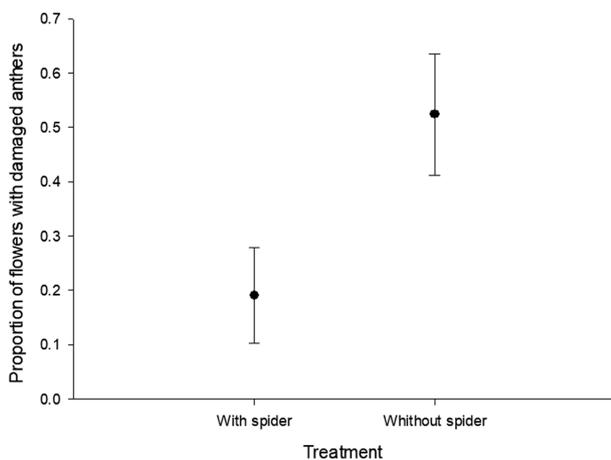


Figure 5. Proportion of flowers with anthers predated by *Trigona* bees in the presence ($N = 21$) and absence ($N = 21$) of spider on flowers. Bars denote standard errors.

out of 59; without spider = 38 out of 82) did not differ between treatments ($\chi^2 = 0.16$, d.f. = 1, $P = 0.75$), and nor did the number of seeds (with spider = 284; without spider = 396; $\chi^2 = 1.61$, d.f. = 1, $P = 0.21$; Fig. 6).

SPECTRAL REFLECTANCE ANALYSES

Analyses of spectral reflectance of floral structures showed that *C. ramosa* is a yellow UV-reflecting species, while spiders are mostly UV-absorbing, presenting the same pattern for males and females. We never observed any other spider colour morph on *Chamaecrista* flowers, and all sampled and observed spiders presented the same pattern to us, in agreement with the results from spectral reflectance curves. Thus, we averaged the spectral reflectance of males and females (Fig. 7). Overall, the colour matching between spiders and the different floral structures (anthers, normal and falcate petals) they used as hunting platforms was poor in both backgrounds (Table 1). Colour contrast produced by spiders against the different floral structures was above the discrimination threshold reported for Hymenoptera (Théry & Casas, 2002; Dyer & Chittka, 2004). Chromatic and achromatic contrast values were similar for both bee species (Table 1).

We also calculated the chromatic and achromatic contrasts produced between the different petals and stamens, given *Xylocopa* behaviour. Achromatic values close to 1 indicate no difference between structures, and thus detection of stamens at long distances is

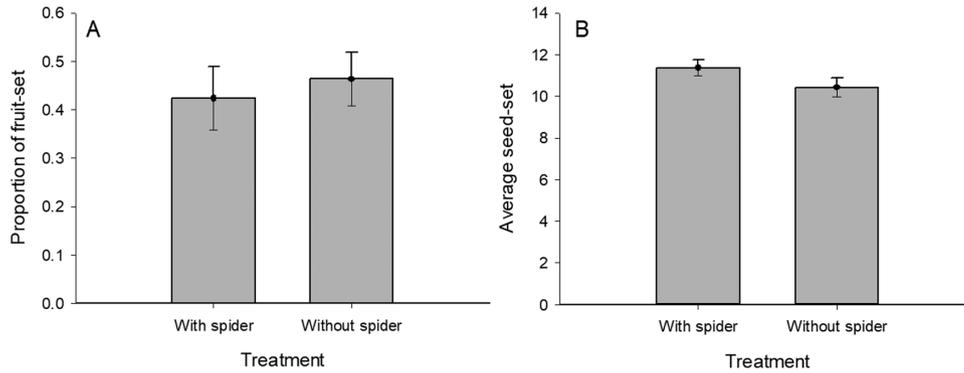


Figure 6. Proportion of (A) fruits ($N_{\text{with}} = 25$ out of 59; $N_{\text{without}} = 38$ out of 82) and (B) average number of seeds ($N_{\text{with}} = 284$; $N_{\text{without}} = 396$) from flowers with and without spiders. Bars denote standard errors.

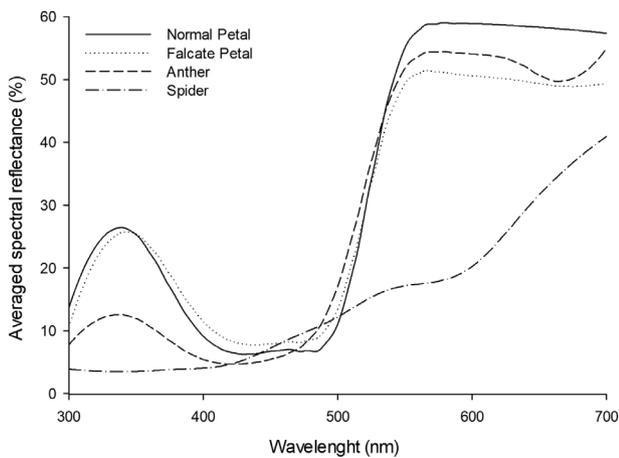


Figure 7. Average spectral reflectance of floral structures and spiders. Normal and falcate petals have similar colours, while anthers reflect less in the UV range and spiders are mostly UV-absorbing yellow.

unlikely considering these values (Table 1). At a close range, the chromatic contrast produced between stamens and petals was enough to allow both *Xylocopa* and *Trigona* bees to notice the presence or absence of stamens (Table 1).

DISCUSSION

Chamaecrista ramosa flowers seem to benefit indirectly from the presence of spiders when the latter affect the behaviour of *Trigona* bees (avoidance and reduced duration of flower handling). *Trigona* bees reduce the number of male gametes and flower attractiveness to pollinators, by chewing anthers. Despite the few observations, our result suggests that spider presence could be neutral to *Xylocopa* bees, which in turn seemed to be attracted to intact flowers, an idea that remains to be tested. Overall, predators had a neutral effect on

the female component of plant fitness. We did not find differences between treatments on fruit and seed set in the presence and absence of spiders, while the male component, measured as the number of undamaged anthers, did differ. However, whether the scent of the glue used by us affected these results is unknown. To what extent the effect of spiders on the male component contributed to plant fitness is uncertain. We define the apparently antagonistic interaction as a commensalism, modulated by differences in the foraging window of activity between pollinators and larcenists, as well as the efficiency of foragers. Regarding body colouration, spiders did not present a colour match with floral structures: detectability of spiders on flowers was achieved in the visual systems of both bees through achromatic and chromatic contrasts.

THE IMPACT OF A FLOWER-DWELLING PREDATOR IN SPECIALIZED POLLINATION SYSTEMS

The effect of ambushing predators on floral visitors and its consequences for plant reproduction seems to be modulated not only by the density of agents, as stated by Higginson (2010), but also by the specificity of the system. In specialized pollination systems, such as those presented by pollen-flowers with poricidal anthers and complex floral morphologies, the effect can vary according to visitor identity and behaviour (legitimate or illegitimate), usually determined by a mismatch between floral structures and visitor body size (Solís-Montero *et al.*, 2015). For instance, regarding one of those systems (*Melastoma malabathricum* L., Melastomataceae), González *et al.* (2013) found a positive indirect effect of weaver ants (*Oecophylla smaragdina* Fabricius, 1775, Formicidae) on plant fitness. Ants removed small and less effective visitors, attracting the main pollinator (*Xylocopa* spp.) and increasing the female component of individuals. In contrast, in a pollen-flower system free of bee predators

Table 1. Chromatic and achromatic contrasts produced by spiders and floral structures on the eyes of *Trigona* and *Xylocopa* bees when their receptors are habituated to two different backgrounds (green and grey)

Bee species	Contrast produced	Green contrast		Colour distance	
		Green	Grey	Green	Grey
<i>Trigona</i>	Spiders × falcate petal	0.77	0.71	0.29	0.29
	Spider × normal petals	0.75	0.69	0.32	0.31
	Spider × stamens	0.75	0.70	0.25	0.21
	Falcate petal × stamens	0.98	0.98	0.12	0.17
	Normal petals × stamens	1.00	1.00	0.11	0.16
<i>Xylocopa</i>	Spiders × falcate petal	0.76	0.71	0.29	0.29
	Spider × normal petals	0.75	0.69	0.32	0.31
	Spider × stamens	0.75	0.70	0.25	0.21
	Falcate petal × stamens	0.98	0.98	0.12	0.17
	Normal petals × stamens	1.00	1.00	0.11	0.16

(*Eriocnema fulva* Naudin, Melastomataceae), robbery by *Trigona fulviventris* (Guérin, 1837) bees had both direct (reduced fecundity as a result of damage to anthers and stigma) and indirect (pollen consumption, reduced flower attractiveness to pollinators) consequences for the male and female components, reducing fruit set to low rates (6.9%) and putting the survival of the species at risk (Rego *et al.*, 2018).

WHY NOT BE CRYPTIC?

According to our visual model calculations, the detection of *Misumenops* spiders by the different bee species should be straightforward, independent of the floral structure used by spiders. Nevertheless, the results of detectability and discrimination provided by visual models should be interpreted with caution: being able to discriminate between two colours does not necessarily imply that bees detect spiders and identify them as predators. Colour match/crypsis by spiders does not seem to increase prey capture of flower-dwelling species, and there is no evidence of profit from adapting the body colouration to that of flowers/inflorences (Brechtbühl *et al.*, 2009; Llandres & Rodríguez-Gironés, 2011). Instead, a widespread ‘spider avoidance hypothesis’ is more consistent with the behaviour of different prey communities. The level of avoidance varies according to the identity of flower visitors, particularly so for species that are more vulnerable to predation (Brechtbühl *et al.*, 2009). Regarding the relationship between body size and predation risk, behavioural avoidance towards spiders was prominent in *Trigona* bees (Online Resource 4). For *Xylocopa* bees, a different behaviour, termed ‘indifference to spiders’, seems to apply. This behaviour has been reported for large visitors acting as pollinators in several systems (Brechtbühl *et al.*, 2009, 2010; González *et al.*, 2013).

In a detailed analysis of prey capture sequence by a crab spider, Brechtbühl *et al.* (2011) showed that spiders often refused to attack apparently suitable and frequently encountered preys. Their explanation for this was based on the high abundance of prey, those on which spiders gathered more experience (Brechtbühl *et al.*, 2011). The over-abundance of *Trigona* bees in our study system could have reduced the spiders’ need to take every opportunity to catch bees, explaining the observed pattern. Because our predation data were mostly based on daily walking searches at the end of focal observations, we cannot establish the predation success rate after an attempt for spiders, nor how many spiders in the community fed during 1 day. Thus, our observations were possibly mainly based on recent hunting events, when we observed the presence of bees hanging from the spider chelicera near the flower and between branches.

FLOWER SIGNAL VERSUS REWARD

Obligate flower visitors, such as bees, have evolved to effectively forage on flowers, including the mechanisms to bypass morphological barriers, as well as to decide on the quality of a reward, based on previous experience (Nicholls & Hempel De Ibarra, 2016). In this context, the sensory and cognitive abilities of pollinators are important in driving subsequent foraging behaviour. In pollen-flowers, the anthers themselves have signalling and reward functions, and their absence modulates floral visitor behaviour (Luo *et al.*, 2008; Papaj *et al.*, 2017). In the field, we observed landing avoidance behaviour of *Xylocopa* bees apparently driven by the presence of damaged anthers. Because *Xylocopa* visits during focal hours were sporadic, we were unable to quantify this behaviour, but the same avoidance has been previously documented (Viana & Kleinert, 2006).

The specific sensory information (e.g. visual, olfactory or both) used by *Xylocopa* and *Trigona* bees to detect anthers or spiders on flowers is unknown. Although we have made inferences about the ability of the bees to visually detect these, whether they specifically recognize anthers and spiders by contrasting them against the different backgrounds, by chemical signals or by a combination of both remains to be tested.

FLORAL VISITOR FORAGING ACTIVITY: DO TEMPORAL VARIATION AND EFFICACY EXPLAIN FEMALE FITNESS?

Trigona bees acting as robbers usually have negative effects on plant fitness (Renner, 1983; Maloof & Inouye, 2000; Rego *et al.*, 2018). In our study, fruit and seed set from treatments with and without spiders did not differ, and were similar to those found in a different restinga area, with reduced pollen theft/robbery (Almeida *et al.*, 2013). The absence of any difference on female success could be explained by temporal segregation of foraging activity, related to resource competition, body size and ambient temperature (Willmer & Corbet, 1981; Pereboom & Biesmeijer, 2003; Willmer & Stone, 2004; Maia-Silva *et al.*, 2014).

Direct competition for pollen seems to lead to different activity periods, through the establishment of distinct activity peaks, among visitor species (Franco *et al.*, 2011). Our focal observations were concentrated during the entire lifespan of flowers. For instance, flowers of *Chamaecrista desvauxii*, a closely related species (Rando, 2014), started to receive visits by pollinators at the bud stage (Nogueira *et al.*, 2018). In this species, the stigma is receptive and pollen grains are viable before flower opening. Both open flowers and buds exposed to pollinators had similar fruit set, indicating that pollination can occur before flowers are fully open. Given that *C. ramosa* constitutes the main pollen resource for carpenter bees in restinga areas (Viana *et al.*, 2002; Figueiredo *et al.*, 2013; Almeida *et al.*, 2013), and that pollen robbery by *T. spinipes* seems to be more prominent in flowers of *C. ramosa* (Viana & Kleinert, 2006), it is possible that *Xylocopa* bees visit flowers before they are fully open, explaining the erratic visits observed during focal hours and the absence of differences on female success (breaking-bud pollination *sensu* Yamaji & Ohsawa, 2015). It is generally accepted that larger bees, such as *Xylocopa*, can be active at lower ambient temperatures (such as during the early morning) than smaller bees (Willmer & Stone, 2004).

FINAL REMARKS

In the literature, there is a bias towards studies of floral antagonists of plants presenting nectar as a reward. Our knowledge of the outcome of multiple-species

interactions involving pollen-flower species, larcenists (thieves and robbers) and mutualists (pollinators) remains poorly understood. Likewise, studies of flower-dwelling predators usually report their negative effects on mutualists. Nevertheless, these studies are generally limited to investigations of predation rates, and few have evaluated the different components of plant fitness, apart from being performed with plants considered to be generalists (e.g. Asteraceae species), receiving visits of many potential pollinators, as well as prey species.

Our results indicate that the by-product of interactions between *Trigona* and *C. ramosa* can be interpreted as commensalism. Although plant populations are thought to diverge over time in response to directional selection, the interaction described here between larcenists and flowers seems unlikely to produce impacts on plant reproduction, for several reasons. From an evolutionary perspective, plants could benefit from small shifts to reduce the impact of larcenists, such as adjusting the receptive floral phase according to temporal variation in the pollination niche. From an ecological perspective (*ad momentum*), we presume that the impact of larcenists and flower-dwelling predators can assume different directions, being highly context-dependent, having the potential to vary across populations, and years. Furthermore, both the ecological and the evolutionary effects of pollen larceny remain largely unexplored.

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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:

Electronic Supplemental Material 1

Online Resource 1. Spectral sensitivity of *Trigona spinipes* (solid line) and *Xylocopa brasiliatorum* (dashed line) used for calculation of chromatic and achromatic parameters in the colour hexagon model (Chittka, 1992). Sensitivities were reconstructed according to the relative spectral positions of photoreceptor peaks, using the *sensmodel* function from the PAVO package (Maia *et al.*, 2013), in the software R (R Core Team, 2013). Maximum sensitivities for *T. spinipes* = UV: 340; blue: 440; green: 536. Maximum sensitivities for *X. brasiliatorum* = UV: 360; blue: 428; green: 544 (Peitsch *et al.*, 1992).

Electronic Supplemental Material 2

Online Resource 2

Table S1. Coefficient estimates from the generalized linear mixed model of the relationship between the approaching/landing decision of *Trigona* bees and the presence/absence of spiders.

Table S2. Coefficient estimates from the linear mixed model of the relationship between the time spent by *Trigona* bees when visiting a flower and the presence/absence of spiders.

Table S3. Coefficient estimates from the generalized linear mixed model of the relationship between male success (anther damaged/undamaged) and the presence/absence of spiders.

Table S4. Coefficient estimates from the generalized linear mixed model of the relationship between fruit set and the presence/absence of spiders.

Table S5. Coefficient estimates from the linear mixed model of the relationship between seed set and the presence/absence of spiders.

Electronic Supplemental Material 3

Online Resource 3. Anthers of *Chamaecrista ramosa* flowers (A) before and (B, C) after predation by *Trigona* bees.