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Plant glandular trichomes mediate protective mutualism in a spider–plant system

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Abstract. 1. Although several species of *Peucetia* (Oxyopidae) live strictly in association with plants bearing glandular trichomes worldwide, to date little is known about whether these associations are mutualistic.

2. In this study we manipulated the presence of *Peucetia flava* on the glandular plant *Rhynchanthera dichotoma* in the rainy and post-rain season, to test the strength of its effects on leaf, bud, and flower damage and plant reproductive output. In addition, we ran independent field experiments to examine whether these sticky structures improve spider fidelity to plants.

3. *Peucetia* suppressed some species of foliar phytophages, but not others. Although spiders have reduced levels of leaf herbivory, this phenomenon was temporally conditional, i.e. occurred only in the post-rain but not in the rainy season. Floral herbivory was also reduced in the presence of spiders, but these predators did not affect plant fitness components.

4. Plants that had their glandular trichomes removed retained fewer insects than those bearing such structures. Spiders remained longer on plants with glandular trichomes than on plants in which these structures had been removed. Isotopic analyses showed that spiders that fed on live and dead labelled flies adhered to the glandular hairs in similar proportions.

5. Spiders incurred no costs to the plants, but can potentially increase individual plant fitness by reducing damage to reproductive tissues. Temporal conditionality probably occurred because plant productivity exceeded herbivore consumption, thus dampening top-down effects. Specialisation to live on glandular plants may have favoured scavenging behaviour in *Peucetia*, possibly an adaptation to periods of food scarcity.

Key words. Conditional outcomes, food web, glandular trichomes, host fidelity, Melastomataceae, Neotropical region, *Peucetia*, protective mutualism, spider–plant interaction, swamp, top-down and bottom-up forces.

Introduction

Mutualisms are interspecific interactions that benefit both partners, maximising their net fitness (Bronstein, 1994a). Mutualisms are ubiquitous in nature (Janzen, 1985) and play a central role in all ecosystems (Boucher *et al*., 1982; Thompson, 1994; Bronstein, 2001a; Stachowicz, 2001). Every organism on Earth is likely associated with one or more mutualistic partners (Bronstein *et al*., 2006). Mutualism can be best viewed as a reciprocal exploitation between partners (Janzen, 1985; Bronstein, 2001b; Yu, 2001). As a consequence, conflicts of interest arise between partners and, if considered in terms of cost–benefit, the responses of these associations are highly dynamic (Bronstein, 1994b). For instance, the direction (i.e. antagonism to mutualism) and strength of these responses, as well as the mechanisms that promote persistence and collapse of mutualisms, can vary in space and time (Bronstein, 1994b;

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Thompson & Cunningham, 2002; Billick & Tonkel, 2003), a phenomenon called conditional outcome.

The main examples of mutualism between animals and plants are represented by interactions between seed-producing plants and pollinators or seed dispersers, as well as ants and mites acting as plant bodyguards (Herrera & Pellmyr, 2002). However, in animal–plant interactions, most studies examine the benefits from the viewpoint of only one species of the interaction; plants are often more studied than their animal partners (Bronstein, 1994a). Spiders are very diverse and are abundant predators on vegetation (Wise, 1993; Foelix, 1996) and can suppress herbivores. Thus, they are considered good biological control agents (Riechert & Lockley, 1984). Moreover, they are influenced by variations in physical and architectural plant traits (Langellotto & Denno, 2004; Romero & Vasconcellos-Neto, 2005a). In addition, some spiders may even feed on plants, consuming pollen, nectar, or food bodies (e.g. Vogelei & Greissl, 1989; Meehan *et al*., 2009), while others maintain specific associations with plant species (e.g. Romero & Vasconcellos-Neto, 2005a; Romero, 2006; Vasconcellos-Neto *et al*., 2007). These independent studies have shown that spiders can interact with plants in diverse ways, and that some of these associations are predictable over space and time; however to date, little is known about mutualistic interactions in spider–plant systems (Whitney, 2004; Romero *et al*., 2006, 2008). This may be explained because most of the studies that have shown some benefit in spider–plant interactions (e.g. Louda, 1982; Ruhren & Handel, 1999; Romero & Vasconcellos-Neto, 2004) analysed only part of the systems. The scarcity of studies on spider–plant mutualisms can also be explained by the wide diet of spiders, which prey on both pollinators and herbivores, as well as other predators (i.e. intra-guild predation), thus reducing their efficiency as mutualists on plants (Whitney, 2004).

For the mutualisms between spiders and plants to occur and evolve, spiders need to be spatiotemporally and closely associated with a particular plant species or at least to a plant type (Romero *et al*., 2008). Currently, the most studied examples of plant structures that strengthen the fidelity of spiders and mediate these spider–plant interactions are leaves arranged in a rosette shape (e.g. Bromeliaceae) (Romero & Vasconcellos-Neto, 2005a,b,c; Romero, 2006; Romero *et al*., 2006) and the presence of glandular trichomes (Romero & Vasconcellos-Neto, 2004; Vasconcellos-Neto *et al*., 2007; Romero *et al*., 2008; Jacobucci *et al*., 2009). Romero and Vasconcellos-Neto (2005a,b,c) showed that the architecture of *Bromelia balansae* provides shelter against predators and fire, foraging, and reproductive sites, as well as nurseries for *Psecas chapoda*. Moreover, Romero *et al*. (2008) demonstrated that spiders *Peucetia rubrolineata* and *P. flava* recognised and selected plants bearing glandular hairs; these sticky structures frequently trap and sometimes kill arthropods (Sugiura & Yamazaki, 2006) that can be used by the spiders.

Up to 10 spider species of the genus *Peucetia* (Oxyopidae) live associated with many plant species bearing glandular trichomes in various vegetation types in Neotropical, Palearctic, Afrotropical, and Neartic regions (Vasconcellos-Neto *et al*., 2007). However, to date little is known about why and how some members of *Peucetia* have specialised in glandular plants. In addition, little is known about whether these associations are mutualistic, i.e. if spiders improve plant fitness by protecting plants against natural enemies, and if glandular hairs provide benefits to the spiders (Romero *et al*., 2008). On the other hand, once upon flowers spiders can disrupt plant–pollinator mutualisms. To better understand these contrasting interactions between *Peucetia* spiders and glandular plants, we ran field experiments by manipulating the presence of the spider *P. flava* (Keyserling) 1877 on *Rhynchanthera dichotoma* (Lam.) DC (Melastomataceae), a shrubby plant that bears glandular trichomes. In some swamp sites from south-eastern Brazil, *P. flava* occurs strictly on this glandular plant species (Morais-Filho & Romero, 2008), representing a suitable system to test cost–benefit relationships in spider–plant interactions, as well as to investigate the role of glandular trichomes as mediators of mutualism between arthropods and plants. The main questions addressed in this study were: (i) Does *P. flava* suppress arthropods and decrease rates of herbivory in *R. dichotoma*? (ii) Is this effect consistent over time? (iii) Does the spider decrease or increase plant fitness? (iv) Does the spider benefit from the presence of glandular trichomes?

Materials and methods

Study areas and organisms

The field experiments were carried out in several swamp areas (20°48′–20°50′S, 49°16′–49°20′W; 494–542 m a.s.l.) at the margins of dams or streams near the city of São José do Rio Preto, northwestern São Paulo state, southeastern Brazil. The climate of the region is the type Cwa-Aw of Köppen, characterised by a season hot and humid in summer (November to March) and drought in winter (June to September). The annual rainfall varies from 1100 to 1250 mm with the rainy season receiving 85% of the annual rainfall (Barcha & Arid, 1971). Climate data were collected from a meteorological station situated 7 km from the place of study.

The plant *Rhynchanthera dichotoma* (Melastomataceae) is a shrub (0.5–2.0 m height) that occurs in temporary aquatic ecosystems (Pinheiro, 1995). This species has abundant glandular trichomes in stems and leaves, and blooms only once a year between March and May, soon after the rainfall season (Morais-filho & Romero, 2009); the flowers are arranged in raceme-like inflorescences. The flowers are visited by Lepidoptera spp. and mainly by some bees of the genus *Bombus* and of tribes Meliponini and Euglossine (J. C. Morais-Filho, pers. obs.). Although this species is self-compatible (evidenced by manual pollination), it only produces seeds in the presence of pollinators; the vibration on anthers caused by pollinators is necessary for pollen release and to its adherence in the stigma (Pinheiro, 1995). Its reproduction is explosive and synchronous, i.e. all individuals of a population having only vegetative branches can produce reproductive branches in less than 10 days (Morais-Filho & Romero, 2008, 2009). This plant species is often inhabited by different guilds of arthropods, including phytophages (e.g. Aphididae sp., Cicadellidae sp., Curculionidae sp., Chrysomelidae sp., Miridae sp., Pentatomidae sp., and larvae of two Lepidoptera species), and predators (spiders and Reduviidae sp.). Also, many insects are eventually adhered to glandular trichomes (e.g. Formicidae spp., Chironomidae sp., Aphididae sp.) (Morais-Filho & Romero, 2008, 2009). Larvae of Geometridae sp., Chrysomelidae sp., and especially larvae and adults of Curculionidae sp., which occur in great abundance and also attack flower buds, cause damage on leaves. Larvae of Lepidoptera spp. attack buttons and fruits, while Chrysomelidae sp. attacks only petals and stamens. Eventually, *Attini* sp. ants can also attack leaves and flowers (J.C. Morais-Filho, pers. obs.).

In São José do Rio Preto, the spider *Peucetia flava* occurs strictly on this plant species, where it forages and reproduces (Morais-Filho & Romero, 2008, 2009). It occurs over the year and in the rainy seasons (November–May); they can reach a density of one spider per branch, and up to 10 spiders in a single plant. Its diet is variable, and includes Cicadellidae sp., larvae of Curculionidae sp., nymphs and adults of Miridae sp., Chrysomelidae sp., Pentatomidae sp., Pollinators (Euglossine bees), other predators (spiders and Reduviidae sp.), and small arthropods attached to glandular trichomes, such as Formicidae spp. and Aphididae sp. (Morais-Filho & Romero, 2008). Individuals of *P. flava* are errant and do not build webs; the females can reach about 12 mm long and males about 9 mm. Using its silk, females join the sides of two or three leaves to produce a ceiling-like shelter, under which it deposits an egg sac and will remain on it for several days, apparently exhibiting maternal care (J. C. Morais-Filho, pers. obs.).

Abundance of arthropods and leaf herbivory

To investigate the effects of spiders on the abundance of arthropods and leaf herbivory, we selected naturally growing plants of *R. dichotoma* using a systematic design (Hurlbert, 1984), i.e. these plants were sequentially numbered according to the order of find along the margin of the stream and then were subjected to two treatments: the even plants had all the spiders removed (control treatment), and for the odd plants we kept the spiders that naturally colonised the plants (>80% of the cases) or introduced spiders on plants that lacked *Peucetia* (experimental treatment). Through daily inspections between 8.00 and 12.00 hours, spiders were included or removed from the plants according to the treatment. The density of spiders on these plants was controlled based on its natural density in the field (one spider per branch, as described above).

The experiment to test the effect of spiders on the abundance of arthropods associated with *R. dichotoma* occurred between December 2005 and March 2006. All the arthropods on the experimental ($n = 21$) and control plants ($n = 21$ plants) were counted 30, 60, and 100 days after the beginning of the experiment. The plants were inspected between 9.00 and 13.00 hours; some arthropods were collected for identifications, but most of them were morphospeciated and identified in the field. Each plant was inspected for 5–7 min.

To verify if *Peucetia* protects the plants against foliar herbivores, we conducted an experiment in the rainy season (from December 2005 to February 2006) and another at the end of the rainy season (April 2006). For the first and second experiments, we used 44 and 34 plants, respectively. For each individual of *R. dichotoma*, we estimated leaf herbivory by randomly selecting and marking three to five young leaves (unexpanded) with thin coloured wires placed on the basis of its petiole. Data on total leaf area and leaf area damaged by phytophages were estimated by using a clear plastic grid. For the first experiment, data were collected on four sampling dates following repeated measures design; the first sampling occurred at the beginning of the experiment (pre-treatment) and the remainders at intervals of 20–30 days until the end of the experiment. For the second experiment, these data were collected in two samples with 20-day intervals, following a repeated measures design.

Floral herbivory and seed set: a cost–benefit analysis

To test if *Peucetia* reduces herbivory on flower buds and flowers, and if they increase plant fitness (i.e. seed set), we ran an experiment during the reproductive season of *R. dichotoma* (April 2008) using 15 pairs (blocks) of randomly chosen plants following a randomised block design. For one plant of the pair we kept the spiders that naturally colonised the plants (>80% of the cases) or introduced spiders on plants that lacked *Peucetia* (experimental treatment), and for the other plant we removed all the spiders (control treatment). A distance of 1–2 m separated paired plants, and each pair was at least 7 m apart from conspecifics. We used the same procedure for the maintenance of spiders as described above.

We randomly selected and marked two to four groups of buds per plant of the block, giving a total of 134 groups in the experiment. Each group had 28.3 ± 1.2 buds (mean ± 1 SE). These groups were monitored throughout the development of buds until the formation of fruits. Data on total number of flower buds and flowers in anthesis per group, and number of buds and flowers that had any herbivory damage, were obtained in five sampling dates at intervals of 5 days. Since many of the flower buds became fruit before the end of the experiment, data on the last two sampling dates were removed from the analysis to avoid missing values and loss in degrees of freedom. However, these data were plotted in the figures. To test the influence of spiders on fruit set, we divided the number of fruits produced by the initial number of buds for each sampling date. The production of flower buds is continuous; each group marked had reproductive structures in different phenological phases. Consequently, in each new sampling period we counted new buds and flowers.

At the end of the experiment, we randomly collected 10 fruits in the initial phase of development from each experimental plant ($n = 26$ plants). The fruits of each plant were stored in polyethylene tubes with lids made by mesh to allow drying and ripening. These fruits were then dissected to extract and count the seeds under a stereomicroscope (Bel Photonics®, Milano, Italy).

Role of glandular trichomes to the spiders

To test whether glandular trichomes of *R. dichotoma* retain arthropods by their adhesive action, we conducted an experiment in August 2008 using 11 pairs (blocks) of *R. dichotoma* plantlets (0.4–0.6 m height) having a single branch. Each plant of the block was randomly designed to receive one of the following treatments: (i) removal of glandular trichomes from both sides of all the leaves (experimental treatment), or (ii) glandular trichomes remained intact (control). Experimental plants had most of the glandular trichomes carefully removed using blade-shave, while the controls were slightly shaken to simulate removal of trichomes. A distance of 0.5–1 m separated paired plants, and each pair was at least 5 m apart from conspecifics. To simulate insects that naturally adhere to the glandular trichomes, we used live *Drosophila melanogaster* vestigial flies; 30 flies were thrown at a height of 0.15 m above each plant of the experiment. Then, we counted the number of retained flies during its application (time 0), and 20, 40, 60, and 80 min after its application.

To test whether spiders select plants of *R. dichotoma* with intact glandular trichomes over those in which these structures were removed, we used 10 of the 11 pairs of plants of the previous experiment. Prior to the beginning of this experiment, all arthropods on the leaves of all the plants were removed. Then, a subadult or adult female spider (see classification in Morais-Filho & Romero, 2008, 2009) of *P. flava* was introduced on each plant. The residence time (in hours) of spiders on these plants was determined.

To test whether *P. flava* feed on arthropods attached to glandular trichomes of *R. dichotoma* and the frequency with which they feed on live and dead prey, we developed an experiment in December 2007 using isotopically labelled (^{15}N) live and dead flies of *D. melanogaster*; no statistical difference of ^{15}N isotopic values between spiders that fed on dead and live flies would indicate that spiders use dead and live prey in similar amounts. The flies were labelled (ca 10 atoms % excess) following the same procedures described in Romero *et al.* (2006). Prior to the beginning of the experiment, 15 live flies were applied in each of nine plantlets of *R. dichotoma* (0.4–0.6 m height) chosen randomly, while other nine plantlets each received 15 dead flies. To avoid traces of ether in the flies, the experiment began only a few minutes after the adherence of the flies on trichomes. Then, each plant received a subadult or adult female spider (see classification in Morais-Filho & Romero, 2008, 2009) of *P. flava*, which remained on the plant for 3 days. To prevent escape of these spiders from the plants, we applied mesh cages above the plants. Prior to the beginning of the experiment, all plants were inspected, and arthropods (dead or live) on the plants were removed. At the end of the experiment, individuals of *Peucetia* as well as *D. melanogaster* were collected, frozen and dried for isotopic analysis. δ^{15}N values were determined in the Stable Isotope Facility at the University of California at Davis. Stable isotope ratios of ^{15}N were determined by continuous flow isotope ratio mass spectrometer (IRMS) (20–20 mass spectrometer, PDZ Europa, Sandbach, England) after sample combustion to N_2 at 1000 °C by an on-line elemental analyser (ANCA-GSL, PDZ Europa). δ^{15}N values below 10 represent natural abundance of ^{15}N; thus, only the spiders that had higher values actually ate the labelled flies.

Statistical analyses

The total number of arthropods and arthropod number of each taxonomic group were compared between treatments (presence or absence of spiders) using standard repeated-measures ANOVA, with treatment (two levels) as a fixed factor and time as a factor of repetition. Leaf herbivory in both experiments (rainy season and end of rainfall) was compared using repeated-measures ANCOVA with treatment presence or absence of spiders (two levels) as fixed factor, time as repeated factor and initial number of leaves per plant as covariate for both experiments. The probabilities for the repeated factors (when more than two repeated measures are analysed) and interactions were corrected with the Greenhouse–Geisser (G–G) approximation procedure to avoid sphericity (Zar, 1996).

Data on proportion of damaged flower buds and flowers, and number of retained *D. melanogaster* vestigial on plants with and without trichomes, were compared using randomised-block, repeated-measures ANOVA with treatment (two levels) as fixed factor, time as repeated factor and blocks treated as random effects. Data on proportion of flower buds that became fruits were compared using repeated-measures ANCOVA with treatment presence or absence of spiders (two levels) as fixed factor, time as a repeated factor and initial number of flower buds as covariate. Number of seeds per fruit was compared using randomised-block ANOVA with treatment presence or absence of spiders (two levels) as fixed factor and blocks treated as random effects. Residence time (in hours) of spiders on plants with and without trichomes was analysed by randomised-block ANOVA with treatment presence or absence of trichomes (two levels) as fixed factor and blocks treated as random effects. δ^{15}N values from spiders that fed on dead or live labelled flies were compared using a t-test.

When necessary, prior to analysis the data of counts were log or $\log(n + 1)$ transformed, and data on proportions were arcsine square-root transformed for normalisation and equalisation of variances.

Results

Abundance of arthropods and leaf herbivory

The total number of arthropods on *R. dichotoma* decreased significantly over the experiment. However, this phenomenon was not related to the presence of spiders (Table 1, Fig. 1a). The number of Curculionidae sp., which represents the most abundant arthropods on these plants, also decreased significantly during the experiment without being affected by the spiders. In contrast, Miridae sp. and Cicadellidae sp. were reduced in the presence of the predator (Table 1, Fig. 1). Although we have not seen *Peucetia* feeding on lepidopteran larvae, these herbivores were also reduced in the presence of predators (Table 1, Fig. 1).

Table 1. Repeated-measures ANOVA examining the effect of the presence of spiders on the number of arthropods per leaf on plants of *Rhynchanthera dichotoma*.

Source of variation	d.f.	MS	*F*	*P*	*G– G*
Total arthropods					
Treatment	1	0.0015	2.99	0.095	–
Error	27	0.0005	–	–	–
Time	2	0.0057	13.51	**<0.001**	**<0.001**
Time × treatment	2	0.0003	0.63	0.537	0.513
Error	54	0.0004	–	–	–
Cicadellidae sp.					
Treatment	1	0.0000	4.33	**0.047**	–
Error	27	0.0000	–	–	–
Time	2	0.0000	0.02	0.975	0.969
Time × treatment	2	0.0000	1.92	0.157	0.160
Error	54	0.0000	–	–	–
Curculionidae sp.					
Treatment	1	0.0000	0.14	0.709	–
Error	27	0.0003	–	–	–
Time	2	0.0020	10.44	**<0.001**	**<0.001**
Time × treatment	2	0.0003	1.69	0.194	0.203
Error	54	0.0002	–	–	–
Larvae of Lepidoptera spp.					
Treatment	1	0.0000	10.57	**0.003**	–
Error	27	0.0000	–	–	–
Time	2	0.0000	0.32	0.729	0.701
Time × treatment	2	0.0000	0.43	0.655	0.629
Error	54	0.0000	–	–	–
Miridae sp.					
Treatment	1	0.0001	6.80	**0.015**	–
Error	27	0.0000	–	–	–
Time	2	0.0001	8.57	**<0.001**	**0.001**
Time × treatment	2	0.0000	0.80	0.455	0.437
Error	54	0.0000	–	–	–

Treatment = presence versus absence of spiders; time = sampling periods. Significant *P*-values are given in bold.

In the rainy season the spiders had no apparent effect on the rates of leaf herbivory in *R. dichotoma*. In contrast, in the post-rain period leaf herbivory on plants with spiders decreased by 74% (time × treatment effect; Table 2, Fig. 2). In the rainy season the number of leaves increased by 47% (repeated-measures ANOVA: time: $F_{2,66} = 15.89$, $P < 0.001$); there was no influence of spiders in the production of leaves (repeated-measures ANOVA: treatment × time: $F_{2,66} = 0.72$, $P = 0.488$). In the post-rain period the number of leaves did not vary over time (repeated-measures ANOVA: $F_{1,29} = 1.52$, $P = 0.226$), and again the spiders had no influence on leaf production (repeated-measures ANOVA: treatment × time: $F_{1,29} = 1.42$, $P = 0.242$).

Floral herbivory and seed set: a cost–benefit analysis

Peucetia flava spiders reduced the proportion of damaged flower buds by 85% (Table 3, Fig. 3a). The damages found in buds were typically small holes or buds eaten entirely. Also, in the presence of spiders there was a reduction of 55% in the proportion of damaged flowers in anthesis (Table 3, Fig. 3b). Small holes in the calyx and petals, stamens partially eaten or flowers eaten entirely characterised the damage on flowers; we have not seen the insects responsible for this damage. Damage found on petals and stamens were probably caused by Chrysomelidae sp.; the larvae of Lepidoptera spp. probably ate such structures entirely.

Although the spiders decreased the rates of floral herbivory, the fruit production was similar in plants with and without spiders (Table 3, Fig. 3). In addition, the number of seeds per fruit produced in plants with spiders (mean ± 1 SE, 62.5 ± 9.5) and without spiders (65.1 ± 10.6) did not differ statistically (randomised-block ANOVA: $F_{1,12} = 0.20$, $P = 0.663$), indicating that spiders also did not affect plant–pollinator mutualism.

Role of glandular trichomes to the spiders

Soon after the application of *Drosophila* vestigial on the experimental plants, a greater number of flies was retained in plants of *R. dichotoma* that had intact glandular trichomes (mean ± 1 SE: 19.9 ± 1.5) than those with trichomes removed (15.2 ± 1.2) (Table 4, Fig. 4). *Peucetia* spiders remained for 4.4 times longer on plants with glandular trichomes intact (191.8 ± 43.1 h) than on plants with trichomes removed (43.2 ± 19.8) (randomised-block ANOVA: $F_{1,9} = 9.03$, $P = 0.015$); some spiders remained on the plant with glandular trichomes intact for up to 314 h (13 days).

The mean δ^{15}N values of spiders that fed on dead and live flies did not differ statistically (mean ± 1 SE; live *Drosophila*: 134.1 ± 75.3; dead *Drosophila*: 89.1 ± 23.9; *t*-test: $F_{1,16} = 0.32$, $P = 0.577$). Of the total number of spiders in treatments with live ($n = 9$) and dead *Drosophila* ($n = 9$), six and seven spiders fed on labelled *Drosophila*, respectively (Fig. 5). The mean δ^{15}N value (±1 SE) of spiders that did not feed on labelled flies was 5.51 ± 1.49.

Discussion

Peucetia flava decreased the abundance of many phytophagous insects, including Miridae sp., Cicadellidae sp., and larvae of Lepidoptera spp. These results show that the effect of the spiders on the arthropods is taxon specific. Similar results were obtained by Romero *et al.* (2008), who showed that only organisms that tended to be more sessile (e.g. larvae of Lepidoptera, Miridae), but not those more active (e.g. *Melanagromyza* sp., Agromyzidae), were affected by the spiders *P. flava* and *P. rubrolineata*. However, in our study *P. flava* did not affect the population of Curculionidae sp., a typically sessile organism and the most abundant on *R. dichotoma*. As they are easily found on the leaves of *R. dichotoma* and apparently have no camouflage or cryptic coloration, we suggest that this beetle probably possesses some kind of physical or chemical defence against predation by *Peucetia*.

Although *P. flava* effectively removed phytophagous insects in the rainy season, this predator had no influence on leaf herbivory caused by insects during this season. In contrast,

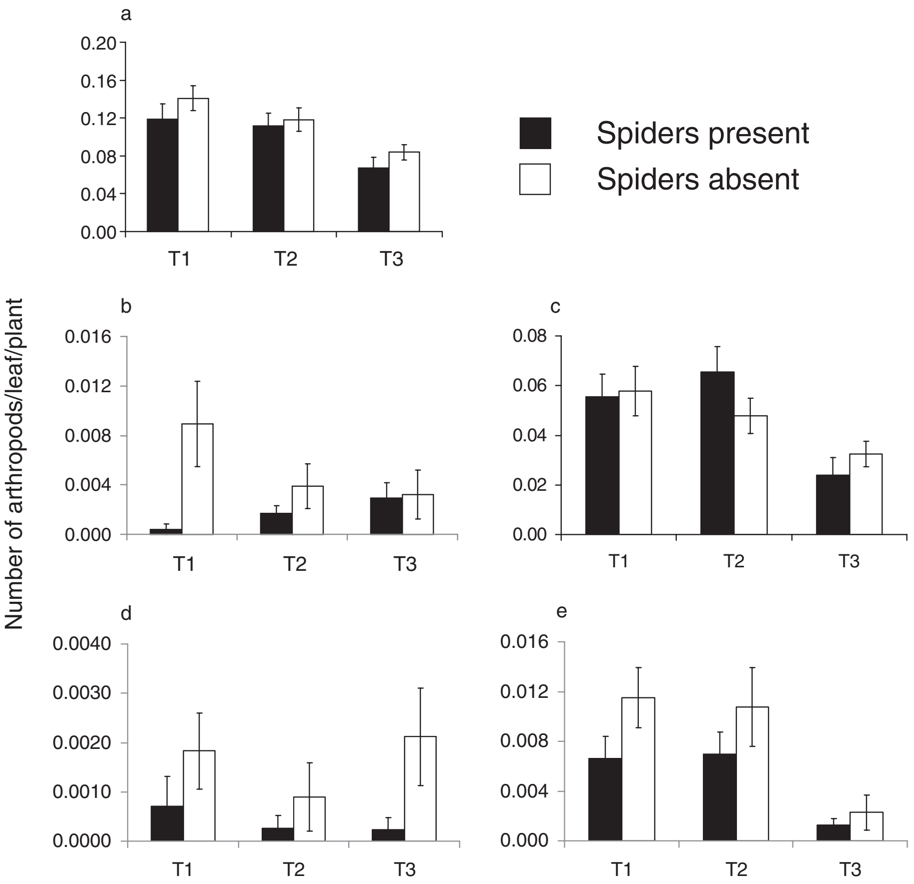

Fig. 1. Total number of arthropods (a), Cicadellidae sp. (b), Curculionidae sp. (c), larvae of Lepidoptera spp. (d), and Miridae sp. (e) on *Rhynchanthera dichotoma* in the presence and absence of *Peucetia flava* spiders. Error bars represent ±1 SE. Sampling dates: T1, 13 January 2006; T2, 13 February 2006; T3, 22 March 2006 .

Table 2. Repeated-measures ANCOVA examining the effects of spiders on leaf herbivory in *Rhynchanthera dichotoma* during the rainy season and post-rain period.

Source of variation	d.f.	MS	*F*	*P*	*G–G*
Foliar herbivory; rainy season					
Treatment	1	0.0000	0.01	0.938	–
No. of leaves (covariate)	1	0.0067	1.60	0.224	–
Error	16	0.0042	–	–	–
Time	3	0.0042	2.51	0.070	0120
Time × treatment	3	0.0007	0.41	0.747	0.587
Time × no. of leaves	3	0.0012	0.74	0.531	0.435
Error	48	0.0017	–	–	–
Foliar herbivory; post-rain					
Treatment	1	0.0174	8.54	**0.006**	–
No. of leaves (covariate)	1	0.0031	1.51	0.229	–
Error	31	0.0020	–	–	–
Time	1	0.0099	5.64	**0.024**	–
Time × treatment	1	0.0166	9.49	**0.004**	–
Time × no. of leaves	1	0.0015	0.83	0.370	–
Error	31	0.0017	–	–	–

Treatment = presence versus absence of spiders; time = sampling periods. Significant *P*-values are given in bold.

in the post-rain period, spiders greatly decreased the rate of leaf herbivory in *R. dichotoma*. Our results indicate that the role of spiders as plant bodyguards is temporally conditioned. During the rainy months the rate of production of new leaves in *R. dichotoma*, added to the rapid expansion of these leaves, were high, a phenomenon probably triggered by bottom-up forces (e.g. rain, mineral input) that improved productivity of the swamp ecosystem studied here. Higher leaf productivity probably supported more herbivores and exceeded their capacity of leaf consumption, thus dampening top-down effects of spiders on herbivory. Similar results were reported by Denno *et al.* (2003) by manipulating plant nutrition and presence of spiders in salt marsh islets from the U.S.A. In contrast, in the post-rain period the plant invests in the production of reproductive branches, and leaf productivity ceases. This allowed a longer exposure time of the spider and herbivores on leaves, thus strengthening top-down effects of spiders in the system. Therefore, the conditional outcome in the spider–plant mutualism may have occurred because bottom-up forces dampened the effects of spiders in the rainy season.

Although the spider *P. flava* greatly reduced damage on buds and flowers of *R. dichotoma*, these effects on floral

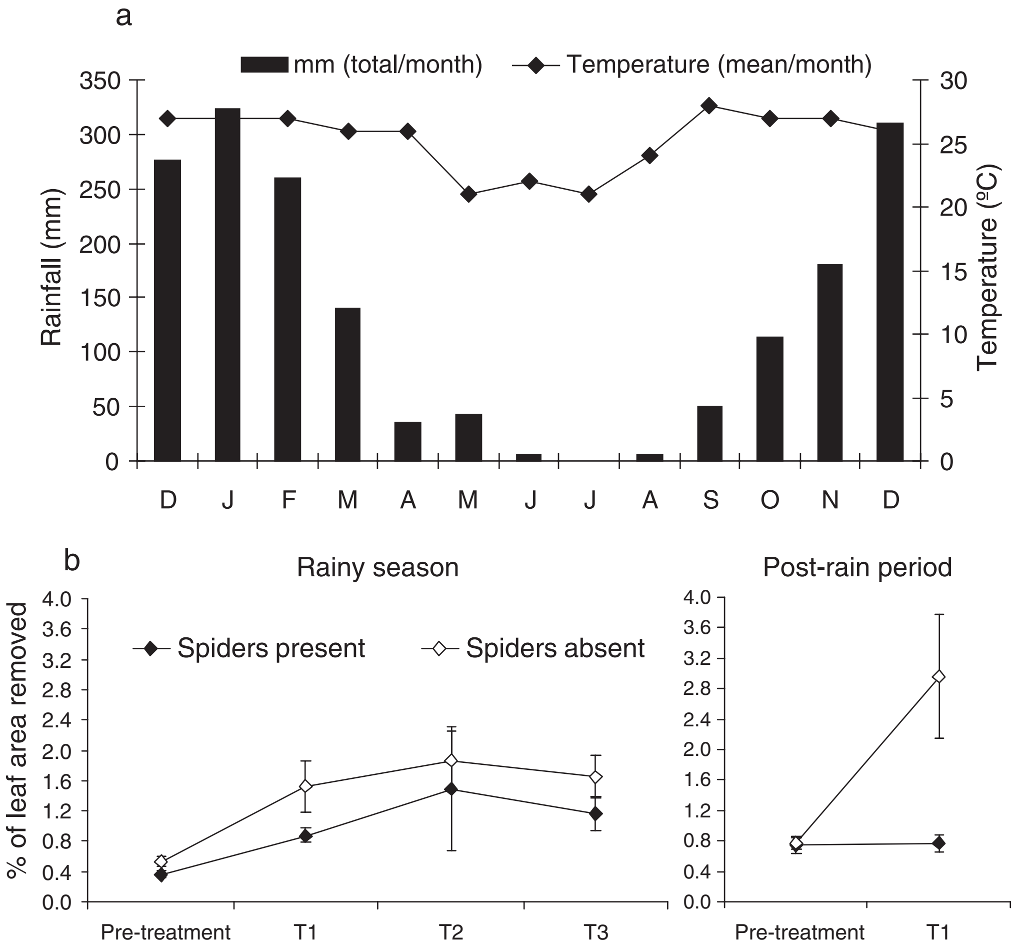

Fig. 2. (a) Monthly rainfall and temperature variation over the experiments of leaf herbivory; (b) mean percentage of leaf area removed by chewing phytophages on *Rhynchanthera dichotoma* in the presence and absence of spiders, during rainy and post-rain periods. Error bars represent ±1 SE. Sampling dates: *rainy season*: pre- treatment, 13 December 2005; T1, 12 January 2006; T2, 2 February 2006; T3, 22 February 2006; *post-rain period*: pre–treatment, 10 April 2006; T1, 30 April 2006 .

herbivory did not translate into fruit production, suggesting that the overall effect of spiders was weak. However, the spiders did not affect plant reproduction by preying on pollinators (similar seed set between treatments), suggesting that in this system *P. flava* may potentially improve plant reproduction with no indirect costs to plant fitness. To date, of the 10 spider species of the genus *Peucetia* that live on glandular plants (Vasconcellos-Neto *et al.*, 2007), three (i.e. *P. viridans*, *P. rubrolineata*, and *P. flava*) were already considered mutualistic (Louda, 1982; Romero *et al.*, 2008; the present study). However, in contrast to our study, Louda (1982) and Romero *et al.* (2008) showed that the spiders *Peucetia* reduced or tended to reduce seed set on their host plants (Asteraceae). Contrasting responses of *Peucetia* on their host plants are probably related to types of pollinators and inflorescence architectures. For example, the main pollinator agents in *R. dichotoma* are bees of the genus *Bombus*, which are too big to be captured by *P. flava* (see also Pinheiro, 1995). In fact, Dukas and Morse (2003, 2005) observed that the success of capturing larger prey by *Misumena vatia* (Thomisidae) was low. In contrast, floral visitors of *T. adenantha*, for example, are smaller and can be captured by spiders (Romero *et al.*, 2008). Moreover, in *Haplopappus venetus* (Asteraceae) the capitula are arranged side by side at the top of the inflorescence (flat-topped inflorescence) (Louda, 1982), allowing the spiders *P. viridans* to forage on a larger number of capitula simultaneously. In *T. adenantha* the capitula are distant from each other. However, the spiders *P. flava* and *P. rubrolineata* can unite them, to increase the foraging area (Romero *et al.*, 2008). In contrast, in *R. dichotoma* the flowers are large and well spread, preventing these spiders from foraging simultaneously on a large number of flowers, thus avoiding the reduction in the number of seeds produced. Thus, prey size and inflorescence architecture seem to determine the strength of the mutualisms involving *Peucetia* and glandular plants.

Whereas taxonomic groups closely related to *P. flava* are composed of web-building spiders (e.g. *Tapinillus*; Santos,

Table 3. Randomised-block, repeated-measures ANOVA examining the effects of the presence of spiders on herbivory of flower buds and flowers in anthesis of *Rhynchanthera dichotoma*.

Source of variation	d.f.	MS	*F*	*P*	*G–G*
Herbivory in flower buds					
Treatment	1	1.5834	20.44	<**0.001**	–
Block	12	0.0352	0.45	0.907	–
Error	12	0.0775	–	–	–
Time	2	0.2001	2.07	0.148	0163
Time × treatment	2	1.7804	18.42	<**0.001**	<**0.001**
Time × block	24	0.0575	0.59	0.895	0.857
Error	24	0.0967	–	–	–
Herbivory in flowers in anthesis					
Treatment	1	0.6364	5.88	**0.031**	–
Block	13	0.2320	2.14	0.091	–
Error	13	0.1083	–	–	–
Time	2	0.5563	5.81	**0.008**	**0.013**
Time × treatment	2	0.5321	5.55	**0.010**	**0.015**
Time × block	26	0.0636	0.66	0.849	0.824
Error	26	0.0958	–	–	–
No. of fruits produced					
Treatment	1	0.023236	1.00891	0.333	–
Block	14	0.025876	1.12353	0.419	–
Initial no. of buds (covariate)	1	0.063561	2.75979	0.120	–
Error	13	0.023031	–	–	–
Time	3	0.005195	2.92811	**0.045**	0.070
Time × treatment	3	0.003827	2.15693	0.108	0.135
Time × covariate	42	0.002441	1.37565	0.158	0.071
Time × block	3	0.005159	2.90736	**0.046**	0.206
Error	39	0.001774	–	–	–

Treatment = presence versus absence of spiders; time = sampling periods. Significant *P*-values are given in bold.

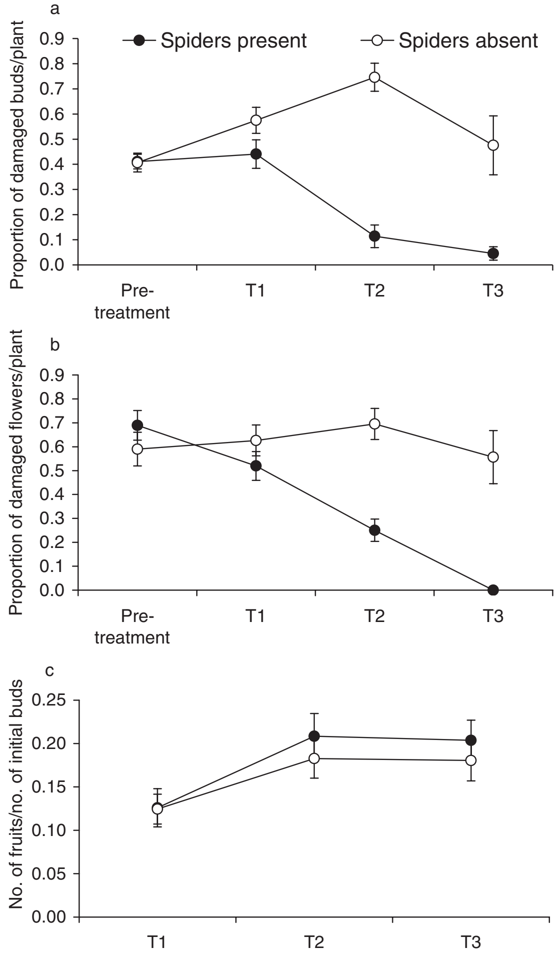

Fig. 3. Mean proportion of damaged buds (a), flowers in anthesis (b), and of buds that becomes fruits (c) of *Rhynchanthera dichotoma* in the presence and absence of spiders. Error bars represent ±1 SE. Sampling dates: pre−treatment, 8 April 2008; T1, 13 April 2008; T2, 18 April 2008; T3, 23 April 2008 .

2004) and use these structures for prey capture, *P. flava* does not build webs or forage actively on the vegetation. As *Peucetia* selected plants having glandular hairs over others without such structures (see also Romero *et al*., 2008), and can capture arthropods adhered to them, we suggest that glandular trichomes may function analogously to webs by capturing small insects (e.g. Chironomidae). In fact, we showed that plants with glandular trichomes retain more prey than plants without trichomes. In the field, we observed *P. flava* feeding on Formicidae spp., Chironomidae sp., and Aphididae sp. attached to such structures. In addition, our experiments showed that *P. flava* can use both live and dead prey adhered to glandular trichomes, thus reinforcing the view that spiders of this genus can be predators, but also scavengers (Romero *et al*., 2008). Romero *et al*. (2008) suggested that the scavenger habit could mean an adaptation to periods of food scarcity. Since several arthropods die after becoming attached to glandular trichomes, if used by spiders it can mean an extra source of energy, which may be important for maintenance in this period. In fact, we observed that the number of individuals of *P. flava* was high even in harsh seasons (Morais-Filho & Romero, 2009). These results suggest that *Peucetia* may have specialised to forage on glandular plants because such adhesive structures contribute to spider nutrition, increasing the chance of finding and capturing prey without the additional costs of producing webs.

Table 4. Randomised-blocks, repeated-measures ANOVA examining the effects of the presence of glandular trichomes in the retention of *D. melanogaster* vestigial flies on leaves of *Rhynchanthera dichotoma*.

Source of variation	d.f.	MS	*F*	*P*	*G–G*
Treatment	1	1.6620	11.19	**0.007**	–
Block	10	0.3743	2.52	0.081	–
Error	10	0.1486		–	–
Time	4	1.3925	60.36	<**0.001**	<**0.001**
Time × treatment	4	0.0911	3.95	**0.008**	**0.019**
Time × block	40	0.0466	2.02	**0.014**	**0.032**
Error	40	0.0231		–	–

Treatment = presence versus absence of glandular trichomes; time = sampling periods. Significant P-values are given in bold.

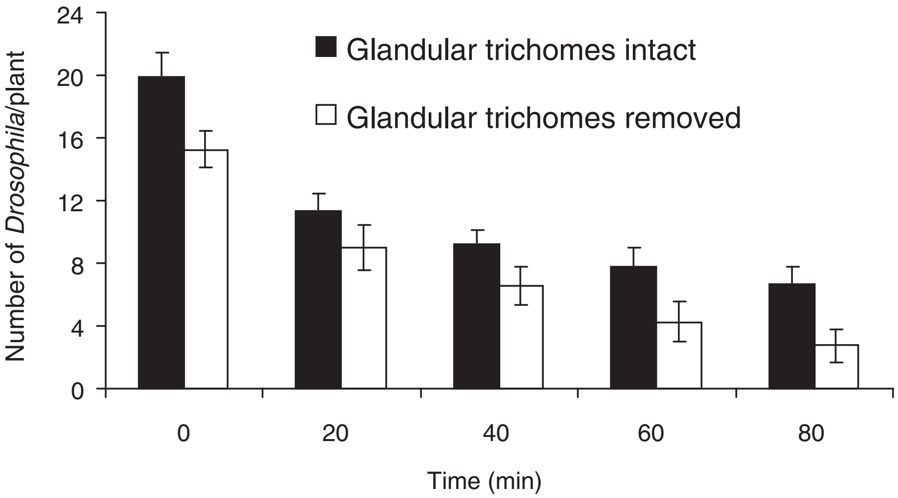

Fig. 4. Mean number of *Drosophila melanogaster* vestigial flies per plant on leaves of *Rhynchanthera dichotoma* with glandular trichomes intact and removed. Error bars represent ±1 SE.

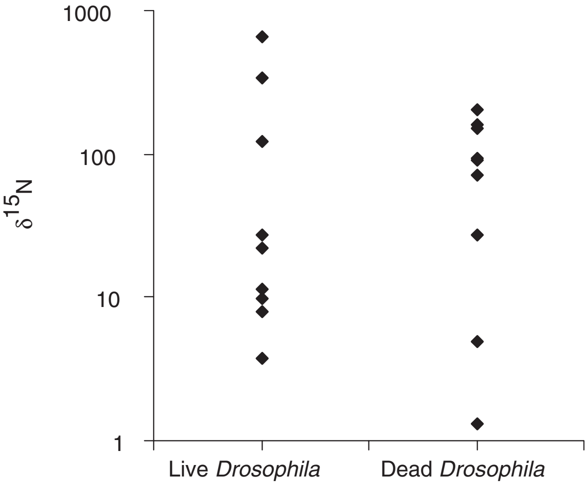

Fig. 5. δ^{15}N values (log scale) of *Peucetia flava* spiders that remained on plants that received live ($n = 9$ spiders) and dead ($n = 9$ spiders) flies (^{15}N). The mean δ^{15}N value of the enriched flies was 1079 ($n = 3$).

To establish mutualisms involving spiders and plants, the spiders should be intimately associated with specific plant species or types of plants (Romero *et al.*, 2008), and such associations should be stable spatiotemporally. And for specific associations to occur, plants must have structures that increase spider fidelity, providing them greater chances of prey capture, shelter, and/or nursery (Romero & Vasconcellos-Neto, 2005a,b,c). Glandular trichomes seem to be a plant attribute that encourages persistence of *Peucetia* on plants. For example, *P. flava* occurs on glandular plants over a large geographic region from South America (Vasconcellos-Neto *et al.*, 2007), and dynamics of their populations are relatively stable seasonally (Morais-Filho & Romero, 2009). Therefore, glandular trichomes probably increase spider–plant fidelity spatiotemporally, although strong bottom-up forces seem to dampen the effects of spiders as plant bodyguards (see above).

In conclusion, the overall effects of spiders on glandular plants were positive – they protected plants against foliar herbivores, but presented no cost to plant reproduction. However, the role of spiders as plant bodyguards was temporally conditional, probably because strong bottom-up forces (i.e. increased vegetative productivity in the rainy season) dampened top-down effects. Glandular trichomes probably exert an analogous effect to a web by retaining small insects and thus reducing spider energy expenditure on prey subjugation and capture. Besides, *Peucetia* can act as scavengers by feeding on dead arthropods stuck on the glandular trichomes. However, it still remains unclear whether *Peucetia* spiders have adaptations to live on glandular hairs. As interactions involving *Peucetia* and glandular plants are distributed worldwide, this protective mutualism may be quite common, but only now are being identified and investigated.

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