



# The role of spider hunting mode on the strength of spider–plant mutualisms

Mônica F. Kersch-Becker<sup>1,2</sup> · Bruno B. Grisolia<sup>3</sup> · Maria J. O. Campos<sup>3</sup> · Gustavo Q. Romero<sup>1</sup>

Received: 5 December 2017 / Accepted: 16 May 2018 / Published online: 4 June 2018  
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

## Abstract

The strength and outcome of mutualistic interactions can be highly dependent on the combination of traits of the species involved. Distinct foraging strategies (e.g., hunting mode) of mutualistic predators may cause predator–prey interactions to vary, potentially affecting the strength of trophic cascades. We evaluate the causes of variation in the strength of spider–plant mutualisms by focusing on contrasting hunting modes of two spiders: an actively hunting lynx spider (*Peucetia* sp.) and a sit-and-wait crab spider (*Misumenops argenteus*). We manipulated spider species composition by assigning each plant to one of the following treatments: (1) no spiders; (2) sit-and-wait spiders only; (3) actively hunting spiders only; (4) actively hunting + sit-and-wait spiders. We then examined the independent and interactive effects of spider species on floral herbivory and fitness of the glandular trichome-bearing plant, *Trichogoniopsis adenantha* (Asteraceae). Both spider species increased plant fitness by suppressing herbivores and increasing ovary fertilization, but the overall net benefit of spiders was contingent on spider hunting mode. Sit-and-wait spiders promoted stronger positive cascading effects compared to actively hunting spiders. The combination of spider species suppressed herbivores in an additive manner; their combined impact on plant fitness, however, was lower than expected, suggesting that the inter-specific interaction between spiders is slightly antagonistic. Thus, both spider species combined weakened the strength of this spider–plant mutualism. Our findings offer a general framework for understanding the critical role of predator foraging mode in trophic cascades.

**Keywords** Flower-dwelling spiders · Functional diversity · Herbivory · Predation · Trophic cascades

## Introduction

Predators often control the structure and dynamics of herbivore prey communities, and trophic cascading effects of predators can extend beyond their prey species (Wootton and Emmerson 2005; Schmitz et al. 2010). Understanding how combinations of species of predators influence lower trophic levels remains a key unresolved issue in the study of trophic interactions (Finke and Snyder 2008). Increasing the number of enemy species generally strengthens prey suppression (Cardinale et al. 2006), but it is the resource partitioning among enemies that may increase resource use and not species diversity per se (Finke and Snyder 2008). The effect of multi-predator species on efficient prey suppression is attributed to two distinct mechanisms: complementarity and the species-identity effect (Ives et al. 2005; Casula et al. 2006). Species complementarity occurs when enemy species partition resources more efficiently among themselves than would be expected from a single species by itself, or when the presence of one species facilitates another (Duffy

---

Communicated by Sven Bacher.

---

Mônica F. Kersch-Becker and Bruno B. Grisolia have contributed equally.

---

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s00442-018-4170-y>) contains supplementary material, which is available to authorized users.

---

✉ Mônica F. Kersch-Becker  
mfkerschbecker@ua.edu

<sup>1</sup> Department of Animal Biology, University of Campinas (UNICAMP), Campinas, SP 13083-970, Brazil

<sup>2</sup> Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL 35487, USA

<sup>3</sup> Department of Ecology, University of Sao Paulo (UNESP), Rio Claro, SP 13506-900, Brazil

et al. 2007). Species-identity effects (sampling effect) refer to the greater likelihood of including key species in species-rich communities (Loreau et al. 2001). Sampling effects are based on the identity of one species and not on the diversity by itself. Neutral effects from multiple predator species may be attributed to neutral complementarity or redundancy. In contrast, negative effects arise from competition or intra-guild predation (Finke and Denno 2005). Identifying species or traits that determine the outcome of species interactions poses a key challenge to contemporary ecology (Finke and Snyder 2008; Schmitz 2008a, b).

Hunting mode is an important trait of predators in the food web, as it often generates distinct responses in prey (Preisser et al. 2007; Romero et al. 2011; Romero and Koricheva 2011). Stationary (sit-and-wait) foraging by predators promotes trait-mediated indirect effects on plants by triggering strong anti-predator behavioral responses in their herbivorous prey. In contrast, widely roaming, actively hunting predators operate mainly on the consumptive component of predators by suppressing prey density and propagating density-mediated indirect effects to plants. These differences can be partially attributed to the predation risk cues evoked by each hunting strategy (Schmitz 2007; Preisser et al. 2007; Schmitz 2008a; Romero and Koricheva 2011). Sit-and-wait predators favor predator-induced responses by providing strong cues of their presence, whereas actively hunting predators promote nonpersistent predation risk cues (Preisser et al. 2007; Romero and Koricheva 2011). Our ability to effectively predict the outcome of predator–prey interactions remains limited, but far less is known on the consequences of species traits on mutualistic associations.

A large body of research shows that diffused mutualistic interactions—when each partner may interact with more than one other partner—depends on the identity of the species involved (Zamora 2000; Stanton 2003; Gove et al. 2007). Because most mutualisms involve more than a pair of species (Zamora 2000; Stanton 2003), a key unresolved issue is whether the combination of species with different traits effectively predicts the strength and outcome of mutualistic associations. Several spider species are strictly associated with plants bearing glandular trichomes (Vasconcellos-Neto et al. 2007; LoPresti et al. 2015). By effectively protecting plants against herbivores, spiders can increase plant fitness (Romero and Vasconcellos-Neto 2004b; Romero et al. 2008). Plants' glandular trichomes may in turn benefit spiders by trapping different arthropods, which may be consumed when prey items are limited (Romero et al. 2008; LoPresti et al. 2015). *Trichogoniopsis adenantha*, a glandular trichome-bearing shrub, is closely associated with the sit-and-wait crab spider *Misumenops argenteus* and the actively hunting lynx spiders *Peucetia flava* and *P. rubrolineata* (Dias et al. 2010); all species are frequently found coexisting on the same plant. These three species of spiders

improve plant fitness by inhibiting herbivore oviposition and removing herbivore larvae (Romero and Vasconcellos-Neto 2004b; Romero et al. 2008). However, they can also prey upon several groups of floral herbivores and beneficial floral visitors (hereafter pollinators). Thus, the benefit of spider predation in reducing herbivory should outweigh the costs of predation on important pollinators.

Here, we explore sources of contingency in the strength of spider–plant mutualistic interactions by focusing on contrasting hunting modes of spiders (actively hunting vs. sit-and-wait). We performed a manipulative field experiment to examine the independent and interactive effects of spider species in reducing floral herbivory and enhancing plant fitness. We investigated whether the combination of spider species with distinct hunting modes would strengthen spider–plant mutualistic interactions. We predicted that the hunting mode of spiders would affect the strength of the spider–plant mutualism by operating distinctively via the consumptive and nonconsumptive effects of predators. Sit-and-wait spiders may increase plant fitness by both consuming herbivores and triggering behavioral changes in herbivores in response to strong predation risk cues (Preisser et al. 2007; Schmitz 2008a). In contrast, actively hunting spiders may benefit plants more through consumption of herbivores as predation risk cues associated with these predators are often nonpersistent (Preisser et al. 2007; Schmitz 2008a). Thus, we expect greater positive cascading effects of sit-and-wait spiders on plant fitness compared to actively hunting spiders. However, negative effects may arise when spiders disrupt plant–pollinator interactions. Because sit-and-wait spiders forage mostly upon flowers, they may capture or scare pollinators more than actively hunting spiders, thus decreasing their overall net benefits (Romero and Koricheva 2011). We also expected plant fitness to increase when spider species complement one another. Conversely, we expected plant fitness to decrease if spider species negatively interact (interference), such that the combined effect of both spider species is less than the sum of effects that each species would achieve by itself.

## Methods

### Study area and system

We carried out the experiments for 45 days from April to May 2011 in a neotropical semi-deciduous forest at the Serra do Japi Ecological Reserve, Jundiá, São Paulo, Brazil (23°11'S, 46°52'W; 1000 m elevation). The climate is seasonal, with a warm rainy season from November to April and cool dry season from June to September. The mean temperatures vary from 13.5 °C in July to 20.3 °C in January.

*Trichogoniopsis adenantha* (DC) (Asteraceae) is a small perennial shrub that is abundant alongside semi-deciduous forest edges and gaps in southeastern Brazil. Its leaves and stems bear glandular trichomes on which small insects adhere to (Romero and Vasconcellos-Neto 2003, 2004a, b, 2005; Romero et al. 2008). Each plant produces ~7 flowerheads per branch. Flowerheads are produced throughout the year, peaking in the rainy season (January–April). Each flowerhead contains an average of 40 flowers. *Trichogoniopsis adenantha* requires pollinators for fertilization (Romero and Vasconcellos-Neto 2004a); upon fertilization, the whitish-colored ovaries turn into black achenes (dry fruits).

There are a myriad of arthropods associated with *T. adenantha*; Ithomiine butterflies, ctenuchine moths, honeybees, and syrphid flies are frequently found visiting *T. adenantha* flowers (Romero and Vasconcellos-Neto 2004a, 2005; Romero et al. 2008). The most common leaf herbivores include chewing herbivores such as Geometridae caterpillars and sucking insects such as *Macrolophus aragarcanus* (Miridae). Pre-dispersal seed and flower predators, including Geometridae larvae, *Trupanea* sp. (Diptera, Tephritidae), *Melanagromyza* sp. (Diptera, Agromyzidae), and the galler *Asphondylla* sp. (Diptera, Cecidomyiidae), attack flowers and developing seeds. Species of the parasitic wasps Braconidae and Pteromalidae (Hymenoptera) frequently parasitize the larvae of the pre-dispersal seed predators *Trupanea* sp. and *Melanagromyza* sp. Two lynx spiders, *Peucetia flava* and *P. rubrolineata* (Oxyopidae), and the crab spider *Misumenops argenteus* (Thomisidae) are frequently found on *T. adenantha* plants preying upon flower visitors and herbivores. The two lynx spiders display similar active hunting behavior and body size (Santos and Brescovi 2003). In contrast, *M. argenteus* is smaller than the two *Peucetia* spiders and shows a sit-and-wait hunting strategy. Prior to the onset of the experiment, we inspected 150 plants and found that *Misumenops argenteus* inhabited 28% of the plants, *Peucetia rubrolineata* 23%, and *Peucetia flava* 14%. Because these species belong to the same feeding guild they may compete for resources, though they are often found spatially separated within the plant. Both intra-guild predation and cannibalism may also occur in this system (G.Q. Romero, personal observations).

## Experimental design

In November 2010, we collected saplings of *T. adenantha* plants from the wild and transplanted them to 25 cm diameter × 20 cm high pots containing homogenized soil collected from the same area where the plants were naturally growing to reduce possible bottom-up effects. We placed the potted plants in a greenhouse at the forest reserve. We transplanted only plants that were 30–60 cm tall and had only one branch. In March 2011, when plants started

producing flower buds, we transferred the potted plants to the margins of the forest and set up 10 blocks ( $N=40$ ). We did not use any type of enclosure; therefore, the plants were exposed to the natural community arthropods present in the area. Within a block, plants were 0.5–1 m apart and between blocks, the distance was at least 3 m. To evaluate the individual and interactive effects of spider hunting mode on *T. adenantha* fitness, we assigned each plant within a block to one of the following treatments: (1) Control, no spiders; (2) two individuals of the sit-and-wait crab spider *Misumenops argenteus*; (3) two individuals of the actively hunting spider, *Peucetia* sp.; (4) One sit-and-wait + one actively hunting spider. We used a substitutive experimental design as we kept the density of spiders the same in all treatments. The number of spiders on each plant was similar to the number of spiders naturally found on *T. adenantha*, which varied from one to three spiders according to the size of the plants (Romero et al. 2008). Five blocks contained *Peucetia flava* and five blocks *P. rubrolineata*. Even though the effects of *Peucetia* species on *T. adenantha* plants and associated insects are similar (Romero et al. 2008; plant attributes: MANOVA  $F_{6,3}=2.69$ ,  $P=0.436$ ; insects: MANOVA  $F_{8,1}=11.23$ ,  $P=0.227$ ), we did not add different *Peucetia* species to the same experimental block. We placed the spiders on flower buds at different branches, checked plants daily, and added or removed spiders as needed. All spiders that moved away were recovered on neighbor branches or plants. We carefully inspected the plants several times a day to ensure the spiders were on the plants. We monitored all plants throughout the duration of the experiment (45 days).

We collected the flowerheads throughout the experiment when they reached the pre-dispersal stage. At this phenophase, achenes would be mature when ovaries were fertilized. We kept the flowerheads on plastic cups for a few days until pre-dispersal seed predators or their parasitoids had emerged. Then, we dissected all flowerheads and counted the number of fertilized and unfertilized ovaries and recorded whether each ovary was damaged or intact. The damage caused by each herbivore differs, and thus, we also quantified the damage caused by each species. We recorded both the abundance of pre-dispersal seed predators and their parasitoids. Because the damage caused by Geometridae was severe it prevented us from recording the number of ovaries. In this case, we recorded the number of ovaries' scars instead of the ovaries per se. To test the individual and interactive effects of spider species on herbivore consumption, we estimated the number of ovaries consumed by each herbivorous larva (*Trupanea* sp. and *Melanagromyza* sp.) as the number of damaged ovary relative to the number of larvae.

## Statistical analyses

### Pre-dispersal seed predators and their parasitoids

To test the individual and interactive effects of spider species on the total number of herbivores, and on the number of *Trupanea* sp., *Melanagromyza* sp., and *Asphondylia* sp. individuals, we performed generalized linear mixed-effect models (GLMMs). We included treatment (four levels) as a fixed effect, number of flowerheads produced as a covariate, and block as a random effect. To investigate the effects of spider hunting mode on the proportion of parasitized herbivores relative to the total number of hosts (*Trupanea* sp. + *Melanagromyza* sp.), we used generalized linear model (GLM) with binomial distribution and logit link function. The number of flowerheads produced per plant was included as a covariate in the model.

To evaluate whether spider hunting mode affects the insect community composition associated with *T. adenantha* flowerheads, we conducted permutation multivariate analysis of variance (PERMANOVA) using distance matrices (adonis) and the Bray–Curtis dissimilarity coefficient; we used Monte Carlo permutation (10,000) to test the significance of these results. The PERMANOVA was performed using quantitative data for each spider group. We followed this analysis with separate pairwise PERMANOVAs to compare the differences in species composition between treatments. We visualized the similarity among insect community composition of treatments with nonmetric multidimensional scaling (NMDS). We conducted this analysis using R version 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria 2014) and the Vegan Package version 2.2-1 (Oksanen et al. 2008).

### Floral herbivory and plant fitness

To investigate whether spider hunting mode influences the total number of flowerheads produced, the number of ovaries per flowerhead, and the number of ovaries damaged by *Trupanea* sp. and *Melanagromyza* sp. larva, we performed GLMMs. In these models, we included treatment (4 levels) as a fixed effect, block as a random effect and the number of flowerheads as a covariate.

To compare (1) the proportion of herbivore infested flowerheads (e.g., *Trupanea*, *Melanagromyza*, and Geometridae sp.) relative to the number of flowerheads produced per plant, (2) the proportion of fertilized ovaries relative to the total number of ovaries (fertilized + unfertilized), and (3) the proportion of damaged ovaries relative to the total number of ovaries produced by each flowerhead, we performed GLMs with binomial distribution and logit link function. We then investigated whether spiders affect plant fitness (seed set) via trophic cascade (spiders removing

herbivores) or through changes in pollination. We tested the effects of spiders and flowerhead condition (damaged vs. intact) and spider-by-flowerhead condition interaction on the proportion of viable seeds (intact and fertilized ovaries) relative to the total number of ovaries (fertilized + unfertilized), using GLM with binomial distribution and logit link function. If spider treatment by itself is significant, then spiders affect pollination likely by keeping the flowerheads undamaged and thus more attractive to pollinators. If flowerhead condition is significant, then floral herbivory (damage) affects seed set. If the interaction term yields a significant result, it indicates that spiders indirectly affect seed set through both trophic cascade and enhanced pollination.

We performed Tukey's HSD post hoc comparison for significant test results, except for GLM with a binomial distribution where pairwise comparisons were performed. We used JMP 12 (SAS Institute Inc, Cary, NC, USA 2012) for all GLMMs and GLMs.

### Path analysis on the indirect effects of spiders on plant fitness

To evaluate whether greater spider diversity (1 vs. 2 species) increase plant fitness, we employed path analyses for each species by itself or with heterospecifics. We examined the strength of each pathway and assessed whether spiders indirectly influence plant fitness through changes in herbivore abundance and fertilization ratio. We ran three separate path models: (A) no spiders vs. sit-and-wait spiders only, (B) no spiders vs. actively hunting spiders only, and (C) spider diversity (no spiders, one species, or two species). For the models A and B, treatments were entered as 0 and 1. For the spider diversity model (C), we entered plants without spiders as 0, the treatments sit-and-wait only and actively hunting spiders only as 1 and the sit-and-wait + actively hunting spider combination as 2. All path models included herbivore abundance per flowerhead, number of fertilized ovaries relative to the total number of ovaries (pollination effect), and seed set (undamaged fertilized ovaries relative to the total number of ovaries). If plant fitness is strongly positively affected by greater spider species diversity (diversity model), then when combined, spiders would consume more than any spider-by-itself (species complementarity). Thus, distinct hunting modes of mutualistic spiders would complement each other and increase the net benefits to plants. In contrast, a negative inter-specific interaction may occur when greater spider diversity reduces the overall positive effect of spiders to plants. We used Systat (Systat Software Inc, Chicago, IL, USA 2004) to run the path analyses.

## Results

### Pre-dispersal seed predators and their parasitoids

We recorded 395 herbivores feeding on *T. adenantha* flowerheads from at least 5 species: three endophagous species [*Trupanea* sp. ( $N=215$ ), *Melanagromyza* sp. ( $N=42$ ), and *Asphondylia* sp. ( $N=41$ )] and 2 exophagous species [Tortricidae ( $N=47$ ) and Geometridae ( $N=9$ )].

We found that the presence of spiders reduced the number of herbivores (pre-dispersal seed predators) relative to the number of flowerheads compared to control plants (Herbivore total:  $F_{[3,25]}=22.80$ ,  $P<0.001$ , *Trupanea* sp.  $F_{[3,25]}=40.34$ ,  $P<0.001$ , *Melanagromyza* sp.:  $F_{[3,25]}=6.78$ ,  $P=0.001$ , Tortricidae  $F_{[3,26]}=4.43$ ,  $P=0.012$ , and *Asphondylia* sp.:  $F_{[3,26]}=3.43$ ,  $P=0.032$ , Fig. 1a). The total number of herbivores, and the abundance of the herbivores *Melanagromyza* sp., Tortricidae, and *Asphondylia* sp. were similar among spider treatments (sit-and-wait only, actively hunting only, or sit-and-wait + actively hunting combination). In contrast, sit-and-wait spiders reduced the abundance of the herbivore *Trupanea* sp. compared to actively hunting predators (Fig. 1a).

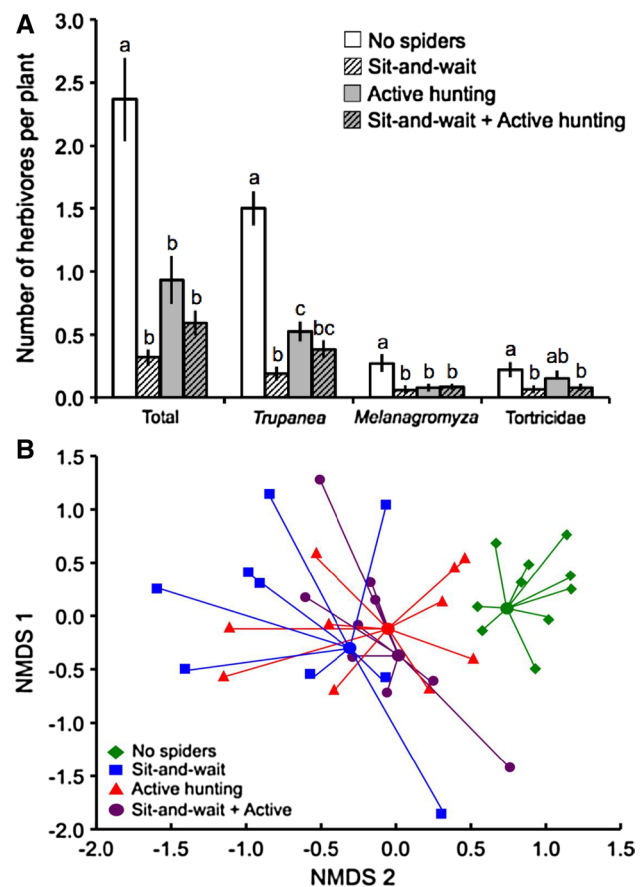
Sit-and-wait spiders reduced herbivore aggregation in *T. adenantha* flowerheads ( $\chi^2_{[3,36]}=12.09$ ,  $P=0.007$ ). Only 5% of the flowerheads with sit-and-wait spiders had more than one herbivore per flowerhead (CI 0.02, 0.12). In contrast, we recorded more than one herbivore per flowerhead in 58% of the flowerheads from control plants (CI 0.47, 0.68), 25% from plants with actively hunting spiders (CI 0.17, 0.35) and 16% from plants with the sit-and-wait + actively hunting spider combination (CI 0.10, 0.26). These results suggest that sit-and-wait spiders strongly affect herbivore distribution patterns by reducing herbivore aggregation on flowerheads.

We found that 62% of the endophagous herbivores (*Trupanea* sp. and *Melanagromyza* sp.) were parasitized by at least two species of parasitoids (Pteromalidae and Braconidae). The proportion of parasitized herbivores was not affected by the treatments ( $\chi^2_{[3,32]}=2.54$ ,  $P=0.468$ ). This suggests that, in this system, intra-guild predation seems to be weak or nonexistent.

The presence of spiders on plants shaped the community composition of insects (herbivores and parasitoids) associated with flowerheads of *T. adenantha* ( $F_{[3,34]}=4.71$ ,  $P<0.001$ , Fig. 1b). Plants with spiders, independent of their hunting mode, harbored a similar community composition of insects. Plant without spiders, however, featured a distinct community composition (Fig. 1b).

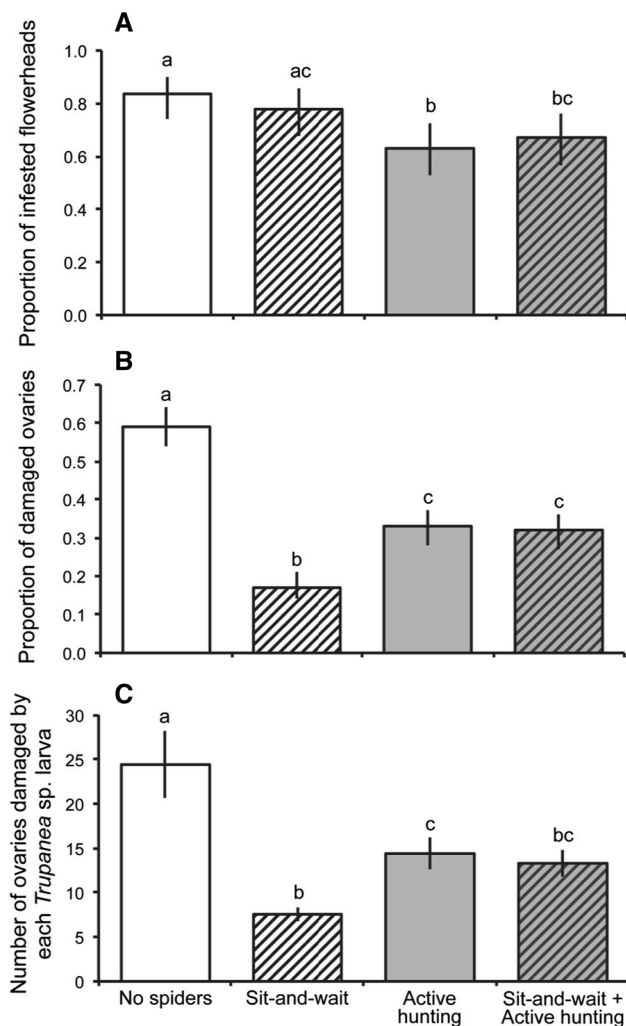
### Floral herbivory and plant fitness

Spider treatment did not affect the total number of flowerheads nor the number of ovaries per flowerhead produced



**Fig. 1** Insect responses to spider treatments. **a** Mean ( $\pm 1$  SE) number of herbivores (total number of herbivores, *Trupanea* sp., *Melanagromyza* sp. and Tortricidae) in response to different spider treatments. Letters above bar indicate significant differences at  $P<0.05$  following Tukey’s HSD post hoc test within each herbivore group. Treatments are: control without spiders (open bars), sit-and-wait spiders (striped bars), actively hunting spiders (gray bars), and sit-and-wait + actively hunting spiders (striped gray bars). **b** Nonmetric multidimensional scaling (NMDS) ordination of insect species composition visiting *T. adenantha* flowerheads on the different spider treatments. Treatments are: control without spiders (green diamonds), sit-and-wait spiders (blue squares), actively hunting spiders (red triangles), and sit-and-wait + actively hunting spiders (purple circles) (color figure online)

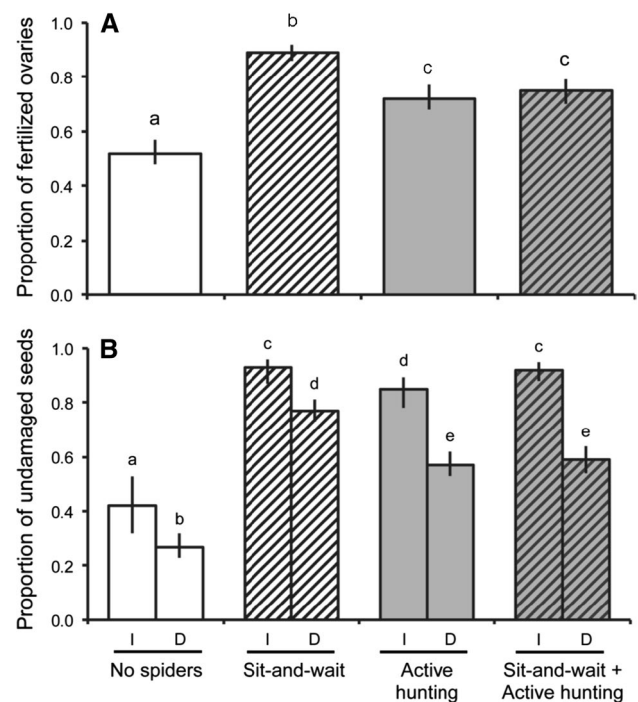
by plants ( $F_{[3,27]}=1.01$ ,  $P=0.404$ ;  $F_{[3,27]}=0.69$ ,  $P=0.568$ , respectively). The presence of spiders reduced the proportion of flowerheads infested by herbivores relative to the total number of flowerheads produced ( $\chi^2_{[3,36]}=12.09$ ,  $P=0.007$ , Fig. 2a). Sit-and-wait spiders reduced the proportion of damaged ovaries relative to the total number of ovaries produced compared to the other treatments ( $\chi^2_{[3,34]}=172.34$ ,  $P<0.001$ , Fig. 2b). The strong effect of sit-and-wait spiders on ovary damage may be driven by the negative effect that these spiders exerted on the feeding of *Trupanea* sp., the most abundant herbivore. Each *Trupanea* sp. larva consumed 108% more ovaries on control plants compared to plants with spiders, and damaged 94% more ovaries on plants with actively



**Fig. 2** Flowerhead infestation and herbivore damage in response to treatments. **a** Mean ( $\pm$  binomial 95% CI) proportion of flowerhead infested by herbivores (infested/total number produced per plant), **b** mean ( $\pm$  binomial 95% CI) proportion of ovaries damaged by herbivores (damaged/total number of ovaries produced per plant), **c** mean ( $\pm$  1 SE) number of ovaries consumed by each *Trupanea* sp. larva in response to treatments: controls without spiders (open bars), sit-and-wait spiders (striped open bars), actively hunting spiders (gray bars), and sit-and-wait + actively hunting spiders (striped gray bars). Letters above the bars indicate significant differences at  $P < 0.05$  following pairwise comparisons for panels **a**, **b** and Tukey's HSD post hoc test for panel **c**

hunting spiders only compared to plants with sit-and-wait spiders only ( $F_{[3,27]} = 21.30$ ,  $P < 0.001$ , Fig. 2c). In contrast, the presence of spiders did not affect ovary consumption by *Melanagromyza* sp. ( $F_{[3,15]} = 1.15$ ,  $P = 0.362$ ).

Spiders increased the proportion of fertilized ovaries ( $\chi^2_{[1,34]} = 144.83$ ,  $P < 0.001$ , Fig. 3a). The proportion of fertilized ovaries was higher on plants receiving sit-and-wait spiders only compared to the plants with actively hunting spiders only or the sit-and-wait + actively hunting spider combination.



**Fig. 3** Effects of spider treatments on ovary fertilization. **a** Proportion of fertilized ovaries (fertilized/total number of ovaries produces per plant); **b** proportion of undamaged seeds (undamaged fertilized ovaries/total number of ovaries produced per plant) on intact [I] and damaged [D] flowerheads. Treatments are: controls without spiders (open bars), sit-and-wait spiders (striped open bars), actively hunting spiders (gray bars), and sit-and-wait + actively hunting spiders (striped gray bars). Bars are means ( $\pm$  binomial 95% CI). Letters above bar indicate significant differences at  $P < 0.05$  following pairwise comparisons

By examining the effect of spiders and flowerhead condition (damaged vs. intact) on seed set (undamaged fertilized ovaries), we found that when flowerheads were damaged, seed set was reduced by 30% compared to undamaged flowerheads ( $\chi^2_{[1,45]} = 112.88$ ,  $P < 0.001$ , Fig. 3b). This indicates that floral herbivory reduces plant fitness. The presence of spiders increased seed set ( $\chi^2_{[3,45]} = 207.18$ ,  $P < 0.001$ , Fig. 3b). The sit-and-wait spiders only treatment showed the strongest positive effects on seed set compared to the other treatments receiving spiders. Despite the potential negative impact of spiders on beneficial floral visitors, we found that spiders increased seed set in both undamaged and damaged flowerheads, suggesting that spiders did not disrupt pollination processes. The spider-by-flowerhead condition interaction term was significant, indicating that spiders, especially the sit-and-wait, attenuated the negative impact of floral herbivory by increasing ovary fertilization ( $\chi^2_{[3,45]} = 16.74$ ,  $P < 0.001$ , Fig. 3b). These results demonstrate that the presence of spiders increases plant fitness by reducing the effects of floral herbivory and increasing ovary fertilization, thus promoting higher seed set.

### Path analysis on the indirect effects of spiders on plant fitness

We evaluated the indirect effects of sit-and-wait spiders (Fig. 4a), actively hunting spiders (Fig. 4b), and spider diversity (0, 1 or 2 species) (Fig. 4c) on seed set through changes in herbivore abundance and ovary fertilization. In all models, herbivore abundance reduced ovary fertilization, suggesting that pollinators may avoid infested flowerheads. Path models independently testing the effects of sit-and-wait (Fig. 4a) and actively hunting spiders (Fig. 4b) showed that spiders indirectly increased seed set by both reducing herbivore abundance and increasing ovary fertilization. Higher ovary fertilization promoted higher seed set in all path models (Fig. 4). The increased diversity of spiders did not affect ovary fertilization, and in this model, the abundance of herbivore did not influence seed set (Fig. 4c). The increased diversity of spiders only affected seed set by decreasing herbivore abundance, which reduced ovary fertilization, suggesting that a negative inter-specific interaction between the two spider species may be occurring. These results showed that in the presence of conspecific spiders, the spiders not only reduced herbivore infestation, but they also increased fertilization rate. This demonstrates that negative effects of spiders on plant fitness are nonexistent in this system. In contrast, the positive effect of spiders on ovary fertilization and seed set is reduced when heterospecific spiders share a

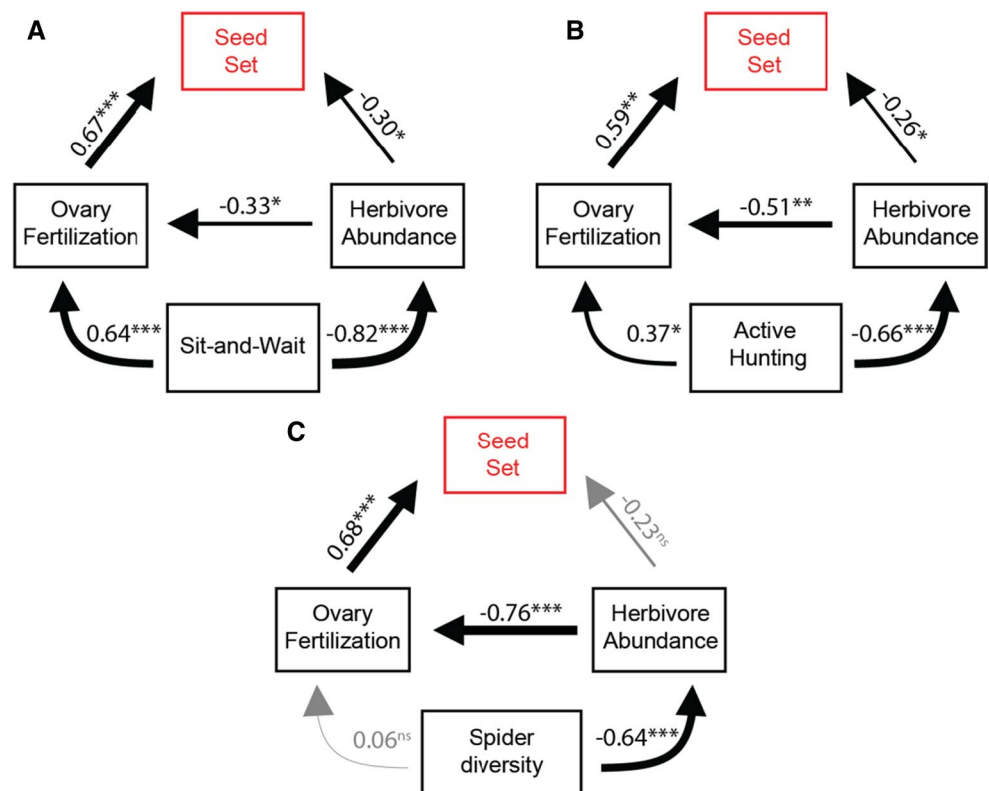
host plant. This suggests that spiders from different species interact negatively, which may affect the overall strength of spider–plant mutualism.

### Discussion

Several studies have successfully demonstrated that increasing the number of predator species often leads to greater herbivore suppression (Finke and Snyder 2008; Straub and Snyder 2008; Schmitz 2009). The current challenge is to identify specific traits of predators that not only strengthen herbivore suppression but also enhance plant fitness. Our results show that although both actively hunting and sit-and-wait spiders dramatically reduced floral herbivory, their combined effect on plant fitness was below the values observed for each species by itself. Thus, the combination of spider species weakened the strength of spider–plant mutualism and greater plant benefit occurred when only one partner was involved.

The magnitude of herbivore suppression and increased plant fitness was contingent on spider hunting mode, underscoring that the strength and not the outcome of this facultative mutualism is variable. It is often expected that the addition of more species of predators will strengthen prey suppression when predator species differ in foraging strategies (Finke and Snyder 2008; Snyder et al. 2008; Schmitz 2009). The stronger effect of multi-predator assemblages on

**Fig. 4** Path diagram of the effect of **a** sit-and-wait spiders, **b** actively hunting spiders, and **c** spider species diversity (sit-and-wait + actively hunting) on ovary fertilization, herbivore abundance, and seed set. Solid black lines denote significant effects, whereas dashed gray lines denote nonsignificant effects. Arrow thickness is scaled to illustrate the relative strength of effects. \* $P < 0.05$ ,  $ns$  not significant



herbivores arises from the increased overall exploitation of available resources when species complement one another or when a key species is found (Chesson 2000). The actively hunting lynx spider *Peucetia* sp. and the sit-and-wait crab spider *Misumenops argenteus* have different body sizes and distinct hunting modes. Although they differ in several traits, their combined impact on herbivore suppression was merely additive (i.e., when the effects of each species by itself or in combination are similar). However, species complementarity occurred on the proportion of damaged ovaries (Fig. 2b), suggesting that although spider diversity had an additive (averaging) effect on herbivore abundance, the combination of spider species reduced herbivory (ovary damage). Because we held spider abundance across all treatments receiving predators constant (all spider treatments received two spiders), it is likely that greater herbivore suppression in this system would arise from higher abundance rather than higher diversity of predators.

In contrast to the additive effect of spider species on herbivore suppression, the combined impact of both spiders on plant fitness was lower than expected by an additive effect. When both spider species shared the same plant, the impact of sit-and-wait spiders was slightly reduced compared to the plants with only sit-and-wait spiders. This antagonistic interspecific effect was revealed in the path analysis and GLMs on plant fitness (Figs. 3b, 4). The effect of spider diversity on seed set was reduced compared to the effect of each species with conspecifics. Predators may interact negatively when they compete for resources or present intra-guild predation (Finke and Denno 2005; Woodcock and Heard 2011). In addition, the total impact of predators relies on both consumptive (i.e., direct predation and density reduction) and nonconsumptive (i.e., predation risk and fear) effects (Schmitz et al. 2004; Preisser et al. 2009; Kersch-Becker and Thaler 2015). In our study, we did not find any evidence for intra-guild predation (consumptive effects), because all spiders were accounted for. However, our results suggest intra-guild predation risk effects. Sit-and-wait spiders may have changed their behavior when predation risk by lynx spiders was perceived (nonconsumptive effects) (Preisser et al. 2009; Miller et al. 2014). In addition, in the presence of heterospecifics, spiders may have displayed adaptive plasticity in their hunting mode (Woodcock and Heard 2011). It is likely that predation risk by actively hunting individuals reduced the benefit of sit-and-wait spiders on plants. This result suggests that the strength of this diffused mutualistic interaction is conditional and that greater plant benefit occurs when only one partner is involved.

Our findings demonstrate that spiders did not influence the total number of flowerheads or the number of ovaries (i.e., flowers) per flowerhead, indicating that these two traits are intrinsic and not conditional on spider protection. The presence of mutualistic spiders, however, increased

seed set. Floral herbivory drastically reduced seed set, but the presence of spiders attenuated this effect. Although spiders may prey upon beneficial floral visitors (Romero et al. 2011; Romero and Koricheva 2011), plants did not appear to suffer fitness costs. This suggests that spiders did not disrupt pollination processes, but likely strengthened plant–pollinator interactions to some extent. Our results indicate that mutualistic spider species enhanced plant fitness by reducing floral herbivory and increasing ovary fertilization.

Our understanding of avoidance behavior of different groups of pollinators in response to predation risk remains limited (Romero et al. 2011), and thus, we cannot identify the specific mechanism associated with increased ovary fertilization. The vulnerability of flower visitors to predators varies (Brechtbühl et al. 2011), which may influence their behavior under predation risk. It is possible that less efficient groups of pollinators (i.e., Coleoptera and Diptera) may have avoided plants with spiders, releasing more specialized groups (i.e., Lepidoptera and Hymenoptera) from competition for plant resources. Alternatively, spider-protected floral buds may be more vigorous, because they are undamaged, thus attracting more floral visitors. We found that the presence of spiders, especially the sit-and-wait species, attenuated the negative impact of floral herbivory on plant fitness.

The stronger impact of sit-and-wait spiders on plants might be attributed to their foraging strategy coupled with architectural traits of plants. During the pre-anthesis phase, flowerhead buds are relatively close to each other; as flowerheads mature, the distance between them increases. Sit-and-wait spiders limit their foraging to a single flowerhead, but the effect of predation risk may still propagate to flowerheads in the vicinity when spatial proximity between them is high. Herbivores oviposit on flowerhead buds, which are spatially close. Pollinators start visiting flowers during anthesis when the distance between flowerheads increases, potentially reducing predation risk cues of sit-and-wait spiders. This may allow pollinators to safely approach the other flowerheads, reducing the potential negative effects of sit-and-wait spiders on pollinators. In contrast, the actively hunting spiders tie two to three flowerheads in anthesis together with silk (see Fig. 1 on Romero et al. 2008), which may confer greater floral protection and stronger negative effects on pollinators compared to sit-and-wait spiders. By tying the flowerheads together, they may promote strong predation risk cues to pollinators. Regardless of the specific mechanism behind the increased ovary fertilization in the presence of spiders, our study shows that the beneficial effect of spiders on ovary fertilization accounts for a great portion of the overall net benefit of mutualistic spiders on seed set. A few studies have attempted to quantify plant fitness costs of defensive mutualisms (Ohm and Miller 2014). Here, we demonstrated that spider partners benefit plants via



herbivore suppression and ovary fertilization, thus enhancing plant fitness through two distinct routes.

We found that the number of ovaries consumed by each *Trupanea* sp. was lower on plants with spiders compared to plants without spiders, and that the reduced consumption was more pronounced on plants occupied by sit-and-wait spiders. We proposed two nonmutually exclusive hypotheses for the reduced consumption of herbivores on plants with spiders. Our first hypothesis is that herbivores may decrease consumption in response to predation risk. Herbivores eat far less when predators are present; this anti-predator behavioral response reduces the likelihood of being detected and potentially killed by a predator (Bernays 1997; Kaplan and Thaler 2010). Lower movement and reduced feeding when predators are present are adaptive responses that have shaped foraging behaviors in herbivores (Stamp and Casey 1993). Thus, herbivores may have perceived the presence of spiders in the vicinity (e.g., volatiles emitted by spiders or spider movement). Our second hypothesis is that reduced herbivore feeding in the presence of spiders may be simply an aggregation artifact. We found that plants without spiders were more heavily infested (i.e., two or more larvae per flowerhead) compared to plants with spiders. More larvae in a flowerhead may cause higher resource competition and, hence, fewer ovaries for individual consumption. Although we cannot identify the specific mechanism behind reduced consumption by herbivores in the presence of spiders, we showed that even though sit-and-wait spiders did not influence flowerhead infestation, they reduced ovary damage by dramatically reducing the consumption rate of herbivores.

Although spiders may prey upon parasitoids (Romero and Vasconcellos-Neto 2004a), we did not detect an effect of spiders on parasitism rate of floral herbivores. This result corroborates the previous findings in this system, showing that the presence of spiders does not affect the parasitism of floral herbivores (Romero and Vasconcellos-Neto 2004a; Romero et al. 2008). This result suggests that potential effects of intra-guild predation between spiders and parasitoids may be negligible or weak in the *T. adenantha* system.

Combined, our results showed that mutualistic spiders benefit plants via positive trophic cascades. Spiders reduced floral herbivory and instead of disrupting pollination processes by preying upon beneficial floral visitors, they enhanced the fertilization of ovaries, promoting greater plant fitness. The hunting mode of predators plays a critical role in trophic cascades (Preisser et al. 2007; Schmitz 2009), and functionally distinct spider species may exert similar or opposing effects on plant partners potentially affecting not only the strength but also the direction of this interaction. We demonstrated that the hunting mode of mutualistic spiders affected the strength, but not the outcome, of the spider–plant mutualism. Overall, plants benefited more from the presence of sit-and-wait

crab spiders than actively hunting lynx spiders, because the former promoted stronger positive cascading effects. Although multiple predator species assemblages may strengthen herbivore suppression, our results pointed to an additive effect of spiders on herbivores. However, the combined effect of spider species on plant fitness was lower-than-additive, suggesting that inter-specific interaction between spiders was slightly antagonistic. Because the magnitude of benefits to plants was reduced when both spider species were present, it is possible that the presence of multiple spider species may carry indirect (“ecological”) costs to spider–plant mutualisms. Thus, our ability to understand and effectively manage natural and agricultural system relies on advancing our knowledge of specific traits of natural enemies that evoke strong trophic cascades by concomitantly suppressing herbivores and enhancing plant fitness.

**Acknowledgements** This study strongly benefitted from comments and suggestions by Oswald Schmitz, Jerome Casas and Shelby Rinehart. This project received financial support the Department of Ecology at State University of Sao Paulo (UNESP) at Rio Claro, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (400892/2014-6), and Fundação de Amparo à Pesquisa do Estado de São Paulo (BPE-FAPESP #2016/01209-9). B.B.G received a fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and M.F.K-B. received the Young Talent Fellowship from CNPq-Brazil (313955/2014-0). G.Q.R. was supported by CNPq-Brazil research grants.

**Author contribution statement** BBG, MJOC, and GQR conceptualized the research; BBG conducted the experiments and collected the data; MFKB analyzed the data; MFKB wrote the manuscript with GQR.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Data accessibility** Data is available as electronic supplementary material.

## References

- Bernays EA (1997) Feeding by lepidopteran larvae is dangerous. *Ecol Entomol* 22:121–123. <https://doi.org/10.1046/j.1365-2311.1997.00042.x>
- Brechbühl R, Casas J, Bacher S (2011) Diet choice of a predator in the wild: overabundance of prey and missed opportunities along the prey capture sequence. *Ecosphere* 2(12):1–15. <https://doi.org/10.1890/ES11-00323.1>
- Cardinale BJ, Srivastava DS, Emmett Duffy J et al (2006) Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature* 443:989–992
- Casula P, Wilby A, Thomas MB (2006) Understanding biodiversity effects on prey in multi-enemy systems. *Ecol Lett* 9:995–1004. <https://doi.org/10.1111/j.1461-0248.2006.00945.x>

- Chesson P (2000) Mechanisms of maintenance of species diversity. *Annu Rev Ecol Syst* 31:343–366. <https://doi.org/10.1146/annurev.ecolsys.31.1.343>
- Dias SC, Carvalho LS, Bonaldo AB, Brescovit AD (2010) Refining the establishment of guilds in Neotropical spiders (Arachnida: Araneae). *J Nat Hist* 44:219–239. <https://doi.org/10.1080/00222930903383503>
- Duffy JE, Cardinale BJ, France KE et al (2007) The functional role of biodiversity in ecosystems: incorporating trophic complexity. *Ecol Lett* 10:522–538
- Finke DL, Denno RF (2005) Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. *Ecol Lett* 8:1299–1306. <https://doi.org/10.1111/j.1461-0248.2005.00832.x>
- Finke DL, Snyder WE (2008) Niche partitioning increases resource exploitation by diverse communities. *Science* 321:1488–1490. <https://doi.org/10.1126/science.1160854>
- Gove AD, Majer JD, Dunn RR (2007) A keystone ant species promotes seed dispersal in a “diffuse” mutualism. *Oecologia* 153:687–697. <https://doi.org/10.1007/s00442-007-0756-5>
- Ives AR, Cardinale BJ, Snyder WE (2005) A synthesis of subdisciplines: predator–prey interactions, and biodiversity and ecosystem functioning. *Ecol Lett* 8:102–116. <https://doi.org/10.1111/j.1461-0248.2004.00698.x>
- Kaplan I, Thaler JS (2010) Plant resistance attenuates the consumptive and non-consumptive impacts of predators on prey. *Oikos* 119:1105–1113. <https://doi.org/10.1111/j.1600-0706.2009.18311.x>
- Kersch-Becker MF, Thaler JS (2015) Plant resistance reduces the strength of consumptive and non-consumptive effects of predators on aphids. *J Anim Ecol* 84:1222–1232. <https://doi.org/10.1111/1365-2656.12371>
- LoPresti EF, Pearse IS, Charles GK (2015) The siren song of a sticky plant: columbines provision mutualist arthropods by attracting and killing passerby insects. *Ecology* 96:2862–2869. <https://doi.org/10.1890/15-0342.1>
- Loreau M, Naeem S, Inchaust P et al (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* 294:804–808. <https://doi.org/10.1126/science.1064088>
- Miller JRB, Ament JM, Schmitz OJ (2014) Fear on the move: predator hunting mode predicts variation in prey mortality and plasticity in prey spatial response. *J Anim Ecol* 83:214–222. <https://doi.org/10.1111/1365-2656.12111>
- Ohm JR, Miller TEX (2014) Balancing anti-herbivore benefits and anti-pollinator costs of defensive mutualists. *Ecology* 95:2924–2935. <https://doi.org/10.1890/13-2309.1>
- Oksanen J, Kindt R, Legendre P et al (2008) *Vegan: community ecology package*. R Foundation for Statistical Computing, Vienna
- Preisser EL, Orrock JL, Schmitz OJ (2007) Predator hunting mode and habitat domain alter nonconsumptive effects in predator–prey interactions. *Ecology* 88:2744–2751. <https://doi.org/10.1890/07-0260.1>
- Preisser EL, Bolnick DI, Benard ME, Benard MF (2009) Scared to death? The effects of intimidation and consumption in predator–prey interactions. *Ecology* 86:501–509. <https://doi.org/10.1890/04-0719>
- Romero GQ, Koricheva J (2011) Contrasting cascade effects of carnivores on plant fitness: a meta-analysis. *J Anim Ecol* 80:696–704. <https://doi.org/10.1111/j.1365-2656.2011.01808.x>
- Romero GQ, Vasconcellos-Neto J (2003) Natural history of *Misumenops argenteus* (Thomisidae): seasonality and the diet on *Trichogoniopsis adenantha* (Asteraceae). *J Arachnol* 31:297–304. <https://doi.org/10.1636/02-19>
- Romero GQ, Vasconcellos-Neto J (2004a) Beneficial effects of flower-dwelling predators on their host plant. *Ecology* 85:446–457. <https://doi.org/10.1890/02-0327>
- Romero GQ, Vasconcellos-Neto J (2004b) Foraging by the flower-dwelling spider, *Misumenops argenteus* (Thomisidae), at high prey density sites. *J Nat Hist* 38:1287–1296. <https://doi.org/10.1080/0022293031000155188>
- Romero GQ, Vasconcellos-Neto J (2005) Flowering phenology, seed set and arthropod guilds in *Trichogoniopsis adenantha* (DC) (Asteraceae) in south-east Brazil. *Rev Bras Botânica* 28:171–178. <https://doi.org/10.1590/S0100-84042005000100014>
- Romero GQ, Souza JC, Vasconcellos-Neto J (2008) Anti-herbivore protection by mutualistic spiders and the role of plant glandular trichomes. *Ecology* 89:3105–3115. <https://doi.org/10.1890/08-0267.1>
- Romero GQ, Antiqueira PAP, Koricheva J (2011) A meta-analysis of predation risk effects on pollinator behaviour. *PLoS ONE* 6:e20689. <https://doi.org/10.1371/journal.pone.0020689>
- Santos AJ, Brescovi AD (2003) A revision of the Neotropical species of the lynx spider genus *Peucetia* Thorell 1869 (Araneae: Oxyopidae). *Insect Syst Evol* 34:95–116
- Schmitz OJ (2007) Predator diversity and trophic interactions. *Ecology* 88:2415–2426. <https://doi.org/10.1890/06-0937.1>
- Schmitz OJ (2008a) Effects of predator hunting mode on grassland ecosystem function. *Science* 319:952–954. <https://doi.org/10.1126/science.1152355>
- Schmitz OJ (2008b) Herbivory from individuals to ecosystems. *Annu Rev Ecol Syst* 39:133–152. <https://doi.org/10.1146/annurev.ecolsys.39.110707.173418>
- Schmitz OJ (2009) Effects of predator functional diversity on grassland ecosystem function. *Ecology* 90:2339–2345. <https://doi.org/10.1890/08-1919.1>
- Schmitz OJ, Krivan V, Ovadia O (2004) Trophic cascades: the primacy of trait-mediated indirect interactions. *Ecol Lett* 7:153–163. <https://doi.org/10.1111/j.1461-0248.2003.00560.x>
- Schmitz OJ, Hawlena D, Trussell GC (2010) Predator control of ecosystem nutrient dynamics. *Ecol Lett* 13:1199–1209. <https://doi.org/10.1111/j.1461-0248.2010.01511.x>
- Snyder GB, Finke DL, Snyder WE (2008) Predator biodiversity strengthens aphid suppression across single- and multiple-species prey communities. *Biol Control* 44:52–60. <https://doi.org/10.1016/j.biocontrol.2007.09.006>
- Stamp NE, Casey TM (1993) *Caterpillars: ecological and evolutionary constraints on foraging*. Chapman & Hall, New York
- Stanton ML (2003) Interacting guilds: moving beyond the pairwise perspective on mutualisms. *Am Nat* 162:S10–S23. <https://doi.org/10.1086/378646>
- Straub CS, Snyder WE (2008) Increasing enemy biodiversity strengthens herbivore suppression on two plant species. *Ecology* 89:1605–1615. <https://doi.org/10.1890/07-0657.1>
- Vasconcellos-Neto J, Romero GQ, Santos AJ, Dippenaar-Schoeman AS (2007) Associations of spiders of the genus *Peucetia* (Oxyopidae) with plants bearing glandular hairs. *Biotropica* 39:221–226. <https://doi.org/10.1111/j.1744-7429.2006.00250.x>
- Woodcock BA, Heard MS (2011) Disentangling the effects of predator hunting mode and habitat domain on the top-down control of insect herbivores. *J Anim Ecol* 80:495–503. <https://doi.org/10.1111/j.1365-2656.2010.01790.x>
- Wootton JT, Emmerson M (2005) Measurement of interaction strength in nature. *Annu Rev Ecol Syst* 36:419–444. <https://doi.org/10.1146/annurev.ecolsys.36.091704.175535>
- Zamora R (2000) Functional equivalence in plant–animal interactions: ecological and evolutionary consequences. *Oikos* 88:442–447. <https://doi.org/10.1034/j.1600-0706.2000.880222.x>