

# Parasitism on seed predators overcomes the detrimental effects of defoliation on plant fitness in a tritrophic system

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**Abstract** It is widely recognised that the interaction between plants and herbivores cannot be completely understood if the natural enemies of the latter are not included. Most studies looking at the effects of herbivores and their enemies on plant fitness only consider one herbivore species or guild; however, plants in nature usually face the attack of more than one herbivore guild simultaneously and these herbivores may have a non-additive effect on the attraction with bodyguards and plant fitness. In this study, we asked whether folivory affects the activity of parasitoids on seed predators and whether this effect cascades down to plant fitness. We assessed these questions in a tritrophic system: the plant *Ruellia nudiflora*, its pre-dispersal seed predators and the parasitoids of the latter. Plants were submitted to either 50 % artificial defoliation or no defoliation (control). The number of seeds, fruit production and parasitoid incidence was assessed periodically in both sets of plants. Parasitoids indirectly and positively affected seed number, while defoliation had a direct negative effect on the number of seeds and an indirect negative effect on parasitoid incidence. However, the combined effect of defoliation and seed predation

increased the indirect positive effect of the parasitoids on seed production, which overcame the negative effects of defoliation.

**Keywords** Folivory · Parasitoid · Pre-dispersal seed predation · *Ruellia nudiflora* · Trophic cascade

## Introduction

Herbivory is a key ecological process with widely recognised effects on primary production, plant population structure and community dynamics (Crawley 1992; Huntly 1991). Herbivores consume a variable amount of photosynthetic and reproductive plant tissue, potentially leading to negative effects on fitness (Blue et al. 2014; Koptur et al. 1996; Lucas-Barbosa 2016; Schwachtje and Baldwin 2008). Plants have several strategies for reducing the impact of herbivores on fitness, and most of them fall within the following categories: direct defences (morphological structures or secondary metabolites which deter herbivores; Chen 2008), indirect defences (plant's alliance with the herbivore's natural enemies; Kessler and Heil 2011) and tolerance (the capacity to reduce the negative effects of damage on plant fitness through compensatory mechanisms; Fornoni 2011; Stowe et al. 2000). Specifically, our study is focused on infochemical-mediated indirect defences (e.g. plant alliance with parasitoids of herbivores), a group of defences that is of recent and growing interest to ecologists. The impact of these defences on plant fitness seems to be variable and is still poorly documented in wild plant species (Hare 2011; Kessler and Heil 2011). Examining the indirect influence of the natural enemies of herbivores on producers may improve our understanding of the fundamental aspects of trophic

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ecology (Price et al. 1980) and is also potentially useful in applied fields such as crop pest management (Fhara-Rheman et al. 2009).

In terrestrial ecosystems, herbivorous insects are extremely species-rich, making up more than a quarter of all described species on Earth (May 1990). Parasitism on herbivorous insects is common in nature (de Meeûs and Renaud 2002) and plays a major role in regulating the host population (Anderson and May 1979). Although there are exceptions (e.g. Xi et al. 2015), most studies have shown that parasitoids reduce plant tissue consumption by herbivores (e.g. Cuautle and Parra-Tabla 2014; Fritzsche-Hoballah and Turlings 2001; van Loon et al. 2000). Therefore, when tissue loss from herbivory has a direct (i.e. seed predation) or indirect effect (i.e. defoliation limits available resources for reproduction) on seed production, the parasitoids of these herbivores may have a positive effect on the producers by reducing the detrimental effects of the consumers on plant fitness (Gomez and Zamora 1994; Halaj and Wise 2001; van Loon et al. 2000). For example, Chattopadhyay et al. (2001) estimated that, on average, seed production in plants attacked by parasitised herbivores is 30 % greater than in plants attacked by unparasitised herbivores. Since the seminal work by Price and colleagues et al. (1980), ecologists have recognised that the interactions between plants and their herbivores cannot be thoroughly understood without considering the enemies of the latter. However, most research on the topic has focused on simplified tritrophic systems where only one species of herbivore is studied (e.g. Abdala-Roberts et al. 2010; Gomez and Zamora 1994; Nakai et al. 2011; Tooker and Hanks 2006; van Loon et al. 2000). In nature, plants are typically attacked by more than one species or guild of herbivores simultaneously (Rodríguez-Saona et al. 2005; Rodríguez-Saona 2012; Walling 2000). A plant's response to one species or guild of herbivores may be affected by the plant's response to another species/guild of herbivores, and this effect, in turn, may cascade up to the third trophic level (i.e. parasitoids) (Rodríguez-Saona et al. 2005; Soler et al. 2007). For example, the size and survival of root herbivores and their parasitoids were negatively affected when the herbivores were reared on plants whose shoots had been damaged previously by aboveground herbivores compared to the size and survival of root herbivores fed undamaged plants (Soler et al. 2007). In another study, the authors found no effect of aphids on folivore caterpillar preference or performance; however, dually damaged plants (aphid and caterpillars) attracted more parasitoids (Rodríguez-Saona et al. 2005). These studies show that plants under attack by more than one species or guild of herbivores may respond in a non-additive manner. However, we know very little about how this interaction affects plant fitness.

Although different herbivore species may elicit a partially different blend of infochemicals in attacked plants (e.g. Turlings et al. 1993), parasitoids usually are unable to discriminate between plants infested only with their herbivore host and plants infested with host and non-host herbivores (De Boer et al. 2008; Moayeri et al. 2007; Vos et al. 2001; but see Zhang et al. 2009). Therefore, it seems that the response of parasitoids to the infochemicals induced by herbivores is frequently not specific (Hare 2011); in fact, some parasitoid species are attracted even by volatiles induced by mechanical damage (Mattiacci et al. 1994; Takabayashi et al. 1991; Whitman and Eller 1990). Plants attacked by multiple herbivores may take advantage of the unspecific response of parasitoids because these plants usually produce a larger quantity of infochemicals, which in turn attracts more parasitoids (relative to a plant attacked by only one herbivore) that eventually will colonise at least a subgroup of the herbivores (De Boer et al. 2008; Moayeri et al. 2007). The effect of this lack of specificity on parasitoids is poorly understood and is probably larger in naive parasitoids, but the energetic cost of the lack of specificity is reduced by parasitoid learning (Hare 2011). In fact, recent research suggests that parasitoid learning is more important for locating their host than hard-wired responses to key stimuli (Hare 2011; Turlings et al. 1993).

In this paper, we asked whether defoliation affects parasitoid incidence (*Bracon* sp. and *Chelonus* sp.) on pre-dispersal seed predators (*Tripudia quadrifera*) and whether this effect cascades down to plant fitness (seed production) in the herb *Ruellia nudiflora*. *T. quadrifera* is the main predator of *R. nudiflora*'s seeds, and its larvae complete all pre-adult stages inside the same fruit where the parasitoid, with the help infochemicals released by the plant, identifies the presence of the larva and oviposits on it (Abdala-Roberts et al. 2010; Cuautle and Parra-Tabla 2014). Both idiobiont (host paralyzing) and koinobiont (non-paralyzing) parasitic wasps use *T. quadrifera* as a host; previous field observations (involving both types of parasitoids) consistently found that fruits attacked by parasitised seed predators had more seeds than those attacked by unparasitised seed predators (Abdala-Roberts et al. 2010; Cuautle and Parra-Tabla 2014). On the other hand, folivory typically reduces fruit and seed production in the study species (Munguía-Rosas et al. 2015). Given that the seeds inside a fruit represent a fixed package of resources available for *T. quadrifera*, it is reasonable to think that seed loss resulting from folivory may affect host performance (i.e. size), which in turn is correlated with parasitoid performance (adult size and larval survivorship) in other systems (Harvey et al. 2003; López et al. 2009; Sznajder and Harvey Sznajder and Harvey 2003). In contrast, the joint effect of herbivores (seed predators and folivores) and the

parasitoids of seed predators on plant fitness is not easily predicted. We would expect a stronger negative effect on fitness when plants are attacked by both types of herbivores; thus, the final effect on seed production would depend on the direct and indirect effects of these herbivores on parasitoids. If parasitoid incidence is lower in plants attacked by the two herbivore guilds, one would expect a net negative effect on seed production. However, if plants attacked by folivores and seed predators exhibit greater parasitoid incidence or effectiveness (i.e. the dually damaged plant may experience a synergistic effect on its infochemical production and attract more parasitoids than singly damaged plants; Rodriguez-Saona et al. 2005) then the net effect on seed production could go from neutral to even positive. We assessed these questions at the plant and fruit levels because this dual approach may provide complementary information about the phenomenon under study. For example, the effect of defoliation on fruit production—which is positively correlated with seed predator abundance (Abdala-Roberts and Mooney 2013)—is a plant-level trait, while the number of seeds at the fruit level is highly relevant to a seed predator, such as *T. quadrifera*, that is unable to move to a different fruit

## Materials and methods

### Study system

*R. nudiflora* (Acanthaceae) is a perennial New World herb which naturally occurs from the south of Texas to the Caribbean (Long 1977; Turner 1991). On the Yucatan Peninsula, it usually inhabits fully exposed or partially shaded habitats like open spaces, road sides and heavily disturbed forests (Cervera and Parra-Tabla 2009; Munguía-Rosas et al. 2012, 2013a). *R. nudiflora* has a dimorphic cleistogamy sexual system (*sensu* Culley and Klooster 2007) meaning that a single plant produces closed obligately self-pollinated cleistogamous (CL) flowers and open potentially outcrossed chasmogamous (CH) flowers (Culley and Klooster 2007). CL flowers are more abundant (ca 85 %) and produced year-round, while CH flowers are far less abundant (15 %) and only produced opportunistically during brief pulses (Munguía-Rosas et al. 2013a). Fruits are dry capsules, and each fruit normally has 10–12 seeds which are attacked before dispersal by the *T. quadrifera* complex (Noctuidae), which has six species and is the only known group of pre-dispersal seed predators of *R. nudiflora* in the Yucatan (Cuautle and Parra-Tabla 2014). The herbivore female oviposits on the fruit, and the larvae spend all pre-adult stages (less than two weeks) inside the same fruit (Abdala-Roberts et al. 2010; Cuautle and Parra-Tabla 2014). This could make it easier for the parasitoids to

identify the seed predator because the latter is virtually immobile (Personal Observation). Typically, only one larva is found per fruit and some parasitic wasp species (*Bracon* sp., *Chelonus* sp.) use *T. quadrifera* as a host (Abdala-Roberts et al. 2010; Cuautle and Parra-Tabla 2014). Parasitoids inspect the outside of the fruit, identify damaged fruits and then lay their eggs on the larvae, introducing the ovipositor through the space between fruit valves (Personal Observation). *Bracon* sp. are idiobiont larval ectoparasite species, and *Chelonus* sp. are koinobiont egg-larval endoparasites. In the study area, both can be found simultaneously parasitising larvae in the same plant or even in the same fruit, and the combined influence of both parasitoid types typically leads to greater seed production relative to plants attacked by un-parasitised larvae (Abdala-Roberts et al. 2010; Munguía-Rosas et al. 2013b). When there are no parasites, larvae may consume from half to all of the seeds inside the fruit before leaving the fruit through an exit hole (Abdala-Roberts et al. 2010; Abdala-Roberts and Mooney 2013; Munguía-Rosas et al. 2013b). It has been estimated that parasitoids may save up to 14 % (average 4 %,  $n = 10$  populations) of *R. nudiflora* seeds from predation under field conditions (Abdala-Roberts et al. 2010). Also, in the study area, *R. nudiflora* leaves are mainly attacked by the larvae of several species of Lepidoptera, the generalists, *Anartia jatrophae* and *Siproeta stelenes* (Nymphalidae) being the folivores with the greatest negative effect on fruit production (Ortegón-Campos et al. 2009). Artificial defoliation also negatively affects fruit and seed production in *R. nudiflora* suggesting that sexual reproduction is compromised by leaf-produced photosynthates in this plant species (Munguía-Rosas et al. 2015)

### Experimental design

#### *Collection and selection of experimental plants*

In early June 2014, we harvested 250 young *R. nudiflora* plants (four or fewer true leaves) from a natural population located on the CINVESTAV campus in the city of Merida, Mexico. Harvested plants were chosen to be as similar as possible in size, reproductive status and number of leaves. The distance from one harvested plant to another was at least 3 m. Plants were taken to a plant nursery located about 1 km away from the original population, on the same campus. This greatly facilitated the logistical aspects of the study and allowed us to conduct the study under semi-natural conditions. Harvested individuals were planted in 1.5 L plastic pots filled with a mixture of soil from the site plus gravel (1:1) to improve drainage. Plants in the nursery were fully exposed to sunlight and watered as needed. We left the plants under these conditions for a couple of

months to acclimatise them to their new environment. During this time, 74 plants died.

### *Defoliation treatment*

In early August 2014, the 176 surviving plants were randomly assigned to an artificial defoliation treatment or to a control group. For plants assigned to the defoliation treatment, 50 % of the leaf lamina was removed from each leaf using scissors ( $n = 87$ ) and this treatment was also applied to new leaves that appeared over the course of the experiment. Plants in the control group were not defoliated ( $n = 89$ ). The defoliation treatment simulated the upper limit of damage intensity observed in some populations a few kilometres away from the study area (Cervera and Parra-Tabla 2009; Munguía-Rosas et al. 2015). As in a previous experiment with the same study species and in the same nursery (Munguía-Rosas et al. 2015), we did not see any folivores on the experimental plants during the study and thus assumed that no additional leaf tissue was removed except that which we removed experimentally. We opted for artificial defoliation because it was quite difficult to control the amount of leaf lamina removed by herbivores, and this may have led to greater error. However, we knew that in the study species damage that is artificially inflicted with scissors affects fruit and seed production in a way similar to damage caused by wild herbivores (Munguía-Rosas et al. 2015). It is also known that, when inflicted regularly, as we did, artificial defoliation also elicits from the plants a blend of infochemicals that attract parasitoids (Takabayashi et al. 1991; Steinberg et al. 1992; Mattiacci et al. 1994).

Owing to the proximity to the collection site, the experimental plants exhibited seed predation rates and parasitoid loads similar to those of un-manipulated plants in the original population.

### *Data collection*

We counted and collected all fully developed fruits two or three times per month. The fruits were stored individually in paper bags. In the laboratory we dissected the fruits, with the help of light microscopes, counted the seeds—only seeds with more than 75 % of their original area—and looked for any evidence of seed predation (remains of larvae, dregs or an exit hole) and parasitoids (cocoons or the parasitoid adult) inside the fruit or, in the case of parasitoids, in the bag where the fruits were stored (Munguía-Rosas et al. 2013b). The presence or absence of parasitoids was recorded per fruit. Parasitoid identity was not recorded because its presence was only indirectly inferred (with cocoons) in a large proportion (>80 %) of the fruits examined. Because only a few CH fruits were collected

from a limited number of plants (<10 %), only the data for CL fruits were included in the analyses (following Abdala-Roberts et al. 2010; Abdala-Roberts and Mooney 2013). Throughout the experiment, any other plant species that germinated in the pot of experimental plants were removed weekly.

## **Data analysis**

### *Plant-level effects*

We assessed the effect of defoliation (a two-level factor) on fruit production per plant (dependant variable) using a generalised linear model (GLM) with a Poisson error distribution and logit link function. The effects of defoliation (factor), the proportion of fruit predated by parasitised seed predators (covariable) and their interaction on the mean number of seeds per fruit (dependent variable) were assessed with an analysis of covariance (ANCOVA). We also assessed the effect of defoliation (factor), fruit production per plant (covariable) and their interaction on the proportion of fruit predated by parasitised seed predators (dependant variable) using a GLM with a binomial error distribution and logit link function.

### *Fruit-level effects*

We assessed the effect of defoliation (factor), parasitoid incidence (binary variable: presence vs. absence) and their interaction on seed number (dependent variable) using a mixed-effects linear model (LME). The effect of defoliation (factor) on parasitoid incidence (dependent binary variable) was also assessed using a mixed-effects generalised linear model (GLMM) with a binomial error distribution and logit link function. The plant was included as a random-effect factor in both models (LME and GLMM).

Seed predation rate was not considered in either of the analyses (plant or fruit level) because predation occurred in almost the entire crop of fruit (99 %); therefore, non-predated fruits were excluded from the analyses. Although different theoretical distributions and nonlinear relationships were tested, the previously described models best fit the data, as confirmed by an *a posteriori* examination of the residuals and deviance (Crawley 2013). All statistical analyses were run in R 3.2.0.

## **Results**

### **Plant-level effects**

Defoliated plants produced significantly less (9.6 %) fruits than plants in the control group (Table 1). However,

**Table 1** Fruit production (Fruit) by *R. nudiflora*, mean number of seeds per Fruit and the proportion of Fruit predated by a pre-dispersal seed predator (*T. quadrifera*) and parasitised by parasitic wasps (proportion of Fruit predated by parasitised seed predators)

Response	Control	Defoliation	Statistic	P value
Fruits	89.28 ± 7.24	81.22 ± 7.47	$X_1^2 = 33.49$	<0.001
Mean number of seeds per fruit	8.95 ± 0.16	8.76 ± 0.16	$F_{1,172} = 0.81$	0.37
Predated fruits with parasitoid	0.96 ± 0.02	0.95 ± 0.01	$X_1^2 = 1.27$	0.26

Plants were randomly allocated to two different groups: a control group (Control) and an experimental group where 50 % of the leaf lamina was artificially removed (Defoliation). Data are mean values ± 1 standard error. The effect of defoliation on fruit production, mean number of seeds per fruit and predated fruits with parasitoids were assessed using GLM–Poisson, ANCOVA and GLM–binomial models, respectively. The results of the statistics and P values are listed in the last two columns

defoliated plants did not differ from control plants in the mean number of seeds per fruit or the proportion of fruits predated by parasitised seed predators (Table 1). There was no interaction effect between the defoliation treatment and the proportion of fruits predated by parasitised seed predators on the mean number of seeds per fruit (Table 2). However, the proportion of fruits predated by parasitised seed predators, alone, was a good predictor of the mean number of seeds per fruit (Table 2); that is, the mean number of seeds per fruit increases with the proportion of fruits predated by parasitised seed predators (Fig. 1). There was no interaction between defoliation and the number of fruits produced per plant on the proportion of fruits attacked by a parasitised seed predator (Table 2). The number of fruits produced per plant is positively correlated with the proportion of fruits predated by parasitised seed predators (Table 2; Fig. 2). However, this relationship exhibited a low degree of fit to the data (explained deviance = 4.5 %) (Fig. 2).

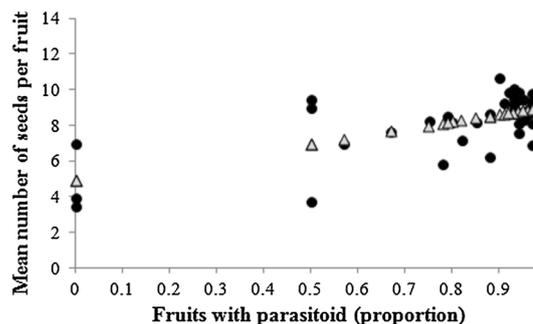
**Fruit-level effects**

There was a non-additive effect of defoliation and parasitoid incidence on the number of seeds per fruit (LME;  $F_{1,1983} = 6.76, P = 0.01$ ); that is, the incidence of parasitoids did not affect seed number (fruit predated by un-

**Table 2** Results of linear models run to assess the influence of the proportion of attacked Fruits by the pre-dispersal seed predator *T. quadrifera* and parasitised by parasitic wasps (predated Fruits with parasitoid) and their interaction with a two-level factor of artificial

Model	Response	Source of variation	Coefficient ± 1 SE	Statistics	P value
1	Mean seed number per fruit	Predated fruits with parasitoid	3.23 ± 1.05	$F_{1,172} = 34$	<0.001
		Predated fruits with parasitoid × Defoliation	1.53 ± 1.41	$F_{1,172} = 1.18$	0.28
2	Predated fruits with parasitoid	Fruits	0.006 ± 0.002	$X_1^2 = 7.82$	0.005
		Fruits × Defoliation	0.004 ± 0.003	$X_1^2 = 1.69$	0.19

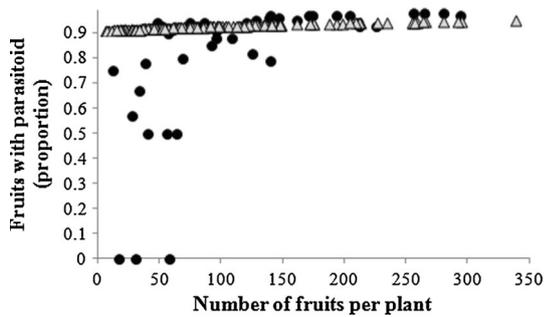
The influence of the number of fruits produced per plant (Fruits) and its interaction with defoliation on the proportion of predated fruits with parasitoids was also assessed in a separate model (Model 2). Model coefficients and their associated statistics are also shown. Model 1 was fitted to an ANCOVA model, and Model 2 to a GLM with a binomial error distribution; therefore, in Model 2, coefficients are in logits. The main effect of defoliation was included in the model, and statistics and coefficients of this term are given in Table 1



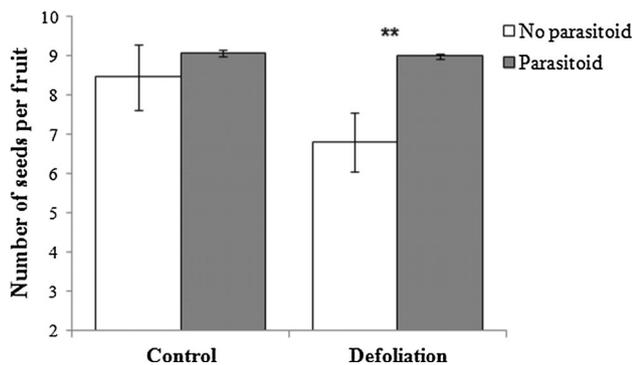
**Fig. 1** Relationship between the mean number of seeds per fruit produced by *R. nudiflora* and the proportion of fruits attacked by a pre-dispersal seed predator (*T. quadrifera*) and parasitised by parasitic wasps (fruits with parasitoid). Black circles represent original data, and grey triangles are the values predicted by a linear model. Analysis was performed at the plant level using linear regression models; the slope is statistically different from zero ( $F_{1,172} = 34, P < 0.001, N = 176$  plants)

parasitised seed predators =  $8.46 ± 0.08$ , fruit predated by parasitised seed predators =  $9.08 ± 0.08$ ) in the plants of the control group. However, in defoliated plants, fruits predated by parasitised seed predators tended to have significantly more seeds ( $9 ± 0.75$ ) than fruits predated by un-parasitised seed predators ( $6.81 ± 0.07$ ) (Fig. 3). The effect of parasitoid incidence alone on the number of seeds was also statistically significant (LME;  $F_{1,1983} = 12.25$ ,

Defoliation (removal of 50 % of the leaf lamina with scissors and a Control group) (Defoliation) on the mean number of seed per Fruit produced by *R. nudiflora* (Model 1)



**Fig. 2** Relationship between the proportion of fruits produced by *R. nudiflora* attacked by a seed predator (*T. quadrifera*) and parasitised by parasitic wasps (fruits with parasitoid) and the number of fruits produced per plant (number of fruits per plant). Black circles represent original data, and grey triangles are back-transformed values predicted by a generalised linear model (GLM). Analysis was performed at the plant level using a GLM with a binomial error distribution and logit link function; the slope is statistically different from zero ( $X_1^2 = 7.82$ ,  $P = 0.005$ ,  $N = 176$  plants)



**Fig. 3** Mean number of seeds in fruits of *R. nudiflora* attacked by a pre-dispersal seed predator. Grey bars represent the number of seeds when the seed predator was parasitised by parasitic wasps, and the white bars show the number of seeds when the seed predator was not parasitised. Plants were also randomly allocated to either a 50 % of artificial defoliation treatment (Defoliation) or a control group (Control). Bars show the mean  $\pm$  1 standard error. Analysis was conducted at the fruit level. Data were fitted to a linear mixed-effects model with the plant as the random factor. The asterisks indicate statistically significant difference (statistics of the interaction Defoliation  $\times$  parasitoid incidence:  $F_{1,1983} = 12.25$ ,  $P < 0.01$ ,  $N = 176$  plants, 2162 fruits)

$P < 0.01$ ), though the effect of defoliation on the number of seeds was not (LME;  $F_{1,1983} = 1.05$ ,  $P = 0.31$ ). Defoliation alone did not affect parasitoid incidence either (GLMM;  $\chi_1^2 = 1.33$ ,  $P = 0.25$ ).

## Discussion

In general, our results suggest that defoliation has a direct and detrimental effect on fruit production in *R. nudiflora*. Defoliation also has a negative effect on parasitoid incidence, but this effect is weak and indirect (via fruit

production). Interestingly, although defoliation directly reduced fruit production and indirectly reduced parasitoid incidence, we did not detect any negative net effect on seed production. Likely, seed loss due to defoliation is compensated by the seeds saved from predation by parasitoids. This suggestion is clearly supported by data. Defoliation actually reduces the number of seeds in fruits attacked by seed predators (Fig. 3); however, if the seed predators are parasitised, seed production is not different between defoliated and control plants (Fig. 3). The suggested mechanism is that plants experiencing damage to their seeds and leaves increase parasitoid efficiency at reducing seed consumption by seed predators by releasing more or qualitatively more effective infochemicals that attract more parasitoids than plants attacked only by seed predators. Because parasitoids indirectly increase plant fitness in the study species, our results support the idea that an association with parasitoids may be ecologically advantageous to the plants, especially when facing the attack of multiple herbivores.

According to the results, on average defoliated plants produced eight fewer fruits than the control plants did (Table 1), corresponding to an approximate seed loss of 88 seeds per plant ( $8 \times 11$  seeds per fruit = 88 seeds). The indirect effects of defoliation via parasitoids may contribute to reported seed loss; however, this indirect effect has only a minor impact on seed production. Using model coefficients shown in Table 2, we predicted that a reduction in eight fruits per plant would reduce parasitoid incidence by only 0.12 % which, in turn, translates into a seed loss of less than half a seed per plant (0.41 seeds). Therefore, we suspect that, as seen in other plant species, the negative impact of defoliation on seed production is mainly due to an allocation trade-off where the removal of leaf lamina reduces available photosynthates and this compromises fruit and seed production (Koptur et al. 1996; Schwachtje and Baldwin 2008; Blue et al. 2014; Lucas-Barbosa 2016). Interestingly, our results also show that parasitoids saved an average of 2.2 seeds per fruit from predation, which corresponds to approximately 169 seeds saved per plant (2.19 seeds saved per fruit  $\times$  81.22 fruits produced per plant on average  $\times$  0.95 [proportion of fruit with parasitised seed predator] = 168.97 seeds). Thus, the predatory action of parasitic wasps on seed predators is big enough to at least cancel out the detrimental effect of defoliation on seed production.

Returning to our research questions: does defoliation affect parasitoid incidence on seed predators? Does this effect cascade down to plant fitness? We would answer yes to both questions but with some concessions. As we predicted, defoliation had a negative, indirect effect on parasitoid incidence (via a reduction in fruit production) but, contrary to expectations, the net effects of defoliation on

seed production range from neutral to positive when the seed predators were parasitised. We suggest that the underlying mechanism is that plants, when damaged by seed predators and folivores, release even more volatiles than plants only damaged by seed predators. The release of a larger quantity of infochemicals has been observed when other plant species are attacked by more than one herbivore guild (Cardoza et al. 2002; Moayeri et al. 2007; Rodriguez-Saona et al. 2005; Shiojiri et al. 2001; but see Abogba and Powell 2007). An extra amount of infochemicals may improve the efficiency of parasitoids in locating the seed predator and stopping seed consumption earlier. While our proposed mechanism should be explicitly tested, future studies could focus on quantifying and characterising the chemical composition of the volatile compounds released by *R. nudiflora* when plants are damaged by one (seed predators) or two herbivore guilds (seed predators and folivores). As mentioned above, we understand that our artificial defoliation treatment may have been a less effective elicitor of these infochemicals. While not as effective as herbivore damage, it is known that artificial defoliation is also an elicitor of infochemicals and attracts parasitoids more effectively than undamaged plants do when damage is inflicted regularly (Mattiacci et al. 1994; Steinberg et al. 1992). We think that the effects shown in this study are conservative and may be even greater when the defoliation is inflicted by herbivores. It may be also interesting to look at whether or not real folivores are parasitised by parasitic wasps attracted by the infochemicals released by *R. nudiflora*; if so, it may amplify the positive indirect effect of parasitoids on plant fitness revealed by this study. The fact that plants damaged by seed predators and mechanical defoliation attract or improve the efficiency of parasitoids at saving seeds supports the idea that the response of parasitoids to the cry for help from the plant (mediated by infochemicals) is not specific (Hare 2011). The energetic cost of this lack of specificity to the parasitoids is probably overcome by a later gain in foraging efficiency by learning and the possibility of finding a suitable, previously unrecognised host (Hare 2011).

Because the alliance between the plant and the natural enemies of the herbivores may affect plant fitness positively, it has been suggested that the plant traits that increase parasitoid effectiveness in searching for herbivore hosts may have some adaptive value (Price et al. 1980). However, some authors have challenged this idea, and one of the main criticisms is that the association between plants and parasitoids does not necessarily lead to greater relative fitness. For example, some parasitoids do not reduce the amount of tissue consumed by their host or the plant may have a high tolerance for herbivory (Kessler and Heil 2011). We argue that the alliance between plants and

parasitoids may affect plant fitness in some plant species but this effect may be context-dependent. For example, in our study system, the positive effect of parasitoids on seed production was more evident in defoliated plants, suggesting that the positive effects of the parasitoids on plant fitness may be stronger in environments with high rates of folivory. The fact that seed predators directly affect a fitness component of *R. nudiflora* (seed set) and that the seed predator has severe mobility restrictions (i.e. parasitoids may locate seed predators more quickly) may have also contributed to our detecting a positive indirect effect of parasitoids on plant fitness.

The study of indirect defences mediated by volatile organic compounds is relatively recent (Kessler and Heil 2011), and most studies have been conducted on domesticated plant species under tightly controlled laboratory conditions (Hare 2011). In this sense, our contribution is important because we studied a wild plant species under semi-natural conditions (parasitoid load, seed predators and relevant abiotic conditions were not manipulated). Also, to our knowledge, no previous study has addressed similar questions at plant and fruit level simultaneously.

In conclusion, parasitoids reduced pre-dispersal seed predation in *R. nudiflora* and this effect was stronger in defoliated plants. Although defoliation had a direct negative effect on seed production and indirect negative effect on parasitoid incidence (via fruit production), defoliation did not have a net negative effect on seed production. The positive indirect effect of parasitoids on seed predation is big enough to at least cancel out the detrimental effects of defoliation. The indirect effect of defoliation on parasitoid incidence by reducing fruit production is very small and led to negligible seed loss. We suggest that the joint influence of seed predators and defoliation has synergistic effect on the effectiveness of parasitoids at reducing seed predation and that this is likely mediated by infochemicals released by the plant.

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