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HOW TO BE A GOOD NON-POLLINATING FIG WASP: GALLING WASPS (*Idarnes* group *flavicollis*) DO NOT INTERFERE WITH THE FLORAL RECEPTIVITY

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Abstract: Mutualistic relationships are open to exploitation by non-cooperative species that can reduce the fitness of one or both cooperating partners. In addition to their obligate agaonid pollinators, a diverse community of non-pollinating fig wasps (NPFW) also uses the figs as a resource for the development of their broods. Some species of NPFW are gall makers and compete with the pollinators for the same pool of pistillate flowers. We experimentally demonstrated that the oviposition of the galling wasp *Idarnes* sp. group *flavicollis* at the beginning of anthesis does not interfere with stigma receptivity or the fig attractiveness of *Ficus citrifolia*. However, in situations of high *Idarnes* sp. infestation, the time window during which pollinators can enter the figs is shortened approximately by half. The enlargement of the *Idarnes* gall compresses the ostiole bracts, leading to an early closing of the fig ostiole, although the figs are still attractive. This negative effect on fig pollination seems to be more important in severely fragmented habitats where heliophilous fig tree species such as *F. citrifolia* are more abundant, favoring population of NPFWs, making over-infestation more frequent.

Keywords: Agaonidae; brood-site pollination; mutualism; nursery pollination; plant-insect interaction.

INTRODUCTION

Mutualistic interactions are powerful source of evolutionary novelty in nature (Sapp 1994). For instance, the mutualistic interaction between pollinating animals, especially flying insects, and plants optimizes the process of cross-pollination in plants (Pellmyr 2002). Therefore, the association with pollinating animals as well as seed dispersers has conferred to flowering plants an impressive adaptive radiation (Price 2002). However, mutualistic relationships are open to exploitation by non-cooperative species that can reduce the fitness of one or both cooperating partners (Yu 2001). Within this context, pollinating mutualisms are explored by nectar-robbing animals such as insects, birds and, more rarely, mammals (Irwin et al. 2010) and non-pollinating competitors/parasites in brood-site pollination mutualisms (Compton et al. 1991, Pellmyr et al. 1996, Yu 2001). The fig-fig wasp mutualism is an excellent model for the study of ecological and evolutionary aspects concerning such ‘parasites of mutualisms’. Fig trees are exclusively pollinated by Agaonidae wasps, which carry pollen into the urn-shaped inflorescence (or fig) that is lined inside with hundreds of unisexual flowers. The female pollinating wasps enter the fig by crawling through an opening formed by bracts (the ostiole), oviposit in the ovaries of some pistillate flowers and pollinate other. Thus, the wasp larva feeds on an ovary of a would-be seed (Galil & Eisikowitch 1968). Besides being visited by pollinators, fig inflorescences are used by a diverse community of chalcid wasps called non-pollinating fig wasps (NPFW), since they do not provide pollination services (Borges 2015). NPFW can interfere with the reproductive success of both fig trees and agaonid pollinators in different ways according to their feeding habits. Some NPFW induce galls in the ovaries of pistillate flowers as pollinators do (Elias *et al.* 2012, Jansen-González *et al.* 2014) or in tissues of the fig receptacle (Bronstein 1999, Ghara *et al.* 2014). Cleptoparasitic NPFWs are phytophagous but are unable to induce their galls, so they lay eggs in galls induced by other wasps and eliminate their larvae in the process (Abdurahiman & Joseph 1978). Other groups of NPFW are parasitoids that feed directly on the larvae of phytophagous fig wasps (Tzeng *et al.* 2008), and some species are facultative (Pereira *et al.* 2007) or obligate seed eaters (Wang *et al.* 2014).

The sexual reproduction of fig trees, and consequently the maintenance of mutualism, is dependent on both the pollination of pistillate flowers (*i.e.*, seed production) and on the development of pollinator offspring in galled ovaries (*i.e.*, production of pollen vectors). However, the receptivity of pistillate flowers occur in a relatively short temporal window that imposes constraints to the mutualism maintenance. The fig trees fail to reproduce if their inflorescences are not visited by pollinators during the receptivity phase (Kjellberg *et al.* 2005). Thus, the selection of facultative floral receptivity prolongation is expected in order to maximize reproductive success. Indeed, experimental studies have demonstrated that, in the absence of pollinators, the pistillate flowers of *Ficus* species remain attractive up to 2-4 weeks. When pollinators normally visit the same figs, the attractiveness of pistillate flowers lasts approximately two days (Khadari *et al.* 1995, Suleman *et al.* 2011, Zhang *et al.* 2012). Shortly after the entry of the pollinating wasps, the release of volatiles responsible for wasp attraction ceases (Hossaert-Mckey *et al.* 2010, Souza *et al.* 2015). The costs of prolonged receptivity seem to be lower for fig inflorescences than for other animal-pollinated flowers. The urceolate inflorescence of *Ficus* protects flowers from damage and stigmas from inappropriate pollen. Moreover, fig trees seem to have a low energetic cost for maintaining prolonged receptivity since the fig has a photosynthetic surface that provides part of the energy expended for its own maintenance (Khadari *et al.* 1995).

Among the NPFWs associated with Neotropical fig trees, the genus *Idarnes* (Hymenoptera: Sycophaginae) is the most diverse one associated with fig trees belonging to *Americanae* section. The genus is divided into three groups of species, namely *I.* gr. *carme*, *I.* gr. *flavicollis* and *I.* gr. *incertus* (Farache *et al.* 2017). Wasps belonging to the *I. carme* species-group oviposit after pollination and are probably cleptoparasites of pollinators' larvae (Pereira *et al.* 2007, Elias *et al.* 2008). The *I. incertus* insects oviposit before pollination and induce galls in tissues of young flowers or fig receptacle, whereas organisms of the *I. flavicollis* species-group lay eggs at the same time that pollinate and induce galls in the ovaries of flowers during anthesis. The *I. flavicollis* wasp's ovipositors are introduced through the flower stigma and style, following the same line as the one followed by the ovipositors of the pollinating wasps (Elias *et al.* 2012). The eggs of *Idarnes* group *flavicollis* are laid at the exact location where the pollinator's egg would have been laid, *i.e.*, between the integument and nucellus (Elias *et al.* 2012, Jansen-González *et al.* 2014).

Since females of *Idarnes* group *flavicollis* mimic the mode by which pollinating wasps lay their eggs (*i.e.*, they insert the ovipositor through the flower stile and lay the egg between the integument and nucellus), one can raise the hypothesis that their oviposition interferes with the flower's receptivity, as done by pollinating wasps. Therefore, the pollination success of an individual fig tree may be negatively affected if the oviposition by *Idarnes* group *flavicollis* reduces the receptivity window. Indeed, in disturbed environments where the population density of NPFWs is relatively high occasionally an entire fig crop of *Ficus citrifolia* Mil. is not pollinated, but *Idarnes* group *flavicollis* and some cleptoparasite and parasitoid species can develop in those figs. Wasps of *Idarnes* group *flavicollis* represent about 40% of all insects developing in unpollinated figs (R. A. S. Pereira unpublished data).

Pollinating fig wasps are attracted to receptive figs by volatile compounds (Grison-Pigé *et al.* 2002, Borges *et al.* 2013) produced by scent glands located on the ostiolar bracts and outer layers of the fig receptacle (Souza *et al.* 2015). The fig's attractiveness and the flower's stigma receptivity are separate physical and possibly physiological events in the fig inflorescences. Thus, these events should be distinctly assessed in order to obtain a comprehensive understanding of the period during which fig inflorescences are visited by pollinating wasps. Within this context, we studied here the role of *Idarnes* sp. group *flavicollis* in the pollination of *F. citrifolia.* Specifically, we intended to determine whether (1) the oviposition of *Idarnes* sp. group *flavicollis* affects the attractiveness of the pollinating fig wasps and the receptivity of flower stigmas, and (2) whether the production of pollinating wasps and seeds varies along the fig receptive period (with and without concurrence of *Idarnes* sp. group *flavicollis*).

MATERIAL AND METHODS

Study site and species

The study was carried out on eitgth *F. citrifolia* trees spontaneously growing on the Ribeirao Preto campus of the University of Sao Paulo (21°10′03.05” S, 47°51′18.23” W, *datum* WGS84). *Ficus citrifolia* is a monoecious hemiepiphytic tree about 3–6 m tall, widespread in the Americas from Florida to northern Argentina (Berg & Villavicencio 2004). It belongs to the subgenus *Urostigma* section *Americanae* and is pollinated in Sao Paulo state by an undescribed *Pegoscapus* species. The studied species of *Idarnes* group *flavicollis* (hereafter referred to as *Idarnes* only) colonizes the figs during the same period when the pollinators visit it and induces galls indiscriminately in the ovaries of pollinated and unpollinated flowers (Elias *et al.* 2008, Jansen-González *et al.* 2014).

Data collection

The fig tree receptivity experiment was repeated three times: February and March 2008, and June 2009. The attractiveness experiment was repeated three times in three fig trees in February, March and June 2009, and the reproductive success experiment was carried out twice in two different trees (March and June 2009). Insects were collected under the permission for collecting zoological material (SISBIO nº 10657-2).

Stigma receptivity

To test stigma receptivity we used a Peroxtesmo esterase indicator (Peroxtesmo KO) paper (Dafni & Maués 1998). The figs to be tested (see below) were cut open and a piece of indicator paper (3 x 3 mm) previously moistened in distilled water was placed directly on the stigma surfaces. We avoided touching indicator paper at the cut borders of the fig since the damaged plant tissues give a positive response because of esterases presence (Dafni & Maués 1998). The forceps used to manipulate the indicator paper was washed in alcohol and distilled water after each test to avoid contamination across treatments. The stigmata were considered receptive (*i.e.*, positive result) when the indicator paper turned blue up to 10 seconds after contact. The indicator paper placed on non-receptive stigmata did not color or became light blue after a period of more than 10 seconds (Figure 1a). When a negative result was recorded, three additional figs from the same treatment were collected and tested for receptiveness to confirm the negative result.

For each fig tree experiment, we bagged 10 branches bearing young developing figs to avoid access by any fig wasp. Bags were made of voile cloth. Five branches were randomly used as control treatment (no wasp access) and five were labeled as *Idarnes* treatment. The beginning of the receptivity period was assumed to correspond to the first detection of pollinating wasps (*i.e.*, foundresses) entering non-bagged figs. On the first day of receptivity, approximately 40 figs from 5 bagged branches were exposed to female *Idarnes* sp. wasps collected at their emergence from figs from other *F. citrifolia* tree in the same area. Each fig was labeled and individually bagged with small voile bags and two *Idarnes* sp. females were introduced into each bag to ensure that all experimental figs were exposed to the wasps. The individual bags were removed after 24 h and the whole branch was bagged again. Two figs from two different branches of each treatment (*i.e.*, control and *Idarnes*) were collected every two days in order to test the receptivity of their stigmata in the laboratory as described above. Figs were collected from 24 h after the day of exposure to *Idarnes* sp. wasps up to the end of the receptivity period.

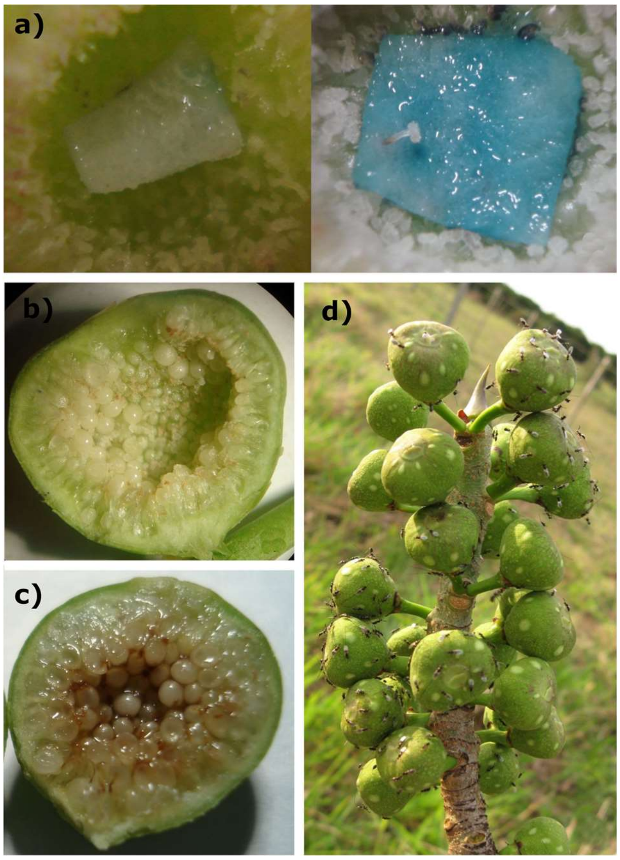

Figure 1. a) Peroxtesmo esterase indicator paper: negative (left) and positive (right) results. b) Fig with galls approximately 15 days after the oviposition of *Idarnes* wasps. c) Fig from the experiment of production of pollinating wasps and seeds under high level of *Idarnes* infestation. d) Figs of *Ficus citrifolia* over-infested by *Idarnes* group *flavicollis* wasps (photo: L. F. M. Coelho).

Fig attractiveness

We observed pollinator's behavior when placed close to the fig ostiole to test fig attractiveness. We placed one pollinating wasp close to the ostiole of a newly unbagged fig using a thin forceps to pick the wasp by its wings. The fig was considered attractive if the wasp attempted to enter it through the ostiole. The wasp was then immediately removed and the fig was bagged again. If the wasp did not attempt to enter it over a period of 5 minutes, the procedure was repeated twice with two different wasps. The fig was considered non-attractive if wasps failed to enter it in the three attempts.

Ten branches bearing young developing figs were previously bagged to avoid access by any fig wasp. Five branches were randomly used as control treatment (no wasp access) and five were used for the *Idarnes* treatment. The beginning of the receptivity period was identified by the first detection of foundress wasps in unbagged figs. On the first day of receptivity, approximately 30 figs from 5 bagged branches were exposed to female *Idarnes* sp. wasps, as described for the receptivity experiment in the previous section. The experiment was started 24 h after the day of exposure of the figs to *Idarnes* sp. wasps, and the figs were monitored up to the end of the attractiveness period. We tested the attractiveness of 30 figs per treatment (*i.e.*, control and *Idarnes*) every two days. To perform the test, we temporarily removed the voile bag and individually bagged each experimental fig (with small voile bags) to avoid natural colonization by pollinating wasps from the wild. To test each individual fig, the small bag was removed and a newly emerged pollinating wasp collected from another *F. citrifolia* tree in the same area was placed close to the fig ostiole, as described above. Immediately after the test, the individual fig was bagged again. After all tests, the individual bags were removed and the whole branch was bagged again.

Production of pollinating wasps and seeds along the receptive period

Ten branches bearing young developing figs were previously bagged to avoid access by any fig wasp. Five branches were randomly used as control treatment (no wasp access) and five were used for the *Idarnes* treatment. On the first day of receptivity, approximately 40 figs from 5 bagged branches were exposed to female *Idarnes* sp. wasps as described for the receptivity experiment. The experiment was started 24 h after the day of exposure of the figs to *Idarnes* sp. wasps, and the figs were monitored up to the end of the receptive period. Every two days, one newly emerged pollinating wasp collected from another *F. citrifolia* tree was manually introduced into each fig, for a total of 6 figs per treatment/day. For pollinator introduction we placed the wasp close to the ostiole of a newly unbagged fig using a fine brush to pick up the wasp. After wasp introduction, the branch was kept bagged until just before offspring emergence. The figs were then collected and placed individually in 50 mL plastic flasks to allow emergence of the wasps, which occurred within 48 h. Figs were cut open to sort the emerged and non-emerged wasps. For each fig we analyzed (1) number of pollinating wasps, (2) number of seeds, (3) number of *Idarnes* sp. wasps, (4) number of bladders (empty galls where the wasp larva has died during its development), and (5) pollinator brood sex ratios.

This experiment was repeated twice in two different fig trees. In the first experiment (March 2009), we exposed each fig to two newly emerged *Idarnes* sp. females. In the second experiment (June 2009), we exposed each fig to five *Idarnes* sp. females to assess the effect of a higher infestation level.

Data analysis

The length of stigma receptivity was estimated by the total number of days during which enzymatic activity was detected. We used a linear model (ANOVA) to test the effects of treatments (*i.e.*, control and *Idarnes*) on the length of fig attractiveness using each fig tree as a repetition. We performed the analyses for each separate experiment repetition (*i.e.*, tree a, b and c), and for the whole dataset, including data of all three experiments. For the whole dataset, we included in the model the tree assignment as a covariate.

The production of wasps and seeds, offspring sex ratios and larval mortality in *Idarnes*-infested and control treatments were graphically compared along the receptive period.

RESULTS

Stigma receptivity

Stigma receptivity lasted seven to ten days according to the period when the experiments were carried out. The oviposition of *Idarnes* sp. wasps at the beginning of the receptive period did not significantly affect stigma receptivity (Table 1) and was confirmed by the presence of developing galls after the receptive period (Figure 1b).

Fig attractiveness

Fig attractiveness lasted, on average, 6.4 to seven days, according to the period when the experiments were carried out. The presence of developed galls after the receptive period indicated oviposition of *Idarnes* sp. wasps that did not affect the total length of fig attractiveness (Table 2). The percentage of attractive figs was high in both treatments up to the fifth day (≥ 90% of attractive figs), but attractiveness decreased to 50% by the seventh to eighth day (Figure 2).

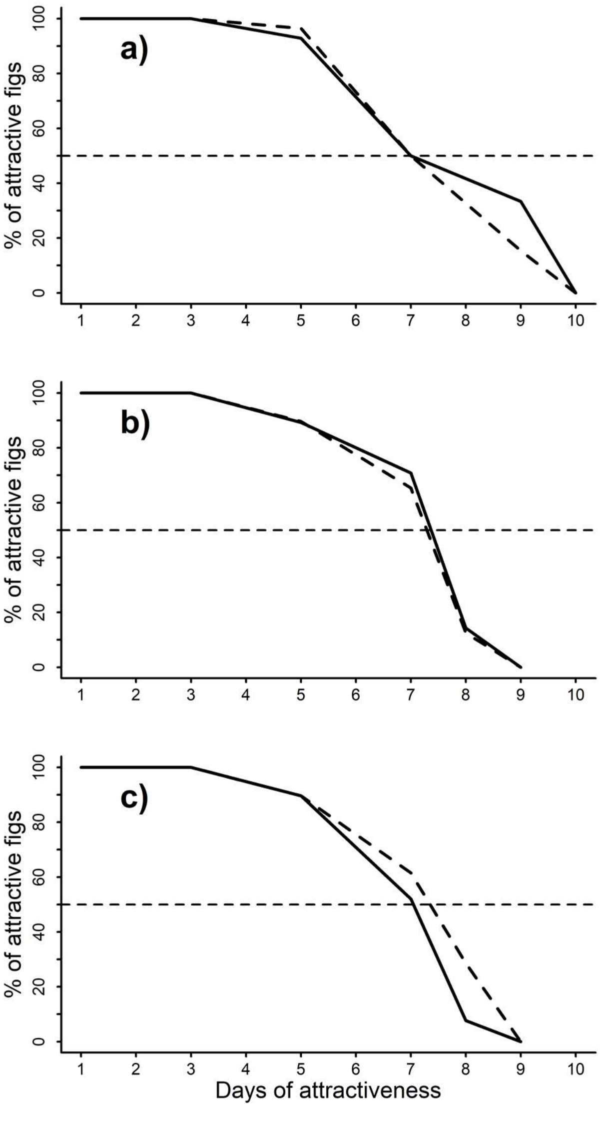

Figure 2. Percentage of attractive non-infested (solid line) and infested figs (dashed line) with *Idarnes* group *flavicollis* wasps along the receptive period. The experiment was carried out in three different trees: a) February/2009; b) March/2009; and c) June/2009. See 'Material and Methods' for sample sizes.

Production of pollinating wasps and seeds along the receptive period

In both experiments (*i.e.*, tree 1: 2 *Idarnes* sp. females/fig; tree 2: 5 *Idarnes* sp. females/fig), figs

Table 1. Length in days of stigma receptivity of non-infested (control) and infested figs with *Idarnes* sp. group *flavicollis* wasps (*Idarnes*). The experiment was carried out in three different trees: a) February/2008; b) March/2008 and; c) June/2009. See 'Material and Methods' for sample sizes.

Experiments	**Control**	***Idarnes***	**ANOVA**
Tree (a)	7	7	-
Tree (b)	9	10	-
Tree (c)	10	10	-
Mean ± SD	8.7 ± 0.9	9 ± 1	$F_{1,4}$ = 0.063, p = 0.814

Table 2. ANOVA results to compare length in days (mean ± SD) of attractiveness of non-infested (control) and infested figs with *Idarnes* sp. group *flavicollis* wasps (*Idarnes*). The experiment was carried out in three different trees: a) February/2009; b) March/2009; and c) June/2009. df = degrees of freedom for effects and residuals, respectively. See 'Material and Methods' for sample sizes.

Effect	**Control**	***Idarnes***	**F**	**p**
Separate experiments				
Tree (a)	6.9 ± 1.4	7.0 ± 1.3	0.009 (df: 1, 53)	0.925
Tree (b)	6.5 ± 1.1	6.5 ± 1.0	0.062 (df: 1, 54)	0.804
Tree (c)	6.4 ± 0.9	6.4 ± 1.12	0.105 (df: 1, 53)	0.748
Complete dataset				
Treatment	-	-	0.005 (df: 1, 162)	0.925
Trees	-	-	3.678 (df: 2, 162)	0.027

at the beginning of the attractive period produced a larger pollinator offspring (Figure 3a-b). In the experiment with a lower level of *Idarnes* sp. infestation (*i.e.*, tree 1; Figure 3a), pollinators introduced on the last attractive day produced markedly smaller offspring. At the higher infestation level (*i.e.*, tree 2), pollinators failed to enter the figs from the fifth day of attractiveness and these figs consequently did not produce any pollinator offspring (Figure 3b). The growth of a large number of *Idarnes* galls modified the internal fig structure, making the fig ostiole less permeable to wasps since the ostiolar bracts were compressed by gall enlargement (Figure 1c). Thus, from the fifth day of attractiveness pollinators were trapped between ostiole bracts when attempting to enter the fig. In general, pollinators produced smaller offspring in figs previously infested with *Idarnes* sp., probably due to the competition for oviposition sites (Figure 3a-b).

The production of seeds varied in the figs that pollinators managed to enter, without a clear pattern along the attractive period (Figure 3e). The pollination process seemed to be less affected by previous *Idarnes* sp. infestation and by the age of the fig flowers since a marked reduction of seed production was not observed in infested or older figs. However, in the experiment with a higher infestation level, the production of seeds was lower in figs previously colonized by *Idarnes* sp. since a portion of the flowers that could produce seeds was probably used by *Idarnes* sp. females to lay their eggs (Figure 3f).

As expected, the number of *Idarnes* sp. offspring in tree 2 was five times larger than in tree 1 (Figure 4a-b). Both pollinator and *Idarnes* sp. sex ratios were seemingly unaffected by fig age or previous *Idarnes* sp. infestation (Figures 3c-d, 4c-d). Larval mortality (*i.e.,* number of bladders) in the experiment with a lower level of *Idarnes* sp. infestation was higher in figs in which pollinators were introduced on the last attractive day (Figure 4e). In the experiment with a higher infestation level, larval mortality was lower in the *Idarnes* treatment probably due to the lower number of produced pollinators (Figure 4f).

DISCUSSION

Fig colonization by the galling wasp *Idarnes* sp. *flavicollis* group does not interfere with stigma receptivity or fig attractiveness of *F. citrifolia.* However, in situations of high *Idarnes* sp.

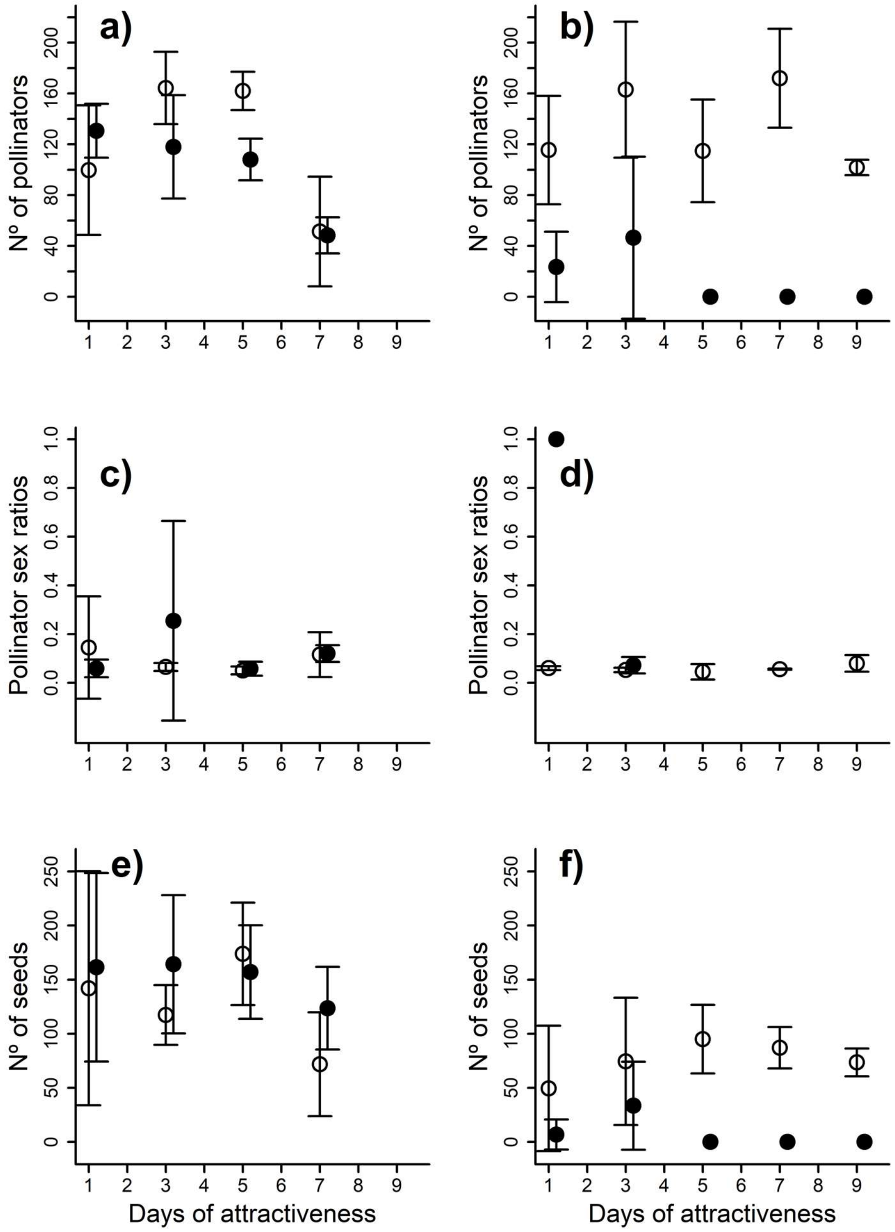

Figure 3. Average number (± SD) of *Pegoscapus* sp. wasps, *Pegoscapus* sex ratios (± SD) and number of seeds (± SD) produced in non-infested (empty circle) and infested figs (solid circle) with *Idarnes* group *flavicollis* wasps. The experiment was carried out in two different trees: a, c and e) March/2009, representing the lowest level of infestation by wasps; b, d and f) June/2009, representing the highest level of infestation by wasps. See 'Material and Methods' for sample sizes.

infestation, the time window during which pollinators can enter the figs was approximately half shortened. This is the consequence of an early closing of the fig ostiole due to the compression of the fig bracts caused by the *Idarnes* sp. gall enlargement. Pollinators failed to enter the figs from the fifth day of attractiveness (*i.e.*, they were trapped between the ostiole bracts), although the figs were still attractive up to the ninth day. Such high level of *Idarnes* sp. infestation (*e.g.*, up to 186 wasps per fig, data not shown) is not common in nature (R. A. S. Pereira, unpublished data), but seems to be more frequent in disturbed areas and forest edges where the density of *F. citrifolia* trees is higher (Elias *et al.* 2007, Coelho *et al.* 2014). A higher density of fig trees probably favors the

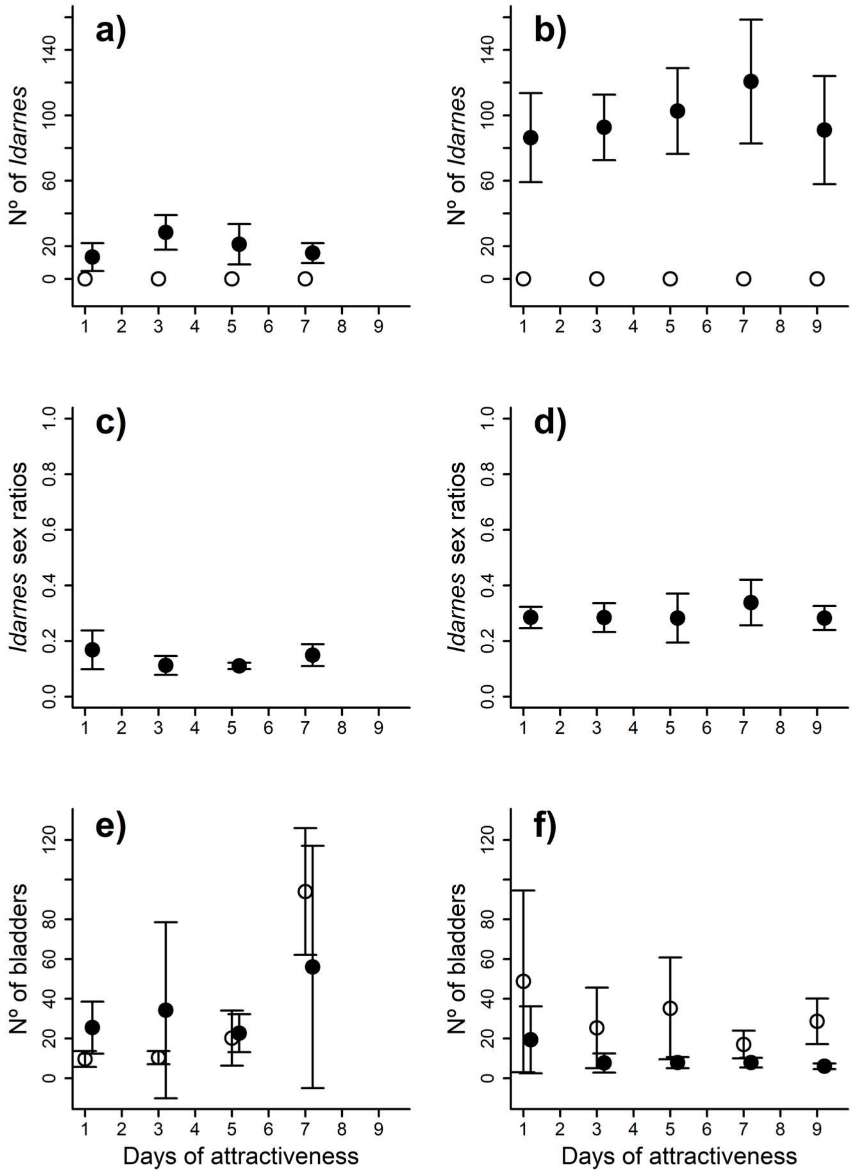

Figure 4. Average number (± SD) of *Idarnes* group *flavicollis* wasps, *Idarnes* sex ratios (± SD) and number of bladders (± SD) produced in non-infested (empty circle) and infested figs (solid circle) with *Idarnes* group *flavicollis* wasps. The experiment was carried out in two different trees: a, c and e) March/2009; b, d and f) June/2009. See 'Material and Methods' for sample sizes.

maintenance of a local population of NPFWs that may result in occasional over-infestation levels (Figure 1d). Our results also showed that the attractive period (6.4 to 7 days on average) is slightly shorter than the length of stigma receptiveness (7 to 10 days), suggesting that these are two distinct floral processes. In fact, the volatile compounds responsible for pollinator attraction are produced and released by scent glands located on the ostiolar bracts and outer layers of the fig receptacle (Souza *et al.* 2015), structurally independent of the pistillate flowers.

Our results provide insights for the understanding of the selective pressures that molded the intricate relationships among *Idarnes* group *flavicollis*, fig trees, and *Pegoscapus* pollinators. This interspecific competition for oviposition sites seems to be driven by a trade-off between accessibility to resources (*i.e.*, flower ovaries) and chances of future reproduction. *Pegoscapus* foundresses have a prompt access to pistillate flowers as they enter the fig cavity to lay their eggs. However, they have limited chances of future reproduction. The probability of a foundress wasp to reemerge from the fig cavity of an *Americanae* species and successfully enter another receptive fig is extremely low, as the foundress wasp has a short lifespan and loses part of its antennae and wings when passing through the ostiole (Kjellberg *et al.* 1988, Dunn *et al.* 2008, Jevanandam *et al.* 2013). Thus, *Pegoscapus* females experience a strong resource competition in figs visited by more than one foundress. In fact, *Pegoscapus* females can fatally fight for ovipositing sites inside the fig cavity (Dunn *et al.* 2015). In contrast, *Idarnes* group *flavicollis* females have a longer lifespan and can avoid resource competition by spreading their eggs among several figs (higher chances of future reproduction). However, they spend more time to gain access to flower ovaries, as they probe the fig cavity from the external fig's surface (Elias *et al.* 2012). In addition, patrolling ants frequently prey NPFWs when they oviposit from the fig surface (Bain *et al.* 2014). Therefore, a shorter time window of fig attractiveness/receptivity potentially favors *Idarnes* group *flavicollis* in the competition for ovipositing sites. Within this context, our findings were unexpected since the ability to shorten the fig attractiveness/receptivity period should be selected for in *Idarnes* sp. wasps. Other ovary fig gallings, *e.g.*, *Walkerella yashiroi* and *Sycobia* sp. associated with the Asian *F. microcarpa* and *F. benjamina*, respectively, do not rely on pollinating fig wasps to complete their life cycles, as they are able to induce galls in unpollinated flowers and their males can chew the exit hole through the fig wall to release the dispersing females. Indeed, these wasps were introduced in the neotropics before their host pollinators (Figueiredo & Motta Junior 1993, Farache *et al.* in press), demonstrating their independence. *Idarnes* sp. group *flavicollis* can also induce galls in unpollinated flowers (Jansen-González *et al.* 2014) and even produces offspring with larger wasps when developing in figs without competition from the pollinators (Elias *et al.* 2012). However, they depend on male pollinators to exit the natal fig, as *Idarnes* sp. males cannot open the exit hole through the fig wall (Elias *et al.* 2008). Thus, the dependence on pollinator offspring to exit the fig may constrain the ability to manipulate the fig attractiveness/receptivity. A shorter time window for pollinator visits therefore can be unfavorable to *Idarnes* sp. wasp fitness since it increases the probability of their offspring to die trapped inside the fig cavity if no pollinator males are available to open the exit hole. The reasons why the capacity to open an exit hole did not evolve in *Idarnes* sp. have not being investigated, but one hypothesis is that the aggressive behavior of *Idarnes* males due to the local mate competition (Pereira & Prado 2005, 2008) constrains the selection of the cooperative traits required to perform the work of chewing an exit hole.

Although it does not interfere with fig attractiveness or stigma receptivity, *Idarnes* sp. group *flavicollis* can negatively affect both male and female fig tree's functions by competing with the pollinating species for flower ovaries that would produce pollinator offspring (*i.e.*, pollen vectors) and seeds. In more preserved habitats, this negative impact seems to be of little evolutionary importance because *Idarnes* wasps occur at low abundance (R. A. S. Pereira, unpublished data). However, our results demonstrated that under high infestation levels the primary components of the fig's reproductive success could be null when the early development of *Idarnes* sp. galls leads to a premature closure of the fig ostiole. In this situation, the foundress wasps are stuck between the ostiole bracts and no seeds or pollinator offspring are produced. In the last century, Brazilian seasonal semi deciduous forests were reduced to less than 10% of their original area and the remaining fragments, usually < 100 ha, are surrounded by extensive sugarcane fields, favoring the population of heliophilous fig trees such as *F. citrifolia* (Coelho *et al.* 2014). This recent fragmentation context caused by man has exposed the fig-fig wasp mutualism to a new selective pressure (*e.g.*, higher population density of NPFWs) never experienced by the involved species during their evolutionary history. Therefore, the long-term effect of galling NPFWs such as *Idarnes* sp. group *flavicollis* on the pollination mutualism and consequently on the other animals associated with fig trees is unpredictable.

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