

# Colonization sequence of non-pollinating fig wasps associated with *Ficus citrifolia* in Brazil

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## Abstract

Mutualisms, such as the fig-agaonid wasp association, are susceptible to colonization by parasitic species, which exploit the resources involved therein. In most cases, they oviposit into the figs from outside without providing any pollination service. In this study, we used several different methods (adhesive traps and direct standardized field observations) to assess the colonization sequence of a diverse fig wasp fauna associated with *Ficus citrifolia*, section *Americana*, in Brazil. They consistently showed a temporal partitioning in colonization among non-pollinating fig wasp species. *Idarnes* species belonging to the *flavicollis* and *incerta* groups colonized figs just before or during the fig receptive phase. In contrast, *Idarnes* females belonging to the *carne* group oviposited one to three weeks later, mainly in the middle of the inter-floral phase. *Eurytoma*, *Heterandrium*, *Physothorax* and *Torymus* were later colonizers, and laid eggs either in the middle or during the late inter-floral phase. The results suggest that these Neotropical fig wasps have different strategies of resource exploitation, even among species belonging to the same genus.

**Keywords:** Agaonidae, *Ficus*, mutualism, resource exploitation

## 1. Introduction

The specialized association between fig trees and their pollinating agaonid wasps is a classical example of mutualism between plants and insects (Weiblen, 2002). Such mutualistic interaction is susceptible to exploitation by parasitic species, which take advantage of the resources involved in the interaction (Yu, 2001). Indeed, several chalcid species that use both mutualists as resources to develop their offspring exploit the fig-fig wasp system (Weiblen, 2002). The natural history of this association can be summarized as follows: winged female agaonid wasps, (a) enter the urn-shaped *Ficus* inflorescences (hereafter called figs) through the ostiole, (b) pollinate the female flowers and simultaneously, (c) oviposit into the ovaries of some of them (Jousselin et al., 2001b). Thus, their larvae develop in galled ovaries. In addition to pollinating wasps, other non-pollinating fig wasp (NPFW) species, that usually oviposit externally through the fig wall (but see

Jousselin et al., 2001a), develop in flower ovaries or parasitize larvae of primary galling wasps (Bronstein, 1992). NPFW includes species with diverse larval biologies: gallers, inquilines (or kleptoparasites) and parasitoids (Kjellberg et al., 2005). Phytophagous gallers colonize figs at the same time or before the pollinating females (foundresses). Inquilines are also phytophagous, but they are not able to induce galls. Therefore, they oviposit in already induced galls and in the process eliminate the galler larvae. Parasitoids feed directly on the galler larvae (Abdurahiman and Joseph, 1978). However, the biology of most of NPFW species is poorly documented (West et al., 1996; Kjellberg et al., 2005). Detailed studies on colonization sequence of NPFW were carried out only in Old World *Ficus* species, subgenus *Sycomorus* and *Urostigma* (Kerdelhué and Rasplus, 1996; Compton, 1993, respectively) and *Sycomorus* (Profitt et al., 2007).

Temporal segregation of colonization time among NPFW is due to the use of different volatile signs produced by figs at different developmental stages (Profitt et al., 2007). The sequence of fig development leads to a

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progressive shift in available resources. Gallers oviposit early in figs, but space constraints probably prohibit later oviposition: the ovules swell rapidly after pollination so that there is no space left for new galls to develop. Indeed, the largest wasps are species that oviposit well before receptivity, which enables galls to grow larger (Cook and Rasplus, 2003; Kjellberg et al., 2005). It seems reasonable to assume that wasps that oviposit after fig pollination should be inquilines or parasitoids. Despite differences on timing during the colonization period, the offspring of all fig wasp species complete their development at the same time, indicating strong variation in developmental length (Kerdelhué and Rasplus, 1996). Therefore, parasitoids that parasitize late-developed larvae must have a very rapid development themselves. Hence, fig development and, consequently, its internal structure strongly restrict wasp strategies.

The effect of NPFW on the fig-fig wasp mutualism differs according to their larval biology (Bronstein, 1992). Gallers exert a negative effect on production of both seeds and pollinators, whereas inquilines and parasitoids affect directly only the production of pollinators and other galler species. However, Pereira et al. (2007a) have demonstrated that a Neotropical inquiline species can also use fig seeds as an alternative low quality resource to develop their offspring. The observation of colonization sequences can indirectly reveal such a broader range of resource utilization. Therefore, not only can colonization sequences bring insights on the NPFW strategies, but also on their impact on the fig-fig wasp mutualism.

In the present study, we used different methods (adhesive traps and direct standardized observations) to assess, for the first time, the colonization sequence of a diverse fig wasp fauna associated with a Neotropical *Ficus* species.

## 2. Materials and Methods

### *Study sites*

The current study was carried out in two Brazilian campuses: at Universidade Estadual de Londrina (UEL), Londrina city (23°23'S, 51°11'W), and at Universidade de São Paulo (USP), Ribeirão Preto city (21°10'S, 47°48'W). Field experiments were carried out in one *Ficus citrifolia* P. Miller tree at UEL from September to November, 1994, and in another two trees at USP from September to November, 2005. Both sites are covered with gardens and lawns where several *F. citrifolia* trees grow spontaneously.

### *Study species*

*Ficus citrifolia* (subgenus *Urostigma*, section

*Americana*) is a monoecious hemi-epiphytic tree, 3–6 meters tall, that frequently develops within disturbed areas (R.A.S. Pereira, personal observation). In Brazil, *F. citrifolia* is pollinated by *Pegoscapus* near *tonduzi* (J.Y. Rasplus, personal information) and is associated with 14 other non-pollinating chalcid wasp species (Pereira et al., 2000, referred to as *F. eximia*).

Among the non-pollinating species, the genus *Idarnes* is the best represented. *Idarnes* species with apterous males are split into the *carne* and *flavicollis* species groups. In contrast, species with winged males belong to the *incerta* group. The remaining NPFW genera associated with *F. citrifolia* are *Aepocerus* (Epichrysomallinae), *Eurytoma* (Eurytomidae), *Heterandrium* (Epichrysomallinae), *Physothorax*, and *Torymus* (both Torymidae).

### *Colonization sequence*

We used three methods to assess the colonization sequence: fig traps (used at UEL) and adhesive traps and monitoring of control-pollinated figs (both used in the two trees at USP).

Fig traps consisted of 30 figs from two branches (15 figs per branch) that were covered with solid Vaseline. After a period of 24 hours, all wasps trapped on the treated figs were collected and identified according to Bouček (1993). We then discarded the previously treated figs and treated 30 other new figs. We monitored the tree daily from pre-floral to post-floral phases (*sensu* Galil and Eisikowitch, 1968). Since *F. citrifolia* crops are within-tree highly synchronized (Pereira et al., 2007b), we collected some non-treated figs to analyze the general developmental phase of the entire tree *in situ*, for each monitoring day.

Adhesive traps were 3-liter, transparent water bottles covered with entomological glue (Biocontrol©). We placed five adhesive traps on each of the two trees. The area of each adhesive trap was 1,176 cm<sup>2</sup>. Both trees were daily monitored from pre-floral to post-floral phases, and all trapped wasps were sampled and identified according to the same method described for the fig traps.

The previous methods assessed the wasp pool that arrived to the fig tree. We observed the oviposition process in experimental figs to determine the actual colonization time. We bagged branches with very young figs (pre-floral phase) to prevent access from any wasp.

When figs reached the receptive phase, we removed the cloth bags and pollinated them by introducing one foundress wasp per fig [see Jousselin et al. (2003) for details]. Thus, we daily observed 30 control-pollinated figs, and the colonization sequence was expressed in “days after pollination” (DAP). We collected and identified all wasps that probed in these figs. As monitoring started after pollination, we did not sample galler species with this method.

Table 1. Number of wasps sampled by the three sampling methods.

Wasps	Fig trap	Adhesive trap		Pollinated figs	
		Tree 1	Tree 2	Tree 1	Tree 2
Pollinator ( <i>P. near tonduzii</i> )	155	7,085	3,265	0	0
<i>Idarnes (carne group)</i>	25	249	1,044	21	10
<i>Idarnes (flavicollis group)</i>	9	422	35	0	0
<i>Idarnes (incerta group)</i>	0	18	0	0	0
<i>Aepocerus</i> sp.	0	1	0	0	0
<i>Eurytoma</i> sp.	1	19	0	0	0
<i>Heterandrium</i> sp.	0	56	28	0	0
<i>Physothorax</i> sp.	4	276	112	4	23
<i>Torymus</i> sp.	7	220	181	1	20

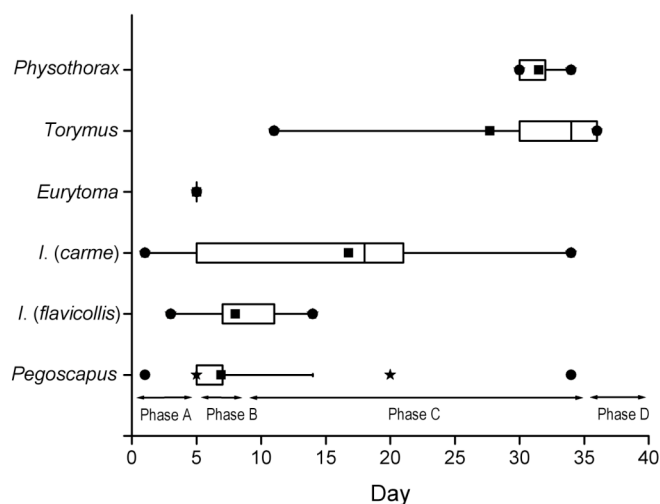


Figure 1. Fig wasp capture sequence on fig traps in *F. citrifolia*. Boxes delimitate 50% of observations, central traces represent medians and squares (■) represent means. Vertical bars indicate the 5th and 95th percentiles and (★) signs represent the 0 and 1st percentiles and (●) signs indicate the total data amplitude. Table 1 shows sample sizes for each species.

### 3. Results

The pollinating and nine other NPFW species were observed as the figs developed. The non-pollinators belonged to the genera *Aepocerus*, *Eurytoma*, *Heterandrium*, *Idarnes (incerta, flavicollis groups and two species of the carne group)*, *Physothorax* and *Torymus* (Table 1).

#### Fig traps

During the figs' receptive phase (when female pollinating wasps were observed being attracted to the figs), around the 8th monitoring day, we collected pollinators, *Idarnes (flavicollis group)* wasps and one individual of

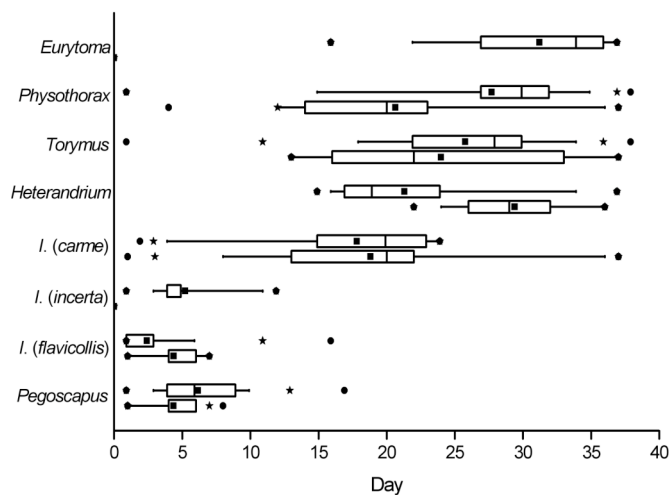


Figure 2. Fig wasp capture sequence on adhesive traps in *F. citrifolia* trees (below = tree 1, above = tree 2). See Fig. 1 for details about the box-plots. Table 1 shows sample sizes for each species.

*Eurytoma* sp. *Idarnes (carne group)* individuals were captured throughout the receptive and inter-floral phases, with a peak around the 17th monitoring day. *Torymus* and *Physothorax* species were the last ones to be sampled in the colonization sequence. They occurred from the middle to the end of the inter-floral phase (Fig. 1).

#### Adhesive traps

Pollinators, *Idarnes (flavicollis group)* and *Idarnes (incerta group)* wasps were captured on adhesive traps at the beginning of the monitoring period. Wasps of the *flavicollis* and *incerta* groups were sampled, in general, just before or at the same time as the pollinating ones (Fig. 2).

Species of *Idarnes (carne group)*, *Heterandrium*, *Eurytoma*, *Torymus*, and *Physothorax* occurred later, with a large overlap in their capture period. However, *Idarnes*

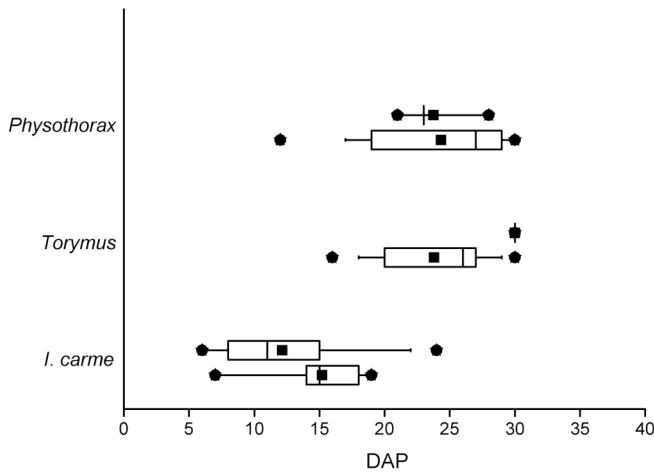


Figure 3. Fig wasp colonization sequence on control-pollinated figs in *F. citrifolia* trees (above = tree 1, below = tree 2). See Fig. 1 for details about the box-plots. Table 1 shows sample sizes for each species.

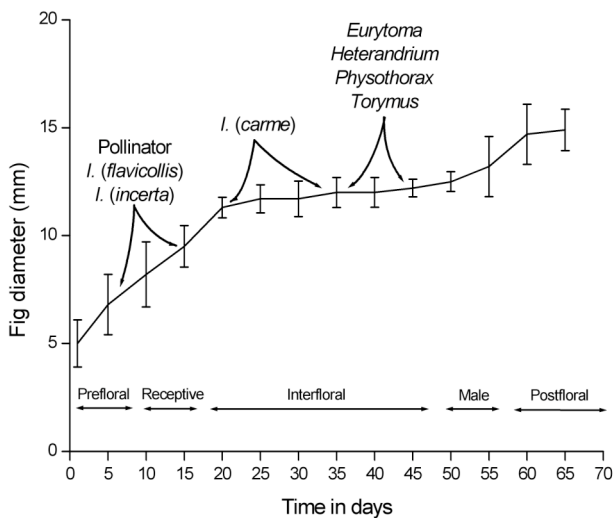


Figure 4. Summary of the colonization sequence of fig wasps in *F. citrifolia*. Bars represent standard mean deviations ( $n = 20$  figs per date).

(*carme* group) wasps occurred before other species (Fig. 2). We trapped one *Aepocerus* male on the 12th monitoring day.

#### Pollinated figs

*Idarnes (carme)* group colonized pollinated figs from the 6th to the 24th DAP, with a peak around the 15th DAP. The colonization period of *Torymus* sp. and *Physothorax*

sp. was later, ranging from the 12th to the 30th DAP (Fig. 3). Six *Heterandrium* sp. wasps were observed visiting, but not ovipositing, the pollinated figs from the 18th to the 23th DAP.

#### 4. Discussion

Our results covered the most common fig wasp genera associated with section *Americana* (Bouček, 1993) and conveyed new data that complements previous suggestions about the biology of these Neotropical fig wasp groups (West et al., 1996; Pereira et al., 2000). All three study methods were consistent and showed a temporal partitioning in colonization among these NPFW species (Fig. 4). *Idarnes* species belonging to the *flavicollis* and *incerta* species groups colonize figs just before or during the fig receptive phase (late pre-floral and female phases, *sensu* Galil and Eisikowitch, 1968). Females of *Idarnes* belonging to the *carme* group probe the figs one to three weeks later, mainly in the middle of the inter-floral phase. *Physothorax* and *Torymus* species are late colonizers, and probe the figs from the second to the fourth week after pollination (the middle to late inter-floral phase). Our results suggest that *Eurytoma* and *Heterandrium* species are late colonizers, but further confirmation is necessary as we did not record any oviposition for those species.

In relation to the genus *Idarnes*, our experiments corroborated a report (Pereira et al., 2007a) that larval biology varies within this genus. Colonization by species of *flavicollis* and *incerta* groups during, or just before, the fig receptive phase supports assumptions that these species are gallers (West et al., 1996; R.A.S. Pereira and L.G. Elias, unpublished data). Moreover, these species were not observed on the control-pollinated figs, thus suggesting that they are neither attracted to nor able to colonize figs in the inter-floral phase. In contrast, *Idarnes (carme)* group species colonized figs later, when there was no space for induction of new galls. This suggests that species of the *carme* group are probably pollinator inquiline. *Idarnes (carme)* group species lay eggs into young galls, which contain small galler larvae or, alternatively, use good seeds to oviposit when free galls are scarce (Pereira et al., 2007a). Therefore, *carme* species can broaden the range of food resources available to their offspring.

The late colonization by *Physothorax* and *Torymus* species supports the suggestion of West et al. (1996) that they are parasitoids. *Heterandrium* females were sampled on adhesive traps at the same time of *Physothorax* and *Torymus* species. This suggests that *Heterandrium* species may also be parasitoids, feeding on galler larvae and not on plant tissues, as Pereira et al. (2000) had previously suggested. Hence, *Eurytoma* sp. may also be a parasitoid, as individuals were trapped at the same time of the late colonizers.

The temporal partitioning in fig colonization and the diversity of larval biologies described here have been reported for other NPFW groups (Kerdelhué and Rasplus, 1996; Kerdelhué et al., 2000). This reinforces that the fig-fig wasp association is an interesting system for the study of both community ecology and evolution of plant-insect interactions, as there are many independently evolved communities to compare (Kerdelhué et al., 2000; Kjellberg et al., 2005).

The results discussed here point out that NPFW biology is complex, with extreme diversity among species from the same genus. Therefore, other studies regarding the evolution of such different biologies and reproductive strategies need to be carried out. Direct histological observations and experiments on feeding capacities are necessary to clearly elucidate NPFW larval biologies and help understand better the impact they have on the fig-fig wasp mutualism.

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