Running head:

Fig tree and fig wasp interaction

Title:

Non-pollinator fig wasp impact on the reproductive success of an invasive fig tree: why so little?

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Abstract

Classical biological control agents fail to achieve an impact on their hosts for a variety of reasons and an understanding of why they fail can help shape decisions on subsequent releases. Ornamental *Ficus microcarpa* is a widely planted avenue fig tree that is invasive in countries where its pollinator (*Eupristina verticillata*) is also introduced. This tree also supports more than 20 species of non-pollinating fig wasps (NPFW) that feed in the figs and have the potential to reduce the plant's reproduction. *Odontofroggatia galili*, one of the most widely introduced NPFW, has larvae that develop in galled ovules that might otherwise develop into seeds or support pollinator larvae. We examined the distribution and relative abundance of the pollinator and *O. galili* on *F. microcarpa* in China, towards the northern limit of the tree's natural range and in Italy, where the two species have been introduced. Where they co-existed, we also recorded the impact of varying densities of *O. galili* on *F. microcarpa* seed and pollinator production. *O. galili* and *E. verticillata* displayed contrasting habitat preferences in China, with *O. galili* almost absent from warmer sites. *O. galili* abundance and sex ratios varied between the natural and introduced ranges. Figs with more *O. galili* contained fewer seeds and pollinator offspring, but reproduction was rarely inhibited totally. Additional species with a greater impact in the figs they occupy are needed if biocontrol of *F. microcarpa* is to be effective.

Key words Biocontrol, fig wasps, fig trees, mutualism, gall, *Odontofroggatia*

1. Introduction

Classical biological control attempts to control weeds that have become invasive using plant-feeding insects or diseases that originate in the plant's natural range (Culliney 2005). Most biological control agents that are released become established, but only a proportion of these have any significant impact on their hosts (Julien, and Griffiths 1998; McFadyen 2003) and an understanding of why established species have little impact can help shape decisions on subsequent releases (Myers 2000). Low-efficacy agents may fail to reach sufficient densities or are otherwise insufficiently damaging to have a significant impact on host plant population dynamics. Reasons given for failure of biological control programs include interference by local natural enemies of agents, poor climate matching and a lack of complementary alternative hosts (Stiling 1993; Rand, Waters, and Shanower 2016). Alternatively, biological features of potential agents may mean that they are never likely to have a noticeable impact on their host plants (McClay, and Balciunas, 2005).

Fig trees (*Ficus*, Moraceae) are a species-rich group distributed in warmer countries throughout the Old and New Worlds (Harrison 2005). They are of great ecological significance because of the many animals that feed on their figs (syconia) (Shanahan, So, Compton, and Corlett 2001), but this wide range of seed dispersal agents also results in the rapid dispersal of any ripe figs produced by fig trees growing outside their natural range (Simberloff, and Von Holle, 1999). Mature figs (and fertile fig seeds) are produced after young figs are pollinated by a fig tree's host-specific pollinator fig wasps (Hymenoptera, Agaonidae). To achieve pollination, adult female fig wasps seek out receptive young figs, using volatile attractant cues (van Noort, Ware, and Compton 1989). Because fig crops are often synchronized within trees, this usual means that they must fly between trees, which can be tens or even hundreds of kilometers apart (Ahmed, Compton, Butlin, and Gilmartin 2009). Foundresses (reproductive female fig wasps) lose their wings and antennae when they enter a fig through its narrow ostiole (Janzen 1979). Once inside a suitable fig they can pollinate some of the flowers and at the same time they gall and lay eggs in some of their ovules. A single pollinator offspring develops inside each galled ovule. The next generation of fig wasps emerge from their galls a few weeks later and after mating and becoming loaded with pollen the female offspring disperse to find receptive figs (Weiblen 2002).

Figs are also exploited by a diverse community of non-pollinating fig wasps (NPFW) that almost never transfer pollen. NPFW exhibit a wide range of trophic relationships, with larvae that feed inside ovules and seeds or in the fig wall. They include gallers, seed predators, secondary gallers, parasitoids (that may also feed on some plant tissue) and specialist hyper-parasitoids (Compton, van Noort, Mcleish, Deeble, and Stone 2009; Chen, Yang, Gu, Compton, and Peng 2013; Wang et al. 2014). Most of these species will have a negative impact on the reproductive success of fig trees because they kill pollinators and reduce seed numbers (Kerdelhué, and Rasplus, 1996), and fig ovules may be particularly easy to be eaten because the plant cannot defend them chemically without harming its pollinators (Cook, and Rasplus, 2003).

Fig trees are widely planted as ornamental and avenue trees outside their native ranges. They can only reproduce sexually if their associated host-specific pollinators are also present, but this has not prevented them from becoming invasive in natural and semi-natural habitats (Stange, and Knight Jr, 1987; McKey 1989). *Ficus microcarpa* L. f.

is the most widely naturalised and invasive fig tree. An Asian native, it is grown in almost every tropical and sub-tropical country world-wide. Its pollinator fig wasp was deliberately introduced into Hawaii (Beardsley 1998) but unauthorised releases have led to pollinators becoming increasingly widely distributed and they are now present throughout most of their host's introduced range. Often the tree remains a minor urban pest, with its seedlings causing architectural damage, but after expansion into natural habitats it has become invasive in Hawaii, Florida, Bermuda and elsewhere (Hilburn, Marsh, and Schauff 1990; Nadel, Frank, and Knight Jr 1992; Simberloff, and Von Holle, 1999; Starr, Starr, and Loope 2003). Increasing numbers of NPFW species associated with *F. microcarpa* have also been introduced outside their natural range. The two most widely introduced NPFW are two species that gall the ovules, *Walkerella microcarpae* Bouček and *Odontofroggatia galili* Wiebes (both Pteromalidae). Interactions between *O. galili* and *F. microcarpa* were investigated by Kobbi et al. (1996) in Tunisia. They confirmed that this NPFW had a negative impact on the numbers of pollinators and seeds present in shared figs.

Biological control of fig trees using insects has never been attempted, though Miao et al. (2011) suggested that a gall midge (Cecidomyiidae) associated with *F. benjamina* might prove effective at reducing seed and pollinator production in that species. It is known that natural enemies with female-biased sex ratios can potentially increase their population sizes more rapidly than species with balanced sex ratios. *O. galili* of *F. microcarpa* has several characteristics that suggest it might be an effective control agent. This species is host specific, has female-biased populations and does not require pollinated figs for development, which should aid population persistence when pollinator numbers are low. Here we address the following questions that together seek to explain why *O. galili* does not have a more significant impact on the reproduction of its host plant. (1) Within and adjacent to the natural distribution of *F. microcarpa,* do *O. galili* and the tree's pollinator display different habitat preferences? (2) How abundant are *O. galili* galls and is their abundance similar in the native and introduced ranges? (3) What is the relationship between *O. galili* gall density and host plant reproductive success?

2. Materials and methods

2.1. Study species

F. microcarpa, the Indian laurel fig or Chinese banyan, (previously often referred to as *F. retusa* L. or *F. retusa* var *nitida* – see Corner 1960) is a medium to large sized tree with a wide natural distribution extending from Australia northwards to Japan and westwards to India, found growing as a hemi-epiphytic strangler or free-standing tree in coastal and riparian forests and on cliffs (Berg, and Corner 2005). *F. microcarpa* is also widely grown as an avenue tree, both in its native and introduced ranges. Within its natural range, *F. microcarpa* figs are produced all year round, usually in discrete crops, but fewer crops are produced in colder seasons (Corlett 1984; Lin, Zhao, and Chen 2008; Yang, Tzeng, and Chou 2013). Its mature figs are pink or purple in colour and average 13 mm in diameter (SE = 0.08, n = 21 figs). They are mainly dispersed by birds (Shanahan, So, Compton, and Corlett 2001), with secondary seed dispersal by ants (Kaufmann, Mckey, Hossaert-Mckey, and Horvitz 1991). Large crops can number many thousands of figs. *F. microcarpa* is a monoecious species, with individual figs capable of supporting both seeds and pollinator fig wasps, as well as NPFWs. The tree's pollinator is recorded as *Euptistina verticillata* Waterston, but this taxon may be a complex of closely related species (Sun, Xiao, Cook, Feng, and Huang 2011). In Yunnan, China there is also an undescribed species of 'cheater' non-pollinating agaonid associated with *F. microcarpa* (Martinson et al. 2014)

F. microcarpa supports a diverse community of NPFW, comprising more than 20 species (Chen, Chuang, and Wu 1999; Wang et al. 2015), several of which have been introduced outside their natural ranges. Amongst these, *O. galili* (Pteromalidae, Epichrysomallinae) is now present in the Pacific (Beardsley 1998), the Americas (Bouček, 1993), Africa (van Noort,Wang, and Compton 2013), Europe (Compton 1989; Lo Verde, Porcelli, and Sinacori 1991) and the Middle East (Galil, and Copland 1991), including areas such as Hawaii where *F. microcarpa* is invasive. *O. galili* is probably restricted to *F. microcarpa,* though there is a single unconfirmed record from a distantly related fig tree (Bouček 1988). *O. galili* females lay their eggs into ovules while standing on the outside of the figs at about the time that pollinator females enter the figs to oviposit (Galil, and Copland 1981). Their larvae develop inside larger galls than pollinator larvae. *Sycophila* (Eurytomidae) species are NPFW with larvae that develop at the expense of epichrysomallines, including *Odontofroggatia* (Compton 1993). These specialist parasitoids have been introduced with *O. galili* into the USA and Greece (Beardsley, 1998; Wang R, unpublished data). One *Sycophila* larva develops inside each ovule galled by *O. galili* and their numbers were combined in some analyses to estimate pre-parasitism densities of *O. galili* in the figs.

2.2. Study sites

The relationship between *O. galili* and its host plant's reproductive success was compared on the basis of collections from Sicily, an island in the Mediterranean Sea where *F. microcarpa* is introduced (Lo Verde, Porcelli, and Sinacori 1991), and several sites in Yunnan Province, south-west China, at and probably beyond the northern limit of the natural distribution of the tree. NPFW in Yunnan are diverse, with around 15 species present, compared with three NPFW species that have been introduced into Sicily, two of which are rare (Wang et al. 2015). Locations of the Yunnan collection sites, with their altitudes and habitats, are given in Table S1. The ten Sicilian collections were all made in July 2012 from street trees in Palermo, at an altitude of approximately 29 m.

2.3. Fig wasp collections

F. microcarpa trees were sampled at times when almost mature figs, without exit holes, were present. The figs were collected haphazardly, then placed individually in netting bags to allow the adult fig wasps to emerge (China), or placed immediately into alcohol for storage (Italy). The figs were opened and the fig wasps and seeds that they had contained were identified using a binocular microscope.

2.4. Data analysis

The differences in number of female pollinator offspring and seeds with and without *O. galili* were determined using a non-parametric Wilcoxon rank sum test.

The relationships between *O. galili* gall numbers and *F. microcarpa* reproduction were modeled using four zero-inflated generalized linear mixed models (GLMM) with negative binomial errors and log links. Crop effects may be present and we therefore included crop identity as a random effect in all the models. The first two models examined the effects of number of *O. galili* (combined with the number of its *Sycophila* parasitoids if present) and the number of non-pollinating fig wasps on seed numbers in China (first model) and Italy (second model). The third and fourth models examined the effects of the number of *O. galili* and the number of non-pollinating fig wasps, and their interaction, on female pollinator offspring numbers in China and Italy. In China, the number of *O. galili* was correlated with the number of non-pollinating fig wasps ($r = 0.5$, $P < 0.001$). Therefore we only included the number of *O. galili* into the model to avoid colinearity. We cannot distinguish males of the two *Eupristina* species morphologically. The males of each species were estimated in proportion to the number of females in figs where females of both species were present.

To determine whether the sex ratio of *O. galili* varied according to the numbers of offspring individuals sharing a fig, we modeled the effects of *O. galili* abundance on the proportion of males produced in China (first model) and Italy (second model) using binomial generalized linear mixed models (GLMM) with logit links. Figs that also contained *Sycophila* spp. were not included in these analyses. Crop identity was again included as a random effect in both models. All analyses were carried out using the statistical software R 3.01 (R Development Core Team 2013).

3. Results

3.1. The distribution of **F. microcarpa** *fig wasps in Yunnan and Sicily*

O. galili was the most common fig wasp in collections of *F. microcarpa* figs from Kunming, where it was present in six of the seven crops. Only one crop had the pollinator *E. verticillata*. In contrast, *O. galili* was rare or absent elsewhere in Yunnan, but the pollinator was common elsewhere (Table S1). In those crops where *O. galili* was present, about 7–100% of the figs were occupied by this species (Table S2). *O. galili* was present in nine of the 10 crops sampled in Sicily (n figs per crop = 10), where it was present in 20–100% of the figs of different crops (Table S2). The pollinator was present in all 10 of the crops sampled in Sicily. Two more species of NPFW were sometimes present in these figs, but in small numbers, occupying between 0% and 20% of the figs in different crops.

3.2. Impact on the pollinator and seed production of **O. galili** *in China and Italy*

In the absence of *O. galili, F. microcarpa* figs in Yunnan were capable of supporting the development of up to 110 female pollinator adult offspring and 137 seeds. Equivalent values for Sicily were 182 female pollinator offspring and 123 seeds. *Sycophila* parasitoids of *O. galili* were absent from the Sicilian fig collections, and were also rare in Yunnan (Table S2). *O. galili* reached high densities in some crops, with a maximum of 126 and 70 *O. galili* recorded from individual figs in Yunnan and Sicily respectively (Table S2). Mean densities of *O. galili* within the figs it occupied ranged from about 5 to over 88 in Yunnan (not including a crop where only one individual was recorded in total, Table S2). The range in densities was lower in Sicily, with crop means ranging between 8 and 54 *O.*

galili per fig (Figure 1; Table S2).

Only three crops in Yunnan had both *O. galili* and *E. verticillata* present (Table S1). Taking these two crops together (not including the crop where only one individual was recorded in total, Table S2) mean ± SE = 9.9 ± 4.5 female pollinator offspring were present in figs shared by the two species (n = 46), compared with 45.5 ± 21.9 offspring in the remaining figs sampled from these crops (n = 6; W = 188.5, $P < 0.05$). The numbers of seeds in the figs shared with *O. galili* were 4.0 ± 1.65 (n = 46), whereas in figs without *O. galili* there were 19.83 ± 12.59 seeds (n = 6; W = 151, $P = 0.67$). In Sicily, the two species co-existed more frequently (9 from 10 crops) and the numbers of female pollinator offspring in figs shared with *O. galili* were 27.9 ± 3.7 (n = 62) compared with 59.6 ± 5.2 pollinator offspring in figs where *O. galili* was absent (n = 35; W = 1690, $P < 0.001$)). The numbers of seeds in the figs where *O. galili* was present were 14.56 ± 2.04 (n = 62), compared to 54.52 ± 5.13 (n = 35; W = 1892; $P < 0.001$)) in figs without *O. galili.* Despite this, figs containing *O. galili* could still release more than 120 female pollinator offspring and more than 60 seeds (Table S2; Figure 2 and 3).

The numbers of female pollinator adult offspring in China decreased significantly with increasing numbers of both *O. galili* (Figure 2A) and other non-pollinators (z = -4.08, $P < 0.01$). Similarly in Italy female pollinator offspring decreased with increasing numbers of *O. galili* (Figure 2B) and other non-pollinators (z = -2.31, $P < 0.05$). The numbers of seeds in the figs in China also decreased significantly with an increase in numbers of *O. galili* (Figure 3A) and with other non-pollinators (z = -4.77, $P < 0.01$). In Italy the numbers of seeds in the figs decreased significantly with an increase in numbers of *O. galili* only (Figure 3B). There were significant differences in seed and pollinator offspring numbers among crops in both countries.

3.3. Sex ratios of **O. galili** *in China and Italy*

Sex ratios in *O. galili* were investigated and were consistently female-biased in Yunnan (Table S2), with a mean proportion of 0.28 ± 0.02 (SE) males (n = 7485 *O. galili* from 222 figs). In Sicily most crops also contained female-biased collections, but a male bias was present in two collections (mean proportion males = 0.48 ± 0.03, n = 1911 *O. galili* from 62 figs, Table S2). The proportion of males decreased significantly with an increase in the number of *O. galili* sharing a fig in China ($z = -3.87$, $P < 0.001$; Figure 4A). However, the proportion of males in Italy did not show any significant difference in relation to density ($z = -0.55$, $P = 0.58$; Figure 4B). There were significant differences in sex ratios between crops in both countries.

4. Discussion

Our results confirm that *O. galili* has a detectable impact on female (seeds) and male (pollinator female) reproductive functions of *F. microcarpa* in both its natural and introduced ranges, but also that it rarely suppresses reproduction entirely. *O. galili* has become established in most of the countries where the pollinator of *F. microcarpa* is also established (Brazil is an exception, Farache, do O, and Pereira 2009), and also in South Africa, where the pollinator has not been recorded (van Noort, Wang, and Compton 2013). This suggests that the two fig wasps have similar climatic preferences, yet at the northern edge of the natural range of *F. microcarpa* in China, *O. galili* is rare or absent from warmer, lowland sites, but frequent in Kunming, a city located at a higher altitude than the other sites, with a cooler climate. Conversely, pollinators were generally absent in Kunming, suggesting that it is less successful than *O. galili* in more seasonal, cooler climates. Alternatively, the pollinator may suffer from competitive displacement in Kunming, because the 'cheater' fig wasp *Eupristina* sp. was common there. The absence of pollinators from Kunming may nonetheless have inflated the apparent fig occupancy rates of *O. galili,* because any figs not utilised by *O. galili* (or *Eupristina* sp.) are likely to have aborted at an early stage of development and only the remaining figs will have been sampled.

The contrasting distribution patterns of *O. galili* and the pollinator meant that they rarely co-existed inside the same figs at the edge of the tree's natural range. In Sicily, where the two species routinely co-existed, opportunities for interactions between the species were much greater. Larvae of *O. galili* and the pollinator of *F. microcarpa* both develop in galled ovules, and therefore compete for oviposition sites. In addition, *O. galili* galls grow quickly and if initiated before pollinator oviposition can distort the fig interior, making entry through the ostiole and oviposition more difficult for pollinator foundresses. Possibly there is also indirect competition for nutrients within the figs, as in other galled plants (Bagatto, Paquette, and Shorthouse 1995). Seed and pollinator offspring numbers in shared figs both declined equally with increasing numbers of *O. galili* galls. This contrasts with the pattern recorded by Segar and Cook (2012), who found that pollinator offspring are usually more greatly impacted by NPFW than seeds. Many NPFW are parasitoids that target pollinator larvae, whereas *O. galili*, as an ovule galler, is preventing ovules from supporting the development of both pollinator larvae and seeds.

O. galili has a demonstrable impact on the reproductive success of *F. microcarpa,* but to provide more effective and ecologically significant control it would need to be present at densities where the reproduction is inhibited more completely. This species often achieved high occupancy rates (the proportion of figs where it was recorded) but the densities required to eliminate host plant reproduction were rarely achieved, in either the natural or introduced ranges, even where the galler's *Sycophila* parasitoids were absent. Factors that prevent *O. galili* from reaching high densities more frequently are unclear, but may include an oviposition strategy that favours the relatively wide dispersal of their eggs by females across several figs. This spreading of offspring across several figs can nonetheless cause mortalities among *O. galili* females in figs where pollinators are absent, because some female offspring develop in figs where no male *O. galili* fig wasps are present, and males are needed to chew the exit holes that allow female fig wasps to escape (Wang et al. 2015).

As well as being a poor use of resources, the release of ineffective agents can add to the potential risks of biological control, without providing benefits (McClay, and Balciunas 2005). Other species of NPFW associated with *F. microcarpa* may have a similarly limited individual impact on *F. microcarpa* reproduction because all fig wasp species have evolved in a close relationship with the fig inflorescence and the pollinator. Therefore, the populations of all NPFW species could be constrained by fig morphology and other features of the pollinator mutualism. As the resources provided by female flowers are limited, some NPFW species may be selected to spread their offspring in several figs, to decrease intra-specific competition (Weiblen 2012). These constraints could select for other NPFWs to disperse their eggs, as seen in *O. galili.* Despite this oviposition behaviour, *O. galili* did reduce both seed and pollinator offspring numbers and its impact could be additive with other NPFW if they are also present. Species, with a greater impact on the reproduction of *F. microcarpa* have been described. They include other species of NPFW, gall midges, beetles and hemipterans, all of which destroy its seeds and/or pollinator larvae (Mia, Yang, Liu, Peng, and Compton 2011).

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Supplementary materials

Table S1 Locations (North-South) and contents of *F. microcarpa* figs in Yunnan. Each collection comprised figs from a single tree, collected on the same date. Kunming is located at N 24º 53', Jinghong at N 22º 00'.

Table S2 The proportion of figs occupied by *O. galili* and its densities within occupied figs in Yunnan (collections 1–6, 19, 16) and Sicily (collections 21–29). *Sycophila* spp. are parasitoids of *O. galili.* Palermo (Sicily) is located at 38º 07' N.

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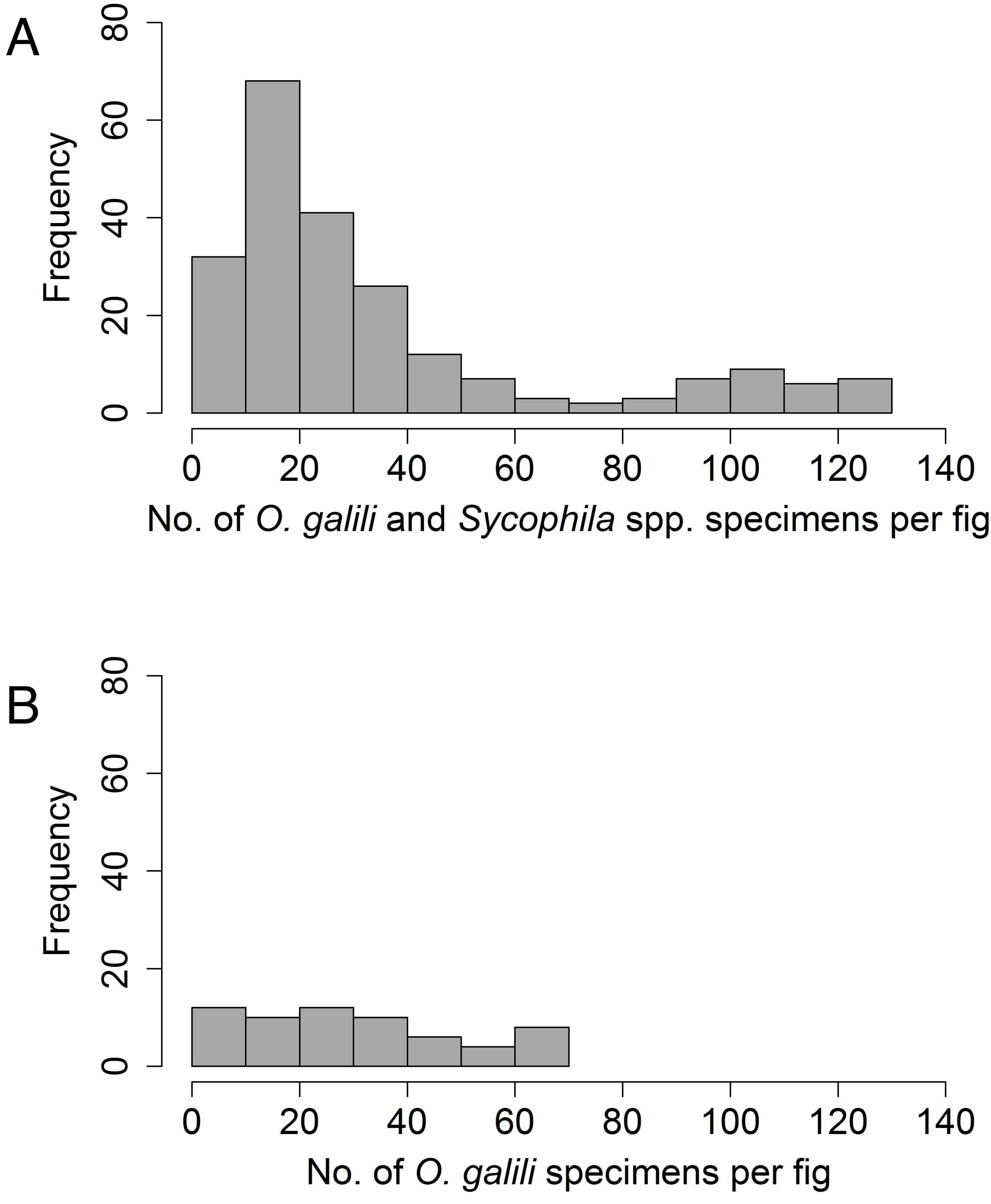
Figure legends

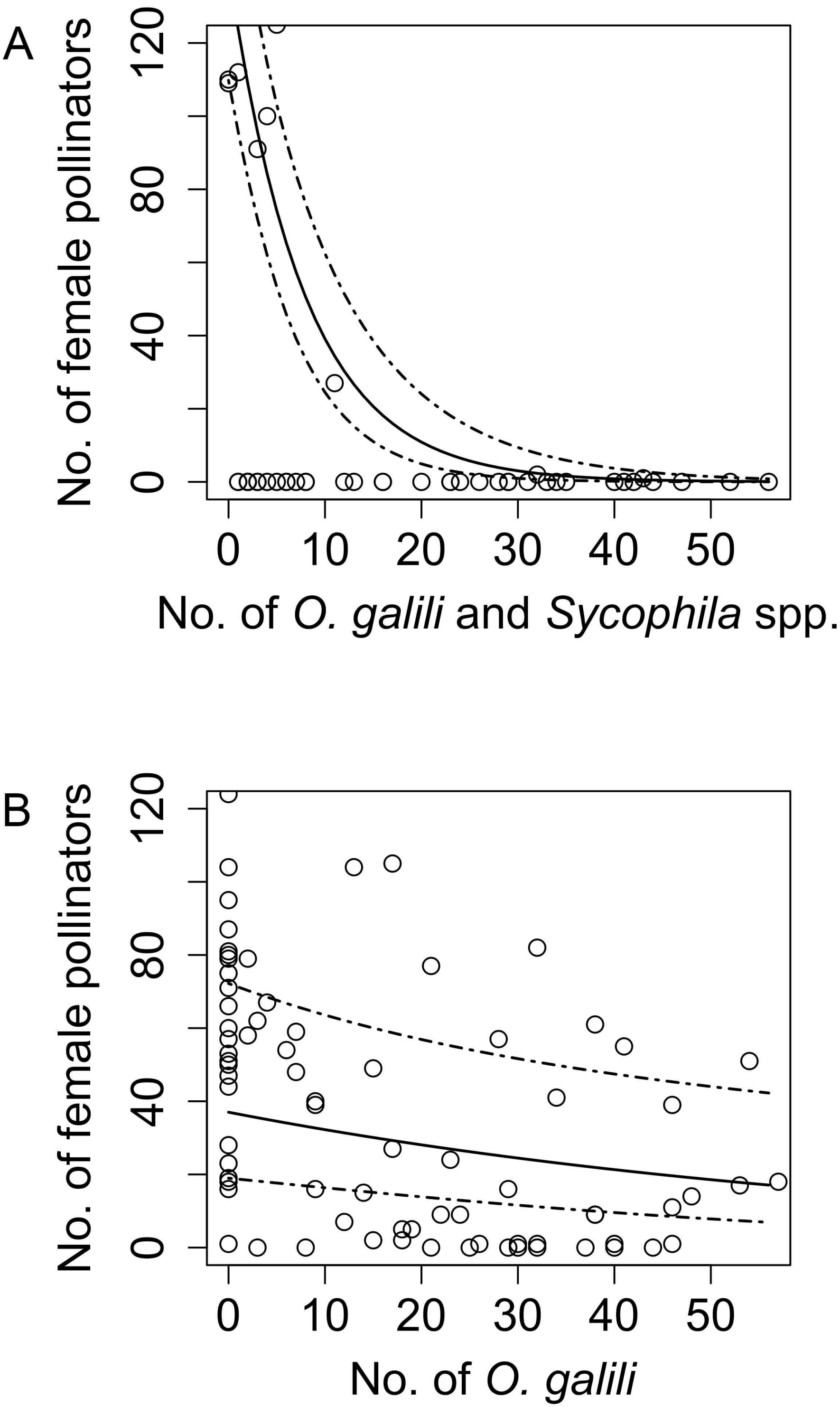
Figure 1 The numbers of *O. galili* present in figs of *F. microcarpa* from A) Yunnan and B) Sicily. *Sycophila* spp. are parasitoids of *O. galili.*

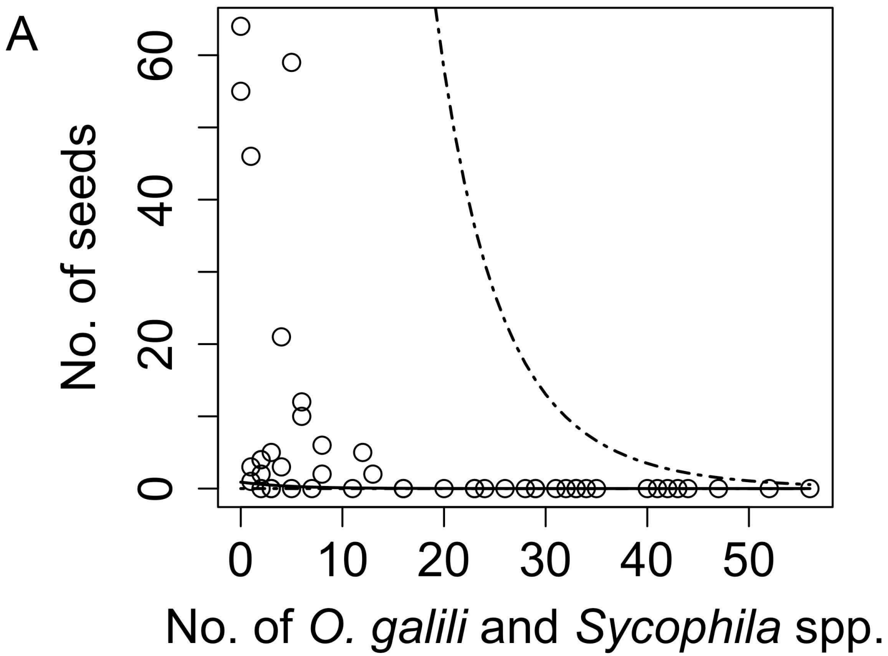
Figure 2 The relationship between densities of *O. galili* and *E. verticillata* pollinators in shared figs of *F. microcarpa* in A) Yunnan (z = -6.88, $P < 0.001$), and B) Sicily (z = -3.34, $P < 0.01$). Only figs that contained *O. galili* and pollinator offspring or seeds are included. Solid lines indicate lines of best fit, dashed lines indicate 95% probabilities.

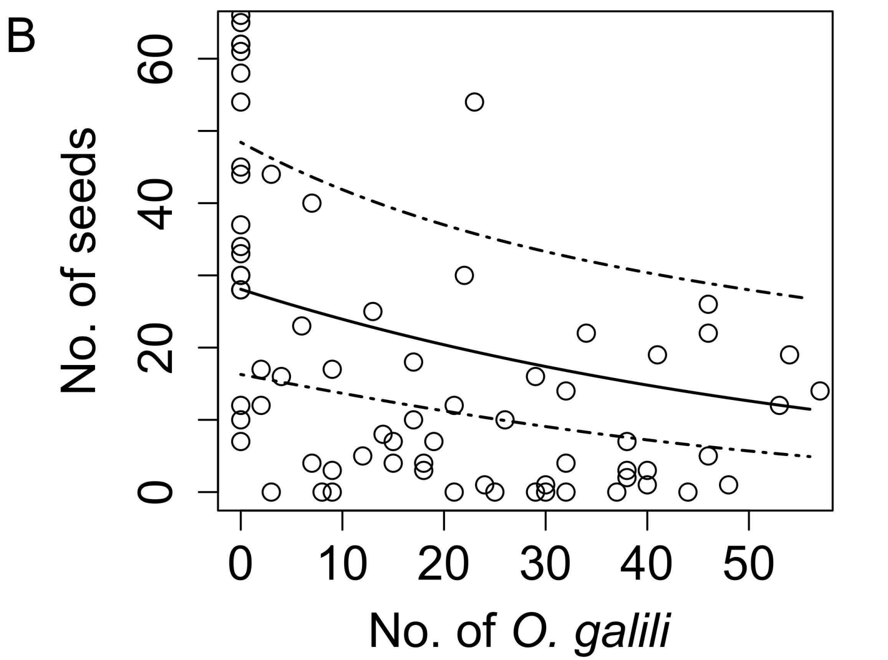
Figure 3 The relationship between densities of *O. galili* and numbers of seeds in shared figs of *F. microcarpa* in A) Yunnan (z = -2.88, $P < 0.01$) , and B) Sicily (z = -6.32, $P < 0.01$) . Only figs that contained *O. galili* and pollinator offspring or seeds are included. Solid lines indicate lines of best fit, dashed lines indicate 95% probabilities.

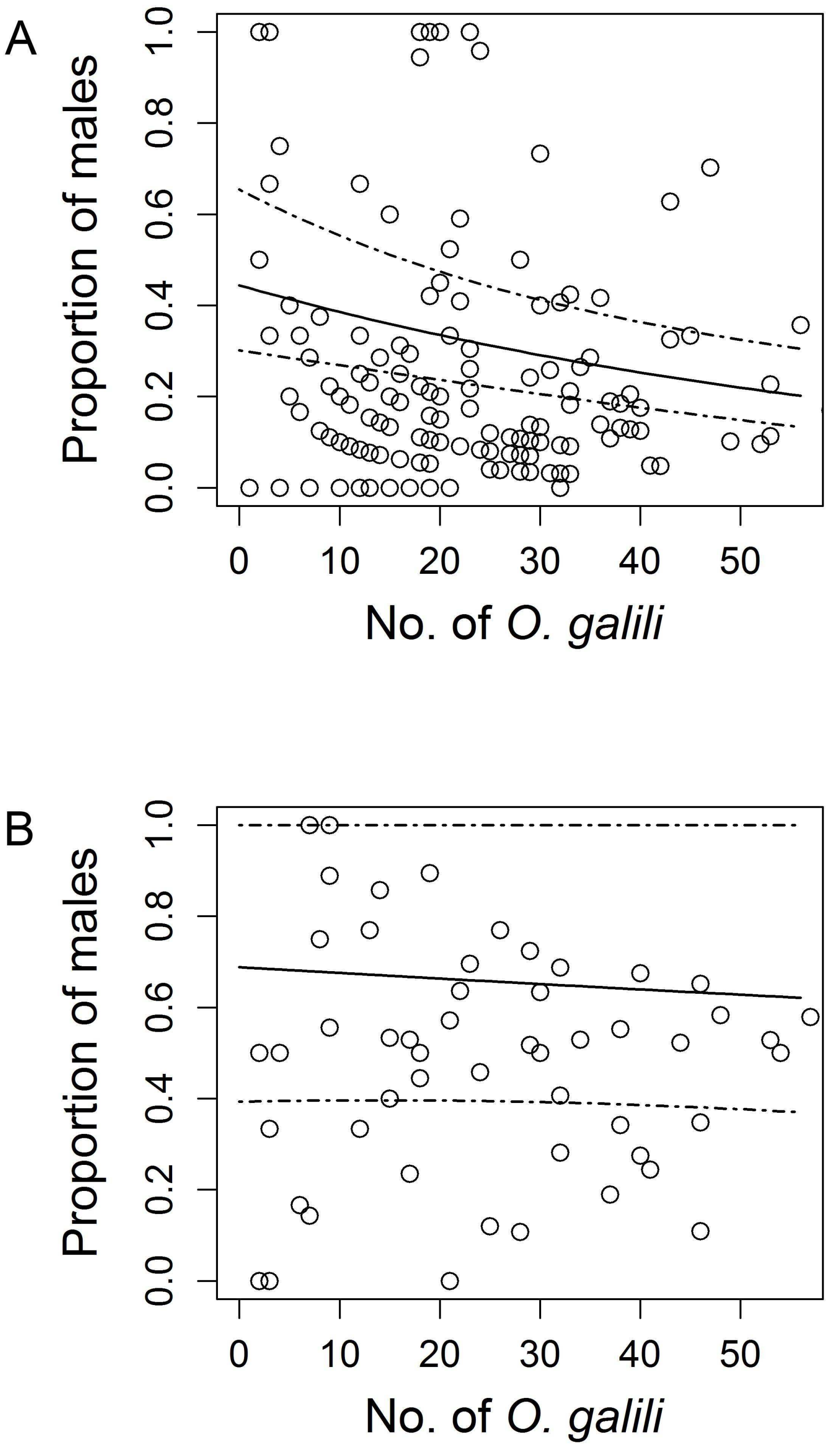
Figure 4 Sex ratios of *O. galili* in relation to densities of this species in figs of *F. microcarpa* in A) Yunnan, and B) Sicily. No figs containing *Sycophila* spp. are included. Solid lines indicate lines of best fit, dashed lines indicate 95% probabilities.


A
Frequency
80
60
40
20
0
0
20
40
60
80
100
120
140
No. of O. galili and Sycophila spp. specimens per fig
B
Frequency
80
60
40
20
0
0
20
40
60
80
100
120
140
No. of O. galili specimens per fig


A
No. of female pollinators
0
40
80
120
0
10
20
30
40
50
No. of *O. galili* and *Sycophila* spp.
B
No. of female pollinators
0
40
80
120
0
10
20
30
40
50
No. of *O. galili*


A
No. of seeds
0
20
40
60
0
10
20
30
40
50
No. of O. galili and Sycophila spp.


B
No. of seeds
0
20
40
60
0
10
20
30
40
50
No. of O. galili


A
Proportion of males
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50
No. of *O. galili*
B
Proportion of males
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50
No. of *O. galili*