The impact of fig wasps (Chalcidoidea), new to the Mediterranean, on reproduction of an invasive fig tree Ficus microcarpa (Moraceae) and their potential for its biological control

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Abstract

Natural enemies that reduce plant reproductive success are often utilized for biological control of invasive species. Reproduction in fig trees depends on host-specific fig wasp pollinators that develop in galled ovules, but there are also many species of non-pollinating fig wasps (NPFWs) that reduce seed and pollinator numbers. Fig wasps associated with an invasive Asian fig tree, Ficus microcarpa (Moraceae), were surveyed around the Mediterranean. Eight NPFW species are now known from the area, three of which are newly-recorded. The impacts of the two most prevalent ovule-galling NPFW species (both Pteromalidae, Epichrysomallinae) on the tree's reproduction were compared: Odontofroggatia galili Wiebes is widely-introduced, whereas Meselatus bicolor Chen has not been recorded previously outside its native range. Both gall-forming NPFWs significantly reduce seed and pollinator production, but M. bicolor has a far greater impact, entirely preventing seeds and pollinators from developing in the figs it occupies. Meselatus bicolor has only been recorded from F. microcarpa and has the potential to be a valuable biological control agent in other countries outside the Mediterranean where F. microcarpa has become invasive.

Key words: Agaonidae, Epichrysomallinae, fig wasp pollinators, galls, mutualism, non-pollinating fig wasps (NPFWs), pollination prevention, Pteromalidae.

1. Introduction

Classical biological control, where natural enemies from the native range of an invasive species are released into its introduced range, assumes that a lack of specific natural enemies has allowed the weed or pest to become more abundant (the enemy release hypothesis) (Keane and Crawley, 2002; Müller-Schärer and Schaffner, 2008; Pearson et al., 2011). Reflecting this, biological control programs normally consider species at higher trophic levels than the invasive organisms when selecting agents for release (van Lenteren, 2012). Phytophagous insects are frequently used to control invasive plant species, which represent one of the major threats to global biodiversity (Garren and Strauss, 2009; Baraibar et al., 2011). Insects that feed on floral structures and seeds are especially useful where established plant species have commercial or aesthetic value, but are also invasive due to prolific seed production (Zimmermann and Neser, 1999). Agents that reduce plant sexual reproduction can provide rapid control of short-lived plants that do not also reproduce asexually (Navntoft et al., 2009; Wilson et al., 2011), although they need to substantially reduce seed production to be effective (Hill et al., 2000; Knochel et al., 2010). Seed reducing agents have also been used successfully against perennial trees, with some having reduced the abundance and extent of their host (Hoffmann & Moran, 1998) and others slowing rates of spread and invasiveness (Dennill, 1985; Dennill and Donnelly, 1991; Le Maitre et al., 2008).

Fig tree species (Ficus, Moraceae) are regarded as 'keystone' species in tropical forests because many animals feed on their fruits (Shanahan et al., 2001; Herre et al., 2008). The genus Ficus contains more than 800 species, mainly in tropical and sub-tropical regions, and is characterized by its unique inflorescences (figs) and a highly specific relationship with species of pollinating fig wasps (Hymenoptera, Chalcidoidea, Agaonidae) (Wiebes, 1979; Cook and Rasplus, 2003; Harrison, 2005). The majority of fig tree species are each pollinated by females of a single, host-specific species of agaonid (Cruaud et al., 2012). Monoecious fig trees have mutualistic relationships with their pollinators, which on entry into the figs pollinate some of the flowers and lay their eggs in others, which become galled. In general, galls are mostly found in centrally-located ovules while peripheral ovules are more likely to develop seeds.

Many fig tree species are widely grown as ornamental species outside their native ranges, where they cannot reproduce sexually because they lack their specific pollinators. However, in some regions pollinator species have reached their hosts in the introduced range and this potentially allows the trees to become invasive (Mckey, 1989; Caughlin et al., 2012). A wide variety of invertebrate species feed in or on figs and can have a negative impact on fig tree reproduction (Compton and Robertson, 1988; Compton and Disney, 1991; Herre, 1993; Jauharlina et al., 2012; Miao et al., 2011). They include nematodes and mites, ants, beetles, moths and gall midges, but the most ubiquitous non-mutualist occupants of figs are non-pollinating fig wasps (NPFWs) belonging to several families of Chalcidoidea. The trophic relationships of NPFWs are diverse, but poorly understood. Traditionally, they are classified as gallers, inquilines (kleptoparasites) and parasitoids (Kerdelhué et al., 2000; Compton et al., 2009; Cook and Segar 2010) but their ways-of-life are proving to be more diverse than previously realized (Compton et al., 2009; Chen et al., 2013).

From the host plant's perspective, NPFWs can be seen as reducing male reproductive success (by reducing the number of pollen-carrying pollinator females), female reproductive success (by reducing seed production) or both. Gall-forming NPFWs can restrict both reproductive functions because they compete with pollinators for oviposition sites and occupy flowers that might have developed seeds (Kobbi et al., 1996). NPFW galls that develop quickly can also limit or prevent pollinator females from entering figs, and there may also be competition for nutrients within figs containing galls of different species. Obligate seed-eating NPFWs appear to be extremely rare, but may be under-reported (Wang et al., 2014). A negative impact of parasitoids on their pollinator hosts has been frequently reported, but their effects have also been seen as helping to stabilize the mutualism, because they preferentially lay their eggs in more peripheral galls, thereby favoring pollinators that lay their eggs more centrally and leave more peripheral ovules to develop into seeds (Dunn et al., 2008; Segar and Cook, 2012; Yu and Compton, 2012; Suleman et al., 2013). NPFWs have the capacity to reduce the reproductive success of fig trees and therefore have the potential to act as biological control agents of invasive fig tree species.

Ficus microcarpa L.f. is the most invasive species of fig tree. Several of its associated fig wasps have been introduced to the Mediterranean area including two species of gall-forming NPFWs (both Pteromalidae, Epichrysomallinae) which are the subject of this investigation. Of the two, Odontofroggatia galili Wiebes is found almost everywhere that F. microcarpa is planted and was the first species reported from the Mediterranean (Galil and Copland, 1981). It has been shown previously to reduce seed and pollinator numbers (Kobbi et al., 1996), but fails to prevent F. microcarpa from becoming invasive. The other, Meselatus bicolor Chen appears to have only recently been introduced to the Mediterranean, and still has a limited distribution, but initial observations suggest that it may have a greater impact on the plant than O. galili.

The main purpose of this study was to (i) describe the distribution and abundance of the fig wasps associated with F. microcarpa in the Mediterranean area; (ii) quantify the effects of the two NPFWs on seed and pollinator production; and (iii) to determine why M. bicolor has a greater impact on its host plant than O. galili, and thus has the potential to be an effective biocontrol agent.

2. Materials and methods

2.1 The tree and its associated fig wasps

Ficus microcarpa (the Malay banyan or Indian laurel) has been referred to previously as F. nitida and F. retusa (Berg and Corner, 2005). It has a broad natural range in tropical and sub-tropical forests from India to Australia where it grows as a hemiepiphytic strangler of other trees, or directly from rocks. The figs (syconia) are small, and are typically produced in largely synchronized crops among the leaves and when they ripen are pink or black (Berg and Corner, 2005). Outside the native range, where fig wasp densities can be low, synchronized fig development is often less pronounced. Development of the figs usually takes 4-8 weeks, depending on temperature (Yang et al., 2013). Frugivorous animals such as birds are mainly responsible for the primary dispersal of its seeds, with ants acting as secondary seed dispersal agents (Kaufmann et al., 1991; Shanahan et al., 2001).

Ficus microcarpa has been widely planted outside its native range in streets, parks and gardens, and in climates ranging from the humid tropics to the strongly seasonal and semi-arid (Nadel et al., 1992; Figueiredo et al., 1995; Kobbi et al., 1996; Beardsley, 1998; Starr et al., 2003; Berg and Corner, 2005; van Noort et al., 2013). It is salt tolerant and is widely-planted in coastal areas (Figueiredo et al., 1995; Kobbi et al., 1996; Beardsley, 1998; van Noort et al., 2013). In Hawaii, Florida and Bermuda, where the tree's pollinator is also introduced, the tree has become invasive (Nadel et al., 1992; Beardsley, 1998; Starr et al., 2003; Caughlin et al., 2012). *Ficus microcarpa* also sets seed around the Mediterranean, where some establishment in natural areas is reported, but it is mainly regarded as an urban nuisance because fallen pollinated figs are messy underfoot and its roots damage walls and buildings (Caughlin et al., 2012).

Eupristina verticillata Waterston (Agaonidae) is the recorded pollinator of *F. microcarpa*. This taxon covers a complex of closely-related cryptic species (Sun et al., 2011), but only one of these is known from outside the plant's natural range (A. Cruaud, J-Y. Rasplus and R. Wang, unpublished). In the early 20th century *E. verticillata* was deliberately introduced into Hawaii (Pemberton, 1939), where the tree was seen as useful at the time, but the insect's subsequent spread elsewhere has been accidental or unsanctioned.

At least 29 NPFW species have been reared from *F. microcarpa* figs in its native range (Chen et al., 1999; Feng & Huang, 2010; Li et al., 2013, R. Wang and S.G. Compton, unpublished results). They include ovule gallers, parasitoids, and *Philotrypesis taiwanensis* Chen, a seed eater (Wang et al., 2014). No officially sanctioned releases of NPFWs are recorded, but several species are now known from their host plant's introduced range. The ovule galler *O. galili* is one of the two most widely introduced NPFWs associated with *F. microcarpa*. The other is *Walkerella microcarpae* Bouček (Pteromalidae, Otitesellinae). *Odontofroggatia galili* has been introduced to most parts of the world where *F. microcarpa* is grown, including the Americas, Europe, Middle East and Pacific (Galil and Copland, 1991; Bouček, 1993; Beardsley, 1998), and also South Africa, despite the absence of the tree's pollinator there (van Noort et al., 2013). *Ficus microcarpa* is likely to be the only host plant of *O. galili* though there is an unconfirmed record from another *Ficus* species (Bouček, 1988). *Sycophila* (Eurytomidae) are parasitoids of *Odontofroggatia* species in *F. microcarpa* figs. Asian *Sycophila* have appeared in Florida and elsewhere (Beardsley, 1998, R. Wang and S.G. Compton, unpublished results) and native African *Sycophila* species have also colonized O. galili in South Africa (van Noort et al., 2013). Several other gall-forming and parasitoid NPFWs have also become established within the introduced range of F. microcarpa, but none are as widespread as O. galili or W. microcarpae.

Ficus microcarpa was introduced around the Mediterranean over the course of the last two centuries (Mifsud et al., 2012). The pollinator of F. microcarpa has probably been in the Mediterranean area since at least the 1980s, allowing the plant to reproduce and colonize both urban and rural areas (Lo Verde et al., 1991; Kobbi et al., 1996; Doğanlar, 2012; Mifsud et al., 2012). The first of its associated NPFWs (O. galili) was recorded from Israel (Galil and Copland, 1981) and then from the Greek Isles (Compton, 1989). Kobbi et al. (1996) subsequently recorded O. galili, together with a second ovule-galler W. microcarpae and the pollinator from Tunisia. More recently, three further NPFWs have been recorded from F. microcarpa figs in the Mediterranean area: Odontofroggatia ishii Wiebes, Philotrypesis emeryi Grandi (sensu Bouček, 1993) and Philotrypesis taiwanensis Chen (Pteromalidae, Sycoryctinae) (Lo Verde and Porcelli, 2010; Doğanlar, 2012). Philotrypesis emeryi is a parasitoid, and P. taiwanensis is a seed eater (Wang et al., 2014). All the fig wasps have larvae that develop in female fig flowers, and only one larva completes its development inside each flower.

Meselatus bicolor is a large ovule gall-forming NPFW previously recorded only in figs of F. microcarpa from China and Taiwan (Chen et al., 1999). It is particularly abundant in north Yunnan and Sichuan, in the northern part of the plant's native range where F. microcarpa is widely planted, suggesting that it prefers seasonal climates (R. Wang and S.G. Compton, unpublished results). Bruchophagus sensoriae Chen (Eurytomidae) is its main parasitoid (R. Wang and S.G. Compton, unpublished results), though it is also attacked by an Ormyrus species (Ormyridae) in the far northern part of its range (Y. Chen personal communication).

2.2 Study sites

Between 2011 and 2013, mature figs were collected from F. microcarpa trees that had been planted in the following Mediterranean locations: Rhodes and Symi (Greece), Sicily (Italy), Tripoli (Libya), Malta (Malta), Majorca (Spain) and Marmaris (Turkey) and from trees in Santa Cruz, Tenerife (Spain), the largest of the Canary Islands (Table 1). These areas have typical Mediterranean climates with mild, rainy winters and hot, dry summers. Their annual precipitation ranges from about 330 mm in semi-arid Tripoli to 1100 mm in Marmaris. Santa Cruz has a warmer climate with mild winters and a low annual rainfall of about 236 mm.

2.3 Sampling methods

The development of monoecious figs has been divided into a series of stages by Galil and Eisikowitch (1968). Pollinator females enter the figs to lay their eggs at B-phase, seeds and pollinator wasp larvae develop during C-phase and the next generation of pollinators emerges from the figs during D-phase. NPFW females oviposit before or during B-phase, or during C-phase, depending on the species. Their adult offspring emerge at the same time as those of the pollinators. At least ten mature figs at late C-/early D-phase were collected haphazardly from different trees at each site and stored in 70% ethanol (Table 1). Each ‘crop’ sample of ten or more mature figs came from one tree on one sampling date (there were no repeat samples from any tree).

Figs at late C-/early D-phase contain all the adult offspring of the fig wasps that had oviposited into the figs. Any figs where some adult offspring had emerged were not included. Each fig was cut into quarters and soaked in water for approximately 10 minutes to soften the galls before dissection. Each flower was checked under a dissecting microscope and was assigned into one of five categories: male flowers; unfertilized and un-galled female flowers; galled female flowers containing wasp larvae; seed bearing; and failed galls (‘bladders’) where fig wasps had not completed their development. Fig wasps extracted from the galls were identified using procedures developed by Chen et al. (1999) and Feng and Huang (2010). Note that figs that lacked fig wasp offspring were not considered.

2.4 The sizes of figs and galls

The lengths and widths of a total of 409 dissected figs (from 35 samples) were measured to the nearest 0.2 mm using a dissecting microscope with an eyepiece graticule. In addition, 138 figs from 9 crops were randomly selected, from which the lengths and widths of 3745 galls containing M. bicolor, O. galili and pollinating agaonids were measured to the nearest 0.04 mm, again using an eyepiece graticule. The volumes of the figs and galls were then estimated using the formula for an ellipsoid, which was their approximate shape (Oliver et al., 2010).

2.5 Statistical analyses

Figs containing any fig wasps other than E. verticillata, M. bicolor and O. galili were excluded from all analyses. All statistical analyses were carried out using R 2.14.2 (R Development Core Team, 2012). Response variables in linear mixed models (LMMs) were square-root or natural logarithm transformed if necessary. Likelihood ratio tests were used to assess the significance of fixed effects in LMMs and Generalized Linear Mixed Models (GLMMs), and multiple tests with Bonferroni correction were applied in pairwise comparisons. Crop identity was set as the random effect in all analyses except for gall size comparisons.

The effects of M. bicolor and O. galili on male and female flower numbers, total and female pollinator offspring abundance, and seed production were quantified using figs sorted into three types: (i) figs where only E. verticillata adult offspring and no other fig wasps were present; (ii) figs containing M. bicolor (with or without pollinator offspring also present); and (iii) figs containing O. galili (with or without pollinator offspring). Differences were tested using LMMs in R package nlme version 3.1 (Pinheiro et al., 2013). The impacts of densities of the two NPFWs on the plant's male (female pollinator abundance) and female (seed production) reproductive functions were also analysed using GLMMs in R package lme4 version 1.0-5 (Bates et al., 2013) assuming Poisson error distributions.

All three fig wasps gall the ovules of their host (with one larva developing in each ovule) and in figs shared by two or more species the galls are potentially competing for nutrients. The identity of the fig wasps that had initiated galls which had failed to complete development (= hollow galled ovules) could not be determined in figs containing mixtures of species. Where adult offspring of just one fig wasp species were present, we assumed that failed galls belonged to the same species. To examine competitive effects, the sizes of successful mature galls, gall failure rates and the sizes of mature figs were compared. Variation in gall size among species was tested using LMMs with fig identity as the random effect. Figs containing a single species of fig wasp were used to test whether gall failure rates (the proportion of galls that failed to generate adult offspring) and fig size varied among species, and whether the relationships between gall failure rate, fig size and total number of galls (per fig) differed between species. GLMMs were used to assess gall failure rates (with binomial error distributions) and LMMs compared fig sizes. Only the combination of E. verticillata and O. galili was included in the analyses comparing gall failure rates, because M. bicolor did not occur in figs that had other species.

3. Results

3.1 Fig wasps associated with Mediterranean F. microcarpa

The contents of 797 mature figs (from 65 samples) were recorded from around the Mediterranean and from Tenerife (Table 1). In addition to the pollinator (E. verticillata), a total of seven NPFW species were recorded, including five ovule gall-forming species: M. bicolor, Micranisa degastris Chen (Pteromalidae, Otitesellinae), O. galili, O. ishii and W. microcarpae and two parasitoids, P. emeryi and Sycophila maculafacies Chen. The former uses pollinator larvae (and possibly other species) as hosts, the latter is a parasitoid of Odontofroggatia species (including O. galili and O. ishii). These are the first records of M. bicolor outside SE Asia and the first records of M. degastris and S. maculafacies in the Mediterranean area. Eupristina verticillata was found in all seven study sites. The most widespread NPFW species was O. galili, which was recorded everywhere except in Marmaris and Tenerife (Table 1). The most diverse fig wasp communities were present in figs from the Greek islands of Rhodes and Symi, where a total of six NPFW species were recorded (five from Rhodes and four from Symi), with three NPFW species recorded from the figs in Sicily and Majorca and just one or no NPFW species recorded from the other areas (Table 1).

The pollinating fig wasp, E. verticillata, was the most abundant species in most collections, emerging from 50.8% (405) of the figs and comprising 54.3% of all the recorded fig wasp adult offspring (33715 individuals). It was noticeably less frequent in collections from the islands of Rhodes and Symi, where only 23.0% of the figs contained this species (Table 2). Meselatus bicolor and O. galili, were the most abundant NPFWs overall, recorded from 25.2% (201) and 47.8% (381) of the figs and comprising 18.4% and 23.5% of the total fig wasp offspring respectively (Table 3). Meselatus bicolor was often present at high densities in the figs it occupied, where it excluded all other fig wasp species, but its distribution was limited to Rhodes and Symi (Table 3; Fig. 1a). On these islands it was recorded from 87.1% of the samples and 60.7% of the figs that were sampled. Odontofroggatia galili was the most prevalent NPFW overall (occupying the most figs), but its offspring were at relatively low densities in the figs where it was present (Table 3; Fig. 1b). The other five NPFW species were always rare, in total emerging from just 7.5% (60) of the figs and in combination comprising only 1.3% of the fig wasp adult offspring.

3.2 Effects of M. bicolor and O. galili on seeds and pollinators

The contents of 737 figs that contained E. verticillata, M. bicolor and O. galili were analysed. The numbers of male flowers in figs containing M. bicolor adult offspring were reduced to about 5% of those in figs occupied by the other species (Tables 4 and 5; Fig. 2). Similarly, female flower numbers in the figs occupied by M. bicolor were less than 25% of those recorded in figs occupied by O. galili or E. verticillata (Tables 4 and 5; Fig. 2). Small but significant differences in male and female flower numbers were also detected between figs where offspring of either O. galili or E. verticillata were present (Tables 4 and 5; Fig. 2).

None of the figs where M. bicolor offspring were present contained any pollinator offspring or seeds, even when as few as six M. bicolor galls were present. O. galili had a smaller, but still significant, influence on host plant reproduction. Female pollinator offspring and seeds were reduced by 61.9% and 73.6% respectively in figs where O. galili offspring were present, relative to figs where there were only E. verticillata offspring (Tables 4 and 5; Fig. 2). Both male (measured as female pollinator abundance) and female (seed production) reproductive successes of the figs were negatively related to O. galili abundance (GLMM: female pollinator abundance: β=-0.030 ± 0.001 (mean ± SE), df=1, Likelihood ratio (LR)=537.66, $p<0.001$; seed production: β=-0.028 ± 0.002, df=1, LR=304.39, $p<0.001$; Fig. 3 a & b). Both

NPFWs therefore had a negative influence on the numbers of pollinator offspring and seeds present in the figs, but the impact of M. bicolor was more emphatic.

3.3 Gall sizes

Meselatus bicolor produced the largest galls (2.95 ± 0.022 mm^3, N galls=1051, N figs=26). They were 9.4 times the volume of E. verticillata galls (0.31 ± 0.004 mm^3, N galls=1184, N figs=50) and 3.0 times the volume of O. galili galls (0.97 ± 0.006 mm^3, N galls=1510, N figs=96). The galls of the three species differed significantly in volume from each other (LMM: fixed effect: species: df=2, LR=3130.46, $p<0.001$; pairwise comparisons: E. verticillata/M. bicolor: df=3605, t=-96.66, $p<0.001$; E. verticillata/O. galili: df=3605, t=-78.57, $p<0.001$; M. bicolor/O. galili: df=3605, t=50.05, $p<0.001$).

3.4 Gall failure rates

A total of 552 figs contained offspring of a single species, and 185 contained a combination of E. verticillata and O. galili. Figs with only E. verticillata offspring or a combination of both E. verticillata and O. galili offspring had high gall failure rates, averaging 15.6% and 16.5%. This was over 3.5 times as high as in figs that contained only offspring of M. bicolor or O. galili. Among the figs where only pollinator offspring were present, 48 (27.4%) had no failed galls compared with 24 (13.0%) of the figs where a combination of E. verticillata and O. galili offspring were present (Tables 6 and 7; Fig. 4). Figs containing only O. galili or M. bicolor had similar gall failure rates, whereas there was a slight but significant increase in gall failure rates between figs containing only E. verticillata and those with a combination of E. verticillata and O. galili.

For all three species, gall failure rates were independent of the total number of galls in a fig, and there was also no variation in the strength of this relationship between species (GLMMs: figs containing only E. verticillata: $\beta=-0.002 \pm 0.001$, z=-1.62, p=0.106; figs containing only M. bicolor: $\beta=0.004 \pm 0.003$, z=1.40, p=0.163; figs containing only O. galili: $\beta=-0.003 \pm 0.006$, z=-0.57, p=0.571; Table 7).

3.5 Fig sizes

Of the 409 figs whose volumes were estimated, 302 contained only one of the three fig wasp species. Figs containing only M. bicolor offspring were 1.9 and 2.2 times as large as those containing only E. verticillata or O. galili offspring, respectively (Tables 6 and 7), whereas figs containing only E. verticillata were similar in volume to those containing only O. galili (Tables 6 and 7). Fig size increased with increasing numbers of fig wasp galls in figs containing only M. bicolor (LMM: slope=0.022 ± 0.002, df=262, t=12.12, $p<0.001$) and only O. galili (LMM: slope=0.009 ± 0.003, df=262, t=3.62, $p<0.001$) with a significantly stronger slope for the former, but the sizes of figs where only E. verticillata was present (and where seeds also contributed to their volume) were independent of total number of galls (LMM: slope=0.001 ± 0.002, df=262, t=0.61, p=0.541; Table 7; Fig. 5).

4. Discussion

The presence in the Mediterranean area of the pollinator of F. microcarpa, together with seven species of NPFW, was recorded. Three of the species were listed from the area for the first time (M. bicolor, M. degastris and S. maculafacies). An eighth species, P. taiwanensis, which was reported recently from Hatay, Turkey (Doğanlar, 2012), was not detected. The fig wasp fauna associated with F. microcarpa in the Mediterranean currently includes over one third of the NPFWs that occur regularly in F. microcarpa figs within its native range (R. Wang and S.G. Compton, unpublished results). An early survey in the Greek Isles detected only O. galili (Compton, 1989). Since then an additional six species appear to have arrived, including the tree's pollinator. The rapid expansion in the fauna is presumably as a result of increasing international trade (Lo Verde et al., 1991; Doğanlar, 2012; Mifsud et al., 2012). Secondary spread around the Mediterranean, either by natural dispersal or human activities, is likely to result in further local enrichment of communities as new species arrive from elsewhere and may lead to eventual homogenization in community composition across the Mediterranean as a whole.

Odontofroggatia galili was the first fig wasp reported from the Mediterranean, whereas M. bicolor has apparently arrived recently and may be restricted to the Greek Isles. Both species reduce the reproductive success of F. microcarpa, but M. bicolor has a much greater impact, totally inhibiting pollinator and seed production in the figs it occupies, even when present in small numbers. Both NPFWs can develop independently of the pollinator, but only M. bicolor prevents pollinator females from developing in figs it has galled. Meselatus bicolor females oviposit before the fig developmental stage when pollinators enter, and the rapid development of their large galls appears to prevent pollinator females from entering the figs or inhibits pollination, oviposition and offspring development in those that do enter.

Rates of flower occupancy by fig wasps were consistently low throughout the Mediterranean, compared with the native range where an average of 45% of the female flowers was occupied in southwestern China (R. Wang & S.G. Compton, unpublished results). This suggests that the figs could have supported more fig wasp larvae and that the impact of O. galili on pollinator offspring numbers was not a result of competition for oviposition sites (Dunn et al., 2008; Segar and Cook, 2012). Failure rates were higher in Eupristina verticillata galls than in either M. bicolor or O. galili, despite its galls being smaller and presumably needing fewer resources. In another fig tree species, failed galls were shown to have been oviposited in and also galled (Ghana et al., 2012). If this is the case within F. microcarpa figs, then failures in larval development are a major mortality factor for E. verticillata.

Both M. bicolor and O. galili are able to generate larger galled ovules, with lower failure rates, than those of the pollinator, suggesting that they are more efficient than the pollinator at directing nutrients to their galls. The increased gall failure rate in figs with a combination of E. verticillata and O. galili is consistent with this, as most of the additional failed galls were small and likely to be those of the pollinator (personal observations). Competition for nutrients among galls in the same figs was expected to result in higher failure rates in figs that contained more galls, but no significant relationship was noted. The mechanisms that allow some galls to be stronger assimilate sinks than others are poorly understood (Dorchin et al., 2006), but the effects of competition between gall inhabitants are well documented (Burstein et al., 1994; McGeoch and Chown, 1997; Hartley, 1998). Further, the larger size of the galls that support M. bicolor development also results in an increase the size of the figs as a whole. Figs containing this species are therefore likely to be extracting more nutrients from the plants than other figs.

Despite its ubiquity and demonstrably negative impact on seed and pollinator offspring numbers, O. galili has failed to prevent F. microcarpa from becoming invasive in areas such as Florida and Hawaii, and the tree is now also becoming established in parts of the Mediterranean (www.maltawildplants.com). Meselatus bicolor offers better prospects for reducing the damage to buildings caused by F. microcarpa seedlings in urban environments, and also for slowing the spread of the tree in natural areas, though destruction of seeds alone is unlikely to provide successful control (Garren and Strauss, 2009). The arrival of M. bicolor in the Mediterranean provides an opportunity to study how its impact on F. microcarpa and other fig wasps changes over time. This species is rare or absent from many sites in the native range of F. microcarpa in China (R. Wang and S.G. Compton, unpublished results), where it seems to prefer areas with seasonal rather than tropical climates.. The Mediterranean is extra-tropical, like many other areas where F. microcarpa is introduced, and M. bicolor is likely to do well there.

Away from the Mediterranean, could NPFWs be used for the biological control of F. microcarpa in areas such as Hawaii, Florida and Bermuda, where this fig tree is invasive? Among weed biological control programs in general there is no history of agents being introduced that target the specific pollinators of the plants, but some NPFWs can reduce not only the numbers of seeds in the figs they occupy, but via their impact on pollinators they also reduce the numbers of flowers that are pollinated. This duel impact on both the male and female components of reproductive success in fig trees suggests that there is the potential for NPFWs to significantly reduce recruitment among invasive fig trees. Ficus microcarpa nonetheless already has several NPFWs that are widely distributed, with no obvious reduction in the invasive capabilities of the plant. Odontofroggatia galili is one of the two most widely distributed NPFWs associated with F. microcarpa, and although previous work showed that it can reduce both seed and pollinator production (Kobbi et al. 1996), it is clearly ineffective. The reason seems to be that O. galili rarely reaches high densities within the figs it occupies, and this allows the figs to continue to support development of pollinators and seeds, albeit in reduced numbers. In contrast to O. galili, M. bicolor has not been widely dispersed beyond its native range, but it usually entirely prevents the development of seeds and pollinators in the figs it colonizes. By causing these figs to grow larger than normal it may also be having effects more widely across the tree

(Dennill, 1985). Meselatus bicolor is also capable of colonizing a large proportion of the figs that are available, has only been recorded from figs of F. microcarpa, and beyond SE Asia is likely to benefit from the absence of its host specific parasitoid, Bruchophagus sensoriae. In conclusion, the use of Meselatus bicolor as a control agent against F. microcarpa merits further consideration.

Acknowledgements

Financial support for R. Wang was provided by the China Scholarship Council. The quality of this publication was significantly enhanced by the work of the editor and two anonymous referees.

Contributions of authors

R. Wang & S.G. Compton contributed to project design, data collection and analysis, and paper writing. R.J. Quinnell contributed to data analysis. R. Aylwin, J. Cobb, L. Craine, S. Ghana & J.A. Reyes-Betancort contributed to data collection.

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Table captions

Table 1. The fig wasps present in samples of F. microcarpa figs collected at seven sites between 2011 and 2013. Each sample of figs was collected from one tree at one time. The fig wasps comprised the tree's pollinator and non-pollinating gallers and parasitoids (NPFW). Abbreviations: Eupristina verticillata: EV, Meselatus bicolor: MB, Micranisa degastris: MD, Odontofroggatia galili: OG, Odontofroggatia ishii: OI, Walkerella microcarpae: WM, Philotrypesis emeryi: PE and Sycophila maculafacies: SM.

Table 2. The numbers of flowers present in figs of F. microcarpa, the percentage of female flowers that contained fig wasp offspring (occupancy rates), and the percentage of galled female flowers where no fig wasp offspring had completed development (gall failures). The numbers of offspring of pollinators and NPFWs per fig are also provided, together with two measures of the plant's reproductive success – the number of female pollinators and the numbers of seeds. The contents of the figs were calculated for all figs at each site. All values are means ± SE per fig.

Table 3. Measures of the abundance of the three most commonly recorded fig wasp species in the figs of F. microcarpa. 'Prevalence' is the percentage of figs where offspring of each species was present. 'Numbers' is the densities of offspring of each species within the figs where they were present and 'Relative abundance' is the percentage of all fig wasp offspring contributed by each species in the figs where they were present. All values are means ± SE per fig.

Table 4. Differences in the numbers of flowers, pollinator offspring and seeds recorded in figs where only the pollinator of F. microcarpa was present and figs that contained M. bicolor or O. galili. These two NPFW species were not recorded sharing figs. All values are means ± SE per fig.

Table 5. LMM comparisons of the numbers of male and female flowers, total and female pollinator offspring and seeds in figs containing only E. verticillata, only M. bicolor or only O. galili.

Table 6. Failure rates(proportion of all galled ovules) and the volumes of mature F. microcarpa figs (cm^3) (means ± SE) that contained offspring of only E. verticillata, of only M. bicolor or only O. galili. M. bicolor and E. verticillata offspring did not coexist in the same figs.

Table 7. The influence of fig wasp species and the total number of galled ovules (per fig) on gall failure rates and the size of mature F. microcarpa figs. GLMMs assumed binomial distributions of residuals.

Table 1.

Country	Site	Location	N figs	N samples	Years	Fig wasp species richness	NPFW species richness	Gallers (n crops)						Parasitoids (n crops)	
								EV	MB	MD	OG	OI	WM	PE	SM
Greece	Rhodes &Symi	N36º10',E27º58', N36º35',E27º50'	331	31	2011-2012	7	6	13	27	0	21	1	3	3	8
Italy	Sicily	N38º07',E13º22'	99	10	2012	4	3	10	0	0	9	0	1	1	0
Libya	Tripoli	N32º51',E13º12'	96	7	2012	2	1	7	0	0	7	0	0	0	0
Malta	Malta	N35º56',E14º23'	130	9	2011	2	1	9	0	0	9	0	0	0	0
Spain	Majorca	N39º35',E02º40'	101	6	2012	4	3	6	0	2	4	0	4	0	0
Spain	Tenerife	N28º29',W16º19'	30	1	2013	2	1	1	0	1	0	0	0	0	0
Turkey	Marmaris	N36º51',E28º15'	10	1	2012	1	0	1	0	0	0	0	0	0	0
Overall			797	65	2011-2013	8	7	47	27	3	50	1	8	4	8

Table 2.

Site	Male flowers	Female flowers	Ovule Occupancy rate (%)	Fig wasp numbers (all species)	Gall failures (%)	Pollinator numbers (per fig)	NPFW numbers (per fig)	Female pollinators (per fig)	Seed numbers (per fig)
Rhodes &Symi	7.1 ± 0.5	91.4 ± 4.1	54.4 ± 1.5	39.2 ± 1.8	7.3 ± 1.9	13.7 ± 1.8	25.4 ± 1.3	11.5 ± 1.6	6.9 ± 1.1
Sicily	20.8 ± 0.8	237.8 ± 4.6	26.5 ± 1.3	64.3 ± 3.5	21.7 ± 1.8	44.5 ± 3.8	19.8 ± 2.2	38.8 ± 3.3	27.5 ± 2.9
Tripoli	19.8 ± 0.6	190.6 ± 3.1	27.6 ± 1.7	51.9 ± 3.2	11.4 ± 1.3	39.4 ± 3.9	12.5 ± 1.3	29.8 ± 3.3	25.0 ± 2.3
Malta	18.4 ± 0.5	190.8 ± 3.6	22.1 ± 1.1	42.6 ± 2.3	4.9 ± 0.8	18.8 ± 2.5	23.8 ± 1.4	14.2 ± 2.3	10.3 ± 1.6
Majorca	19.1 ± 0.6	199.3 ± 4.0	8.6 ± 0.5	16.7 ± 1.1	18.1 ± 1.9	9.7 ± 1.0	7.1 ± 0.9	7.2 ± 0.8	7.7 ± 1.2
Tenerife	15.7 ± 1.0	168.6 ± 6.1	25.5 ± 1.9	41.6 ± 2.9	19.1 ± 1.8	40.3 ± 3.0	1.3 ± 0.4	27.2 ± 1.9	31.1 ± 2.3
Marmaris	26.3 ± 2.2	263.0 ± 6.1	35.7 ± 4.3	93.1 ± 10.8	1.6 ± 0.8	93.1 ± 10.8	0	85.0 ± 9.5	113.3 ± 8.9
Overall	14.2 ± 0.3	156.5 ± 2.8	35.3 ± 0.9	42.3 ± 1.2	10.9 ± 0.9	23.0 ± 1.2	19.3 ± 0.7	18.5 ± 1.1	14.5 ± 0.9

Table 3.

Site	E. verticillata			M. bicolor			O. galili		
	Prevalence (%)	Numbers (N occupied figs)	Relative abundance (%)	Prevalence (%)	Numbers (N occupied figs)	Relative abundance (%)	Prevalence (%)	Numbers(N occupied figs)	Relative abundance (%)
Rhodes &Symi	23.0	59.8 ± 4.3 (76)	82.9 ± 2.8	60.7	30.9 ± 1.7 (201)	100 ± 0	25.1	13.8 ± 0.9 (83)	48.6 ± 4.3
Sicily	87.9	50.6 ± 4.0 (87)	70.3 ± 3.6	0	NA	NA	62.6	31.3 ± 2.2 (62)	59.4 ± 4.2
Tripoli	78.1	50.5 ± 4.4 (75)	79.9 ± 2.9	0	NA	NA	76.0	16.4 ± 1.5 (73)	49.4 ± 4.5
Malta	43.8	42.9 ± 3.8 (57)	65.8 ± 3.4	0	NA	NA	94.6	25.1 ± 1.4 (123)	75.2 ± 3.0
Majorca	69.3	13.9 ± 3.3(70)	83.6 ± 3.2	0	NA	NA	39.6	14.1 ± 1.5 (40)	92.0 ± 2.5
Tenerife	100	40.3 ± 3.0 (30)	96.1 ± 1.2	0	NA	NA	0	NA	NA
Marmaris	100	93.1 ± 10.8 (10)	100 ± 0	0	NA	NA	0	NA	NA
Overall	50.8	45.2 ± 1.8 (405)	78.7 ±1.4	25.2	30.9 ± 1.7 (201)	100 ± 0	47.8	20.8 ± 0.8 (381)	63.6 ± 1.9

Table 4.

Fig wasp	N crops	N figs	Male flowers (per fig)	Female flowers (per fig)	Pollinators (per fig)	Female pollinators (per fig)	Seeds (per fig)
E. verticillata only	37	179	20.3 ± 0.5	206.4 ± 4.0	54.0 ± 3.2	44.4 ± 2.9	39.4 ± 2.5
M. bicolor	27	201	0.9 ± 0.1	43.2 ± 1.8	0	0	0
O. galili	48	357	18.4 ± 0.3	191.5 ± 2.6	20.9 ± 1.6	16.9 ± 1.4	10.4 ± 0.9

Table 5.

Response variable	Fixed effect	df	Likelihood ratio	Pairwise comparisons	df	t value
Male flowers	Fig wasp sp.	2	454.38 ***	E. verticillata vs. M. bicolor	671	25.91 ***
				E. verticillata vs. O. galili	671	3.92 ***
				M. bicolor vs. O. galili	671	-25.73 ***
Female flowers	Fig wasp sp.	2	379.32 ***	E. verticillata vs. M. bicolor	671	22.27 ***
				E. verticillata vs. O. galili	671	3.18 **
				M. bicolor vs. O. galili	671	-22.20 ***
Pollinators	Fig wasp sp.	2	286.68 ***	E. verticillata vs. M. bicolor	671	18.31 ***
				E. verticillata vs. O. galili	671	13.59 ***
				M. bicolor vs. O. galili	671	-9.22 ***
Female pollinators	Fig wasp sp.	2	253.44 ***	E. verticillata vs. M. bicolor	631	17.15 ***
				E. verticillata vs. O. galili	631	12.43 ***
				M. bicolor vs. O. galili	631	-8.77 ***
Seed production	Fig wasp sp.	2	286.34 ***	E. verticillata vs. M. bicolor	671	18.22 ***
				E. verticillata vs. O. galili	671	14.62 ***
				M. bicolor vs. O. galili	671	-8.23 ***

**: p<0.01 ***: p<0.001.

Table 6.

Fig wasps present	Gall failure rate (N crops, N figs)	Fig size (N crops, N figs)
Only E. verticillata	0.156 ± 0.013 (37, 179)	0.482 ± 0.028 (16, 60)
Only M. bicolor	0.039 ± 0.006 (27, 201)	0.928 ± 0.063 (15, 112)
Only O. galili	0.045 ± 0.007 (37, 172)	0.420 ± 0.016 (23, 130)
E. verticillata and O. galili	0.175 ± 0.011 (36, 185)	--

Table 7.

Response variable	Fixed effect(s)	Model	df	Likelihood ratio	Pairwise comparisons	df	z/t value
Gall failure rate	Fig wasp presence	GLMM	2	379.05***	Only E. verticillata vs. Only M. bicolor	--	9.83***
					Only E. verticillata vs. Only O. galili	--	10.46***
					Only E. verticillata vs. E. verticillata and O. galili	--	-6.68 ***
					Only M. bicolor vs. Only O. galili	--	-1.14 ^{NS}
					Only M. bicolor vs. E. verticillata and O. galili	--	-12.57 ***
					Only O. galili vs. E. verticillata and O. galili	--	-15.34***
	Fig wasp presence × total number of galls	GLMM	2	3.40^{NS}	Only E. verticillata vs. Only M. bicolor	--	-1.84^{NS}
					Only E. verticillata vs. Only O. galili	--	0.01^{NS}
					Only M. bicolor vs. Only O. galili	--	1.19^{NS}
Fig size	Fig wasp presence	LMM	2	29.96 ***	Only E. verticillata vs. Only M. bicolor	265	-4.44 ***
					Only E. verticillata vs. Only O. galili	265	1.65 ^{NS}
					Only M. bicolor vs. Only O. galili	265	7.30 ***
	Fig wasp presence × total number of galls	LMM	2	62.56***	Only E. verticillata vs. Only M. bicolor	262	-8.53***
					Only E. verticillata vs. Only O. galili	262	-2.80*
					Only M. bicolor vs. Only O. galili	262	3.99***

^{NS}: not significant, *: $p<0.05$, ***: $p<0.001$.

Figure legends

Fig. 1. The abundance of M. bicolor (a) and O. galili (b) adult offspring in the figs where they were present. Note the different X axis scales.

Fig. 2. Numbers of male and female flowers, total and female pollinator adult offspring and seeds in figs containing M. bicolor (hatched bars), O. galili (grey bars) and only E. verticillata (open bars). In the box-plot, lines, boxes, whiskers, black squares and black triangles represent the median, the range from the first to third quartiles, 1.5 times lower and upper quartiles, mean, minimum and maximum values respectively.

Fig. 3. The effects of O. galili on measures of (a) male (female pollinator offspring abundance) and (b) female (seed production) reproductive successes of F. microcarpa figs.

Fig. 4. Gall failure rates among figs containing only E. verticillata, only M. bicolor, only O. galili and combination of E. verticillata and O. galili adult offspring. Lines, boxes, whiskers, black squares and black triangles represent median, range from the first to third quartile, 1.5 times lower and upper quartiles, mean and minimum and maximum values respectively.

Fig. 5. Differences in the linear relationship between fig size and total number of galls in figs containing only E. verticillata (black dashed line (linear mixed model analysis - non-significant) and squares), only M. bicolor (grey line and circles) and only O. galili (light grey line and triangles).

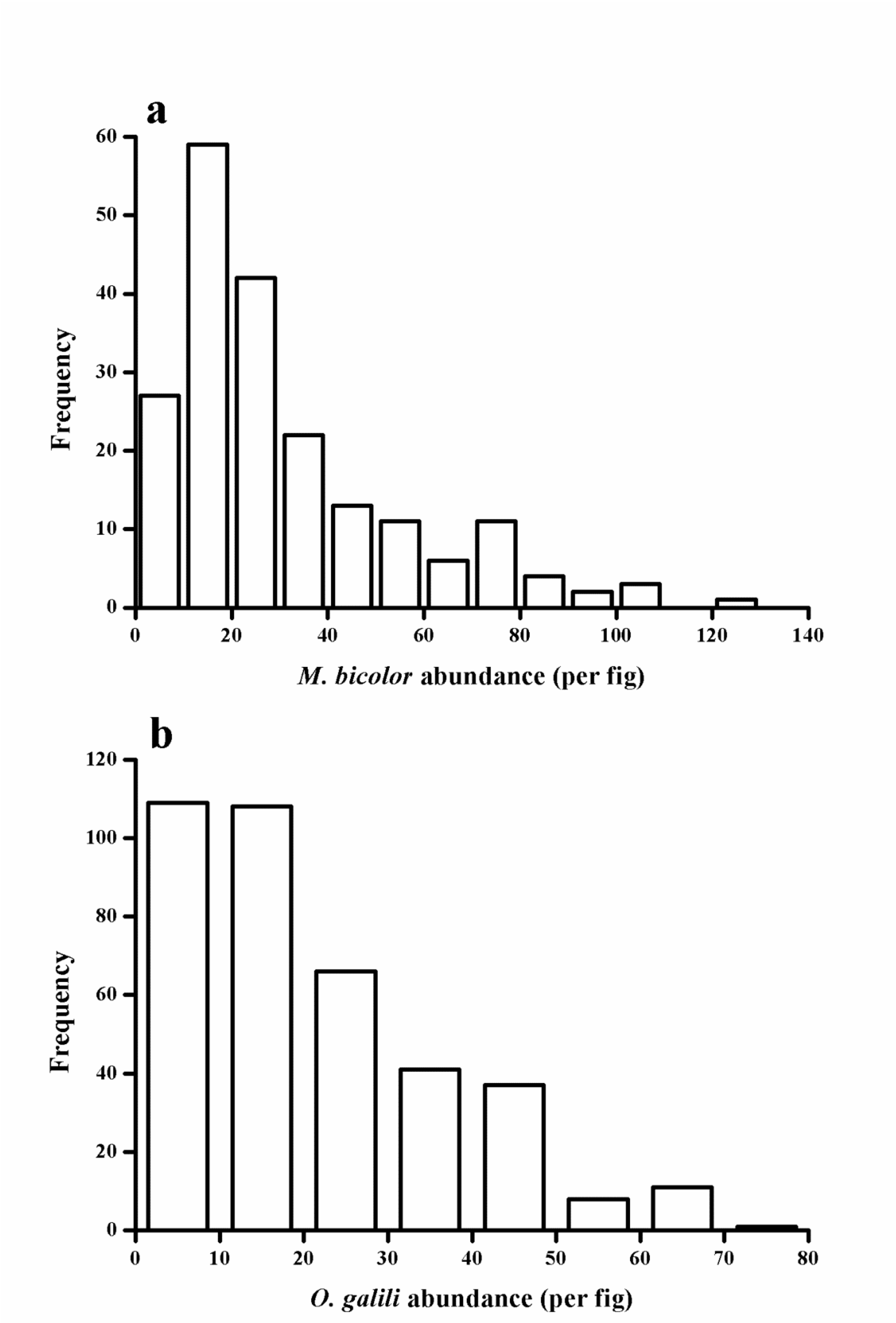
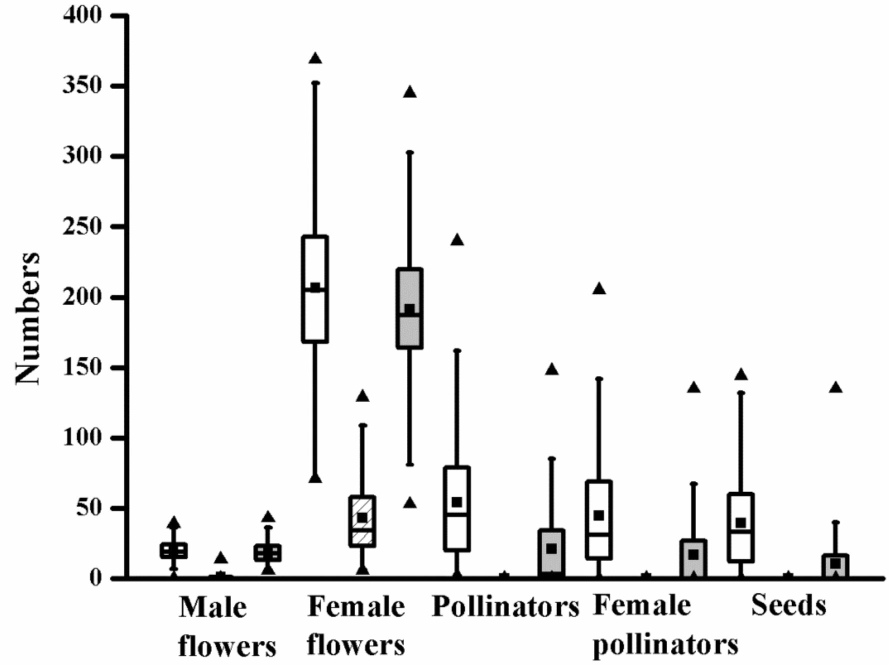

a
Frequency
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
M. bicolor abundance (per fig)
b
Frequency
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
O. galili abundance (per fig)

Fig. 1.



Numbers
400
350
300
250
200
150
100
50
0
Male flowers
Female flowers
Pollinators
Female pollinators
Seeds

Fig. 2.

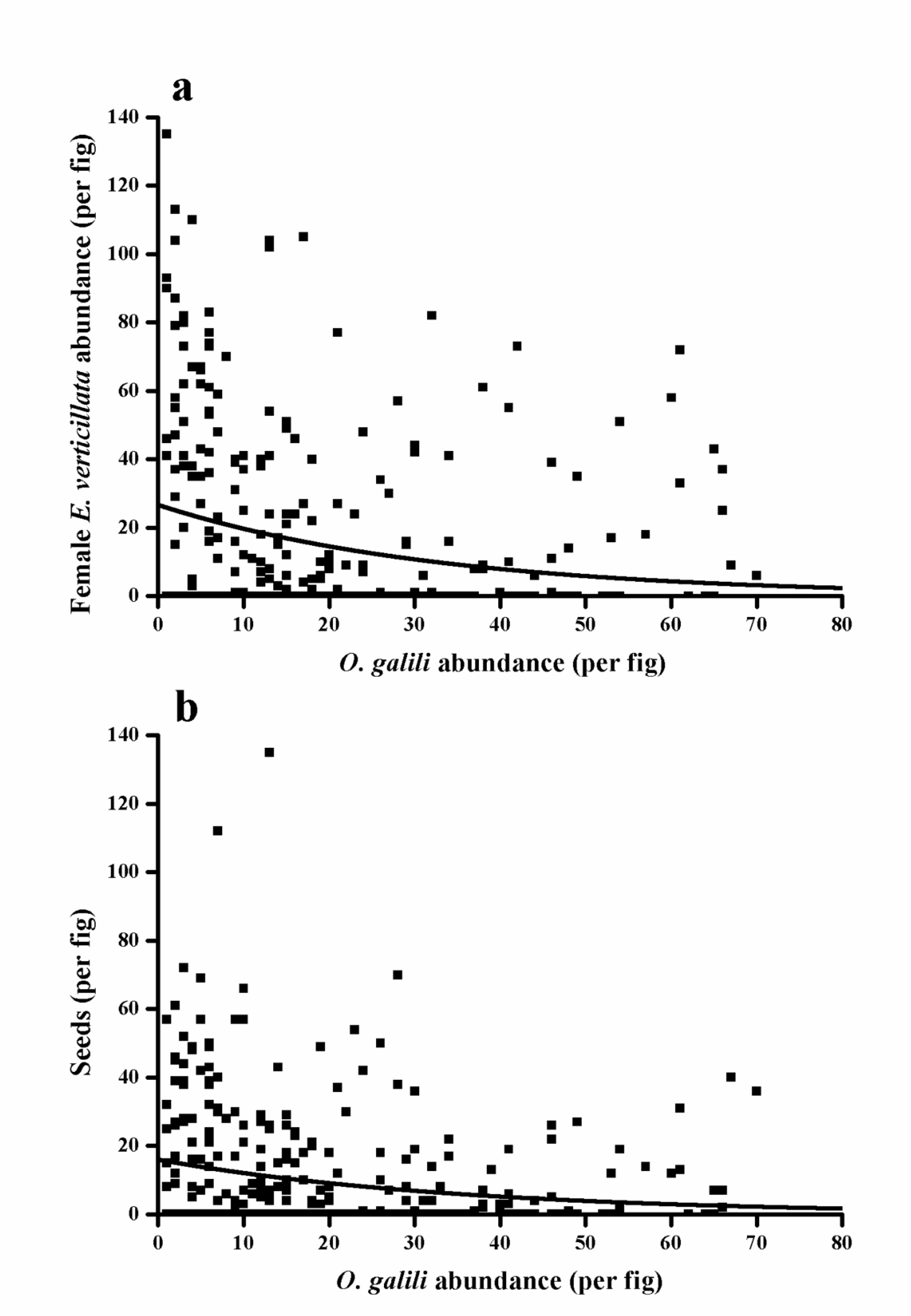

a
Female E. verticillata abundance (per fig)
O. galili abundance (per fig)
b
Seeds (per fig)
O. galili abundance (per fig)
0
20
40
60
80
100
120
140
0
10
20
30
40
50
60
70
80

Fig. 3.

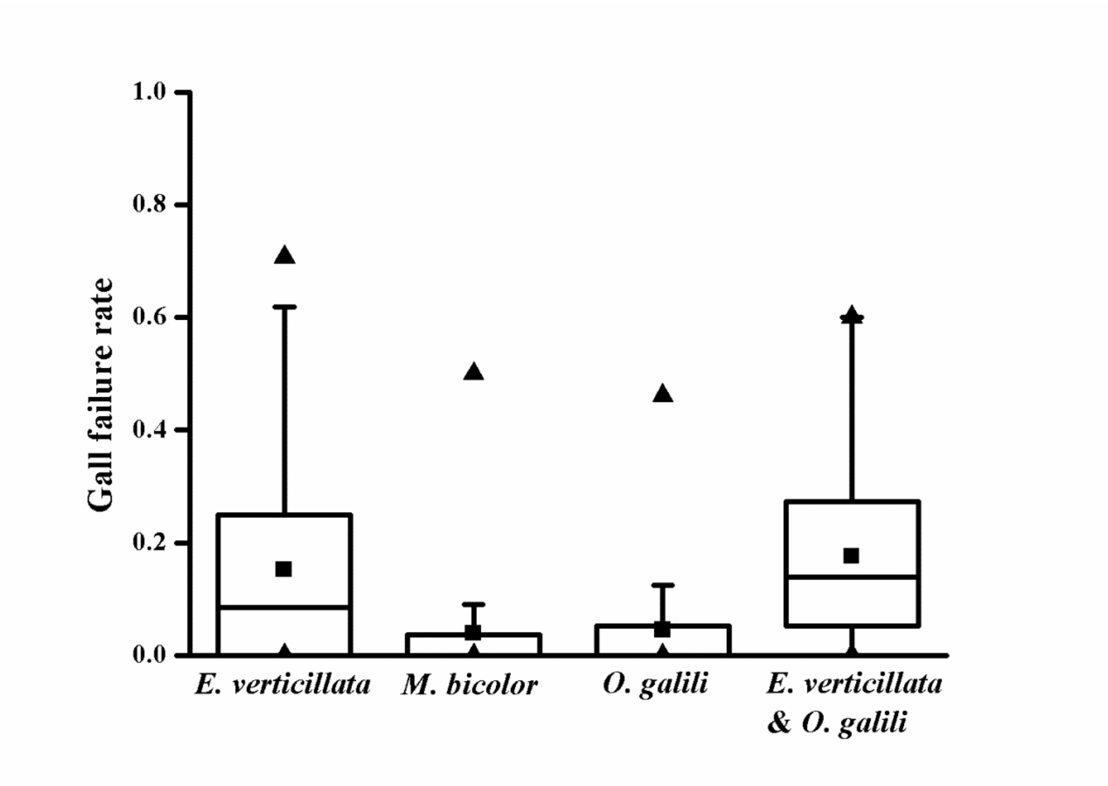

Gall failure rate
1.0
0.8
0.6
0.4
0.2
0.0
E. verticillata
M. bicolor
O. galili
E. verticillata & O. galili

Fig. 4.

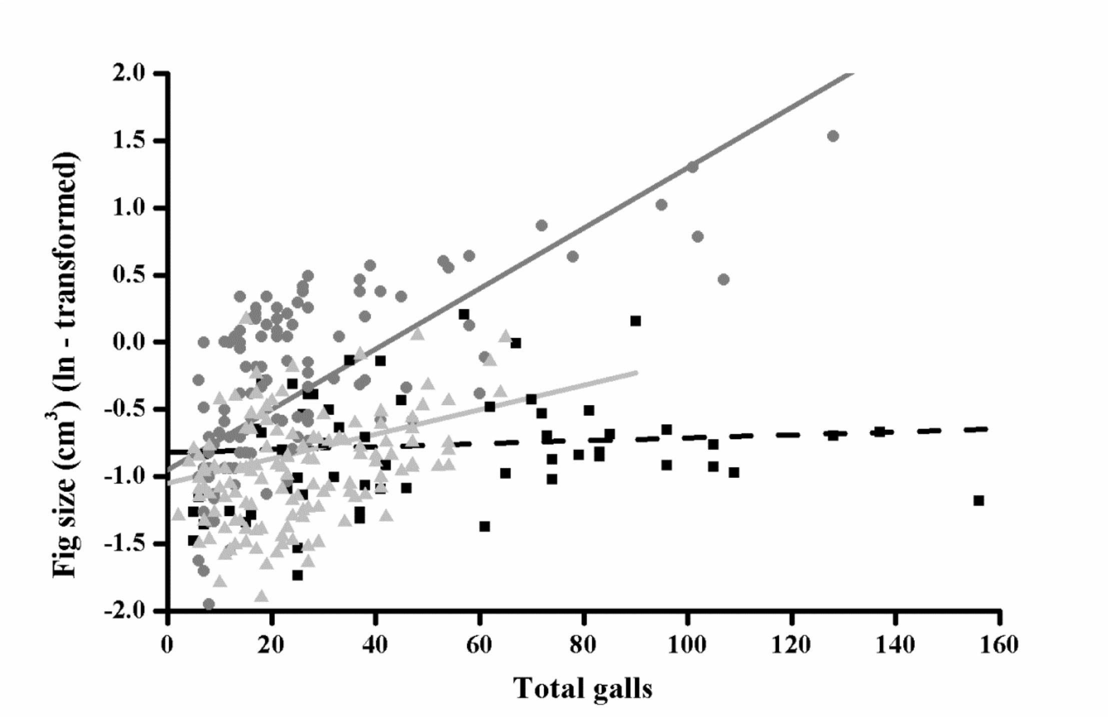


Fig size (cm3) (ln - transformed)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
0
20
40
60
80
100
120
140
160
Total galls

Fig. 5.