SYMBIOSIS (2008) 45, 29–32 ©2008 Balaban, Philadelphia/Rehovot ISSN 0334-5114

The benefits of pollination for a fig wasp

Yaowanit Tarachai[1,2*], Stephen G. Compton[3], and Chusie Trisonthi[1]

[1]Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand, Email. yaowanit@mju.ac.th;
[2]Graduate School of Chiang Mai University, Chiang Mai, Thailand;
[3]Faculty of Biological Sciences, University of Leeds, UK

(Received February 20, 2007; Accepted June 18, 2007)

Abstract

We describe aspects of the mutualistic relationship between the dioecious SE Asian fig tree *Ficus montana* and its pollinator, *Liporrhopalum tentacularis*. Female wasps actively collect pollen, which they later deposit inside receptive figs that they have entered. Inside male figs, we found that the reproductive success of lone females that did not carry pollen was lower than that of females that carried pollen. Figs entered by pollen-free fig wasps were more likely to abort. Furthermore, in those figs that did not abort, there were fewer pollinator progeny than in pollinated figs. When pollen-carrying lone females were prevented from ovipositing in male figs, by having the tips of their ovipositors removed, they appeared to be unharmed, but all the figs aborted. This suggests either that male figs may require oviposition, not pollen, in order to be retained by the trees, or that behavioral changes in the wasps prevented pollination from occurring.

Keywords: Agaonidae, Dioecy, *Ficus*, mutualism, pollination

1. Introduction

The relationship between *Ficus* and their highly specific pollinators is one of the best-studied obligate mutualisms (Kjellberg and Maurice, 1989). There are over 750 species of fig tree distributed mainly in tropical areas, each of which is associated with one or more species of highly-specialized pollinator fig wasps (Bronstein and McKey, 1989; Cook and Rasplus, 2003). Adult female pollinators (foundresses) deposit pollen in figs at about the same time as they attempt to lay their eggs in the flowers and gall them. Pollination may be passive, where pollen from numerous male flowers is distributed over the wasps as they prepare to leave their natal figs, or active, where females both collect pollen into thoracic pollen pockets and subsequently 'paint' with pollen the stigmas inside receptive figs that they have entered. Active pollination is likely to be much more efficient at transferring pollen than passive pollen, and reflecting this the ratio of male to female flowers inside figs is generally a good predictor of the pollination method of the wasps (Kjellberg et al., 2001). Fig wasp behavior, and associated plant traits such as male flower numbers, appear to be highly labile, with frequent gains and losses of active pollination behavior (Cook et al., 2004; Kjellberg et al., 2001).

Figs of monoecious fig tree species produce both seeds and pollen-carrying fig wasps, whereas functionally dioecious fig trees have female plants that produce only seeds and male plants that produce both pollen and the pollinator wasps to disperse it (Nefdt and Compton, 1996; Bronstein and McKey, 1989). The former produce only seeds because, after attracting the pollinators, they prevent them from ovipositing. Female figs achieve this by having female flowers with much longer styles than those in male figs, and there are also differences in the structure of the stigma, making ovipositor penetration more difficult.

Here we address two questions that underpin the relationship between fig wasps and their dioecious fig tree hosts. Jousellin et al. (2003) showed that pollinator species can benefit from active pollination of figs, probably because larval survivorship is higher in fertilized seeds. Benefits were less clear for a passively-pollinating species. Here, we similarly examine whether females that carry pollen into male figs of *F. montana* are at a reproductive advantage, relative to those that do not. This fig tree species is unusual in that plants have figs with a wide range of male flower numbers, encompassing the range that is typical of

**The author to whom correspondence should be sent.*

Presented at the 7th International Fig Wasp Symposium, July 23–26, 2006, Yunnan, China

both actively and passively pollinated *Ficus* species (N. Suleman and S. Raja, unpublished). By preventing fig wasps from ovipositing after entry into receptive figs, we also examine whether the absence of seed production in the male figs of this species reflects a physiological inability to develop seeds and whether pollen carrying into male figs is sufficient to stimulate their further development.

2. Materials and Methods

Species and study site

F. montana Burm. f. (section *Sycidium*) is a dioecious fig tree distributed in South East Asia (Berg, 2003). It is a small shrub that is sometimes a climber, reaching 1–3 m tall. Its specific, active, pollinator is *Liporrhopalum tentacularis* (Grandi). The University of Leeds glasshouse populations of *L. tentacularis* and *F. montana* were used for two experiments that were carried out between November 2005 and June 2006.

Experiment one. Pollen-free L. tentacularis

The reproductive success of pollen-free foundresses was compared with that of typical, pollen-carrying females. To produce pollen-free foundresses, female flowers in male figs that contained recently-mated female wasps were isolated from the male flowers shortly before the females would normally have emerged from their galls. Control foundresses were obtained from figs that had been collected at the same stage and placed in vials covered with a fine mesh. They were allowed to emerge as normal from the figs, and so will have had the opportunity to fill their pollen pockets. Prior to this, 120 pre-receptive phase male figs had been enclosed in fine mesh bags while still attached to the trees, in order to prevent pollination. Once at the receptive stage, 60 figs were allowed to be entered by a single pollen-free wasp and 60 by control wasps. This was achieved by placing the wasps at the ostioles using a fine paint brush. Treatment and control figs were located on the same plants. The bags were replaced after wasp entry to prevent further pollinators entering and also to prevent attacks by parasitoids. The abortion rates of the figs were monitored, and the remaining ripe figs were harvested and their contents were recorded: pollinator progeny, male and female flowers and bladders (unoccupied, empty but galled female flowers).

Experiment two. Oviposition prevention

Male figs were bagged at the pre-receptive stage as before. Foundresses were allowed to emerge naturally from figs that had been collected the day previously and placed in vials covered with fine mesh. The wasps were then cooled briefly in a freezer before half were placed above an ice-chamber and had the tips of their (excerted) ovipositors cut transversely with a scalpel. The wasps were then left for about 30 minutes to recover at room temperature and their longevity appeared unaffected by the treatment. Twenty control and 20 maimed foundresses were then introduced singly, as before, into the previously bagged figs, and the bags were replaced. Treatment and control figs were located on the same plants. The figs were monitored as in experiment one and their contents at maturity recorded.

3. Results

Experiment one

Abortions occurred in figs entered by both pollen-carrying and pollen-free foundresses, but were significantly more frequent in figs that had not been pollinated (χ^2 = 11.87, P<0.01, df = 1; Fig. 1). The figs that survived through to maturity included one that produced no fig wasps, but contained numerous bladders. Overall, the figs entered by pollen-carrying wasps that reached maturity produced over twice as many pollinator progeny as those that did not (Table 1, Z test, z (30,12) = 5.27, P<0.001). The sex ratios amongst the progeny did not differ significantly (Z test, z (30, 11) = 0.932, P>0.05). This difference in the numbers of progeny produced was reflected in the presence of far more bladders in those figs that lacked pollen. Bladders are hollow, but enlarged female flowers and are likely to be galls where pollinator larvae failed to develop successfully. The numbers of undeveloped flowers were similar in the two groups of figs, suggesting that foundress activity had been unaffected.

Table 1. The contents of *Ficus montana* figs that completed their development following the introduction of a single pollen-carrying or pollen-free foundress.

Contents	Pollinated (N = 30)		Without pollen (N = 12)	
	Mean	SD	Mean	SD
Pollinator progeny	56.60	15.26	27.75	16.31
Male progeny	8.70	9.52	5.92	8.33
Female progeny	47.90	13.49	21.83	16.21
% male progeny	15.37	–	21.32	–
Seeds	0	0	0	0
Bladders	30.47	15.33	52.50	23.16
Female flowers	94.30	13.98	93.50	16.58
Male flowers	14.88	4.00	14.00	3.25
Non pollinated flowers	7.23	7.08	13.08	13.66

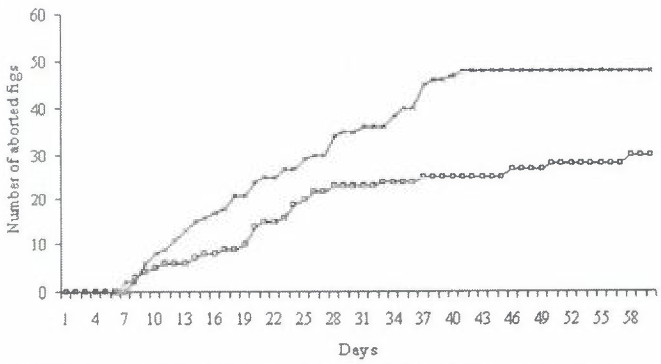

Figure 1. The cumulative numbers of *Ficus montana* male figs aborting after entry by single pollen-free (x–line, n = 60) or pollen-carrying foundresses (□ –line, n = 60).

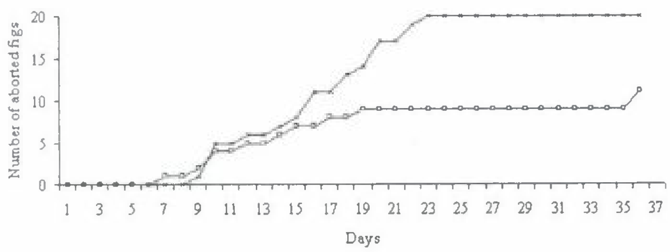

Figure 2. Cumulative abortions amongst *Ficus montana* male figs after entry by single foundresses with (□–line, n = 20) and without intact ovipositors (x–line, n = 20).

Experiment two

Foundresses with cut ovipositors showed typical behavior at the ostioles, readily entering the figs. All of the 20 figs entered by these foundresses had aborted after three weeks and when the figs were opened they were found to contain only undeveloped male and female flowers (Fig. 2). Eleven of the control figs also aborted, but the rate of abortion was nonetheless significantly lower in these figs ($\chi^2 = 11.61$, $P<0.01$, df = 1).

4. Discussion

Those species of fig trees that are actively pollinated typically produce far fewer male flowers than those species that rely on passive pollination (Kjellberg et al., 2005). This improvement in efficiency has clear benefits for the plant, as it can direct resources and more of the limited space within the fig to the production of additional female flowers, but the benefits of active pollination for the pollinators have been less clear. Some studies have detected increased mortality amongst progeny in pollen-free figs, others have not (Nefdt, 1989; Jousselin et al., 2003; Kjellberg et al., 2005). *Ficus montana* is unusual in that individual plants vary widely in the proportion of male flowers present in their figs, covering the range seen in both active and passively pollinated species (Kjellberg et al., 2005; N. Suleman and S. Raja, unpublished). Our results show that there are strong sanctions against *L. tentacularis* foundresses that fail to collect pollen, at least in those figs where they are the only females to enter. Pollen-free figs were more likely to abort, and when they did not abort, far fewer progeny were produced. The associated increase in the numbers of bladders suggests that flowers were being galled, but a smaller proportion of their larvae were surviving.

Whereas pollination has a significant impact on fig wasp reproductive success, it may not be the main stimulus responsible for the retention of male figs by the plant, as all the figs where oviposition was prevented aborted within three weeks. The act of oviposition (or gall production) may therefore be essential for figs to be retained, with pollination increasing the likelihood that retention occurs. It must be borne in mind however that relatively little pollen may have been dispersed by the maimed wasps, even though they appeared to be as vigorous as control females. This is because it is normally dispersed at about the same time as oviposition. Repetition of this experiment using a passively pollinating fig wasp and its host plant would be valuable, and if confirmed, then male fig development contrasts with the situation in female figs, where no oviposition and galling take place and pollination must be the stimulus for floral development to continue. Seeds have never been detected in any male figs of *F. montana*, strongly suggesting that female flowers in male figs are physiologically incapable of producing seeds, even if they do receive pollen.

Acknowledgements

We are grateful to the Commission on Higher Education of Thailand and Maejo University, Graduate School of Chiang Mai University, Chiang Mai, Thailand and the TRF/BIOTEC Special Program for Biodiversity Research and Training grant T_148011 for financial support. Thanks also to Dr. Nazia Suleman for her support throughout and to an anonymous referee for greatly improving the manuscript.

REFERENCES

Berg, C.C. 2003. Flora Malesiana Precursor for the treatment of MORACEAE: *Ficus* subgenus *Sycidium*. *Blumea* **48**: 573–579.

Bronstein, J.L. and McKey, D. 1989. The fig/pollinator mutualism: A model system for comparative biology. *Experientia* **45**: 601–605.

Cook, J.M., Bean, D., Power, S.A., and Dixon, D.J. 2004. Evolution of a complex coevolved trait: active pollination in a genus of fig wasps. *Journal of Evolutionary Biology* **17**: 238–246.

Cook, J.M. and Rasplus, J.Y. 2003. Mutualists with attitude: coevolving fig wasps and figs. *Trends in Ecology and Evolution* **18**: 241–248.

Jousselin, E., Hossaert-McKey M., Herre, E.A., and Kjellberg, F. 2003. Why do fig wasps actively pollinate monoecious figs? *Oecologia* **134**: 381–387.

Kjellberg, F., Jousselin, E., Bronstein, J.L., Patel, A., Yokoyama, J., and Rasplus, J.Y. 2001. Pollination mode in fig wasps: the predictive power of correlated traits. *Proceeding of the Royal Society of London B* **268**: 1113–1121.

Kjellberg, F., Jousselin, E., Hossaert Mckey, M., and Rasplus, J.Y. 2005. Biology, ecology and evolution of fig-pollinating wasps (Chalcidoidea, Agaonidae). In: *Biology, Ecology, and Evolution of Gall-inducing Arthropods*. Raman, A., Schaefer, C.W., and Withers, T.M., eds. Science Publishers, Inc, Enfield (NH), USA, and Plymouth, UK, pp. 539–572.

Kjellberg, F. and Maurice, S. 1989. Seasonality in the reproductive phenology of *Ficus*: its evolution and consequences. *Experientia* **45**: 653–660.

Nefdt, R.J.C. 1989. Interactions between fig wasps and their host figs. M.Sc Thesis, Rhodes University, South Africa.

Nefdt R.J.C and Compton S.G. 1996. Regulation of seed and pollinator production in the fig-fig wasp mutualism. *Journal of Animal Ecology* **65**: 170–182.