# CHAPTER V

## INTERACTION BETWEEN FIG AND THEIR POLLINATORS

### 5.1 DIOECIOUS FIGS

**The benefits of pollination for a fig wasp**

### 5.1.1 Introduction

The relationship between *Ficus* and their highly specific pollinators is one of the best-studied obligate mutualisms (Bronstein and McKey, 1989; Kjellberg and Maurice, 1989; Berg and Wiebes, 1992). There are over 750 fig species distributed mainly in the 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 (Kjellberg *et al*., 2001; Cook *et al*., 2004).

Monoecious fig 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 (Bronstein and McKey, 1989; Nefdt and Compton, 1996). 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. 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 passively-pollinating species.

For dioecious fig, one of the wild species, *Ficus montana* Burm.f., was selected. The fig tree population in glasshouse was examined so the fig trees could be closely monitored. The studies similarly examined whether the females that carry pollen into male figs of *F. montana* are at a reproductive advantage in relation 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 both actively and passively pollinated *Ficus* species (Suleman and Raja, unpublished). By preventing fig wasps from ovipositing after entry into receptive figs, another examination was 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.

**5.1.2 Literature review**

*F. montana* Burm.f. is one of the member of *Sycidium* section in the subgenus *Sycidium* (Berg 2003b; Noort and Rasplus, 2005) It has well-developed pistillodes in staminated flowers. In several species, the 'staminate' flowers even have pistils similar to the short-styled pistillate flowers and can be regarded as (morphologically) bisexual. For the ostiolar bract, this section has developed it differently from other groups that they lack distinct bracts (Berg, 1989).

This fig species distributes in South East Asia. It is a small climbing shrub, 1-3 m high with alternate leaves and small oval syconium. Its distribution is in limestone mountains in moist forests (Berg and Corner, 2005). Its flowering is about 4-6 times annually. Most of *Sycidium* section have pollinators in the genus *Kradibia*, except *F. montana*, of which its pollinators is *Liporrhopalum* (Berg, 2005). The characters of *F. montana* were described as follows (Berg, 2003b);

***Ficus montana* Burm. f.** Subgenus *Sycidium*

**Shrub**: 1-3 m tall; **Leaf**: spiral, alternate, margin of the lamina coarsely crenate-dentate to subentire; base of the lamina cuneate to rounded; basal lateral vein up to 1/2-1/3 of the length of the lamina; waxy glands confined to the axils of the basal lateral veins; leafy twig and laminas (densely or sparsely), whitish hair or indumentum absent, hair straight, curved or ± crinkled, leafy twig usually hollow; epidermis of the petiole persistent. **Figs**: axillary or just below the leaves; in pairs or solitary; figs usually with distinct peduncle (0.1)-0.2-0.8 cm long; ostiole c. 1 mm diam., surrounded by a sublobate rim; fruit (or endocarp body) distinctly tuberculate; stipule up to 1-1.2 cm long, subovate to lanceolate and chartaceous, not striate, cauducous. **Male flowers**: near apical pore; stamens 2; anthers oblong. **Gall flowers**: calyx lobes 4 or 5, linear-lanceolate; style lateral, short. **Female flowers**: basal bracteole 1; calyx lobes 4 or 5; ovary ovoid-ellipsoid; style lateral, long; stigma cylindric, shallowly 2-lobed. Achenes broadly ovoid, apically slightly concave on one side, with ± small tubercles. **Flowering and Fruiting** year-round.

The specific pollinator is *Liporrhopalum tentacularis* (Grandi). It was described by Wiebes (1994) as follow;

*Liporrhopalum tentacularis* (Grandi)

The female head is subquadrate, with protruding eyes; the cheek is nearly equal to the length of the eye. The antennae is very long: when reflexed over the body. The apical segment projects posterior to the gaster; the numberous sensilla are very short and hooked, interspersed with many setae. The mandible is bidentate, and it has one gland; the appendage bear four or five ventral lamellae; the maxilla has a distinct bacilliform process. The total length is 1.4-1.5 mm; the vales of the ovipositor are one-quarter to one-third of the length of the gaster.

The male head is almost triangular in outline, because it is rather wide posteriorly: 1 ½ times as wide as it is long; the eyes are quite large, equal in length to the cheek, and ca. one-seventh of the length of the head. The antennal scrobes are slightly exposed anteriorly and separated by a narrow, three-pointed clypeus. The mandible is tridentate, and it has two glands.

The pronotum has a large, strongly curved collar, and the posterior corners are tapering; the metanotal plates are distinctly contiguous. The fore tibia bears three dorso-apical teeth. The total length is ca. 0.8 mm.

**The abortion hypothesis**

This hypothesis suggested that fig tree abort figs that contain a very high proportion of wasp larvae (Janzen 1979; Axelrod and Hamilton 1981; Murray 1985 in Katharia-Gupta, 1999). Nefdt (1989) reported the experiments with pollen-free wasp of *F. burtt-davyi* (monoeciuos fig) some of larva could survive and some non-pollinator fig wasps developed in seed-free monoecious figs.

Furthermore, comparison between wasp numbers in aborted and unaborted figs showed no difference between the two (Bronstein, 1988). Male figs in dioecious species complete development without producing seeds. However, it may be possible that some of the ovaries harbouring wasp offspring in heavily exploited figs were aborted, giving rise to some of the vacant ovaries (bladders) observed in mature figs. All data available so far suggest that most pollinated figs reach maturity and abortion of figs was seen only under strong resource limitation (Bronstein, 1988).

### 5.1.3 Materials and methods

**Species and study site**

The University of Leeds glasshouse populations of *L. tentacularis* and *F. montana* (Figure 5.1) were used for two experiments that were carried out between November 2005 and June 2006.

The experiments were set to three times, the first one was carried out from November to December 2005, the second one was from January to March 2006 and the last one was from May to June 2006. Although the climate in the glasshouse was controlled, in the winter it was still cold (18-20 ºC). In summer, the glasshouse was open. The average temperature in the period of the study was 21.1 ºC (Table 5.1)

a. b.

**Figure 5.1** *Ficus montana* Burm. f.; *a.* plant, *b.* fig (syconium)

**Table 5.1** Temperature (ºC) of the University of Leeds glasshouse during the study from November 2005 to June 2006

| Periods of the study | Mean max Temperature (ºC) | Mean min Temperature (ºC) | Average Temperature (ºC) |
|---|---|---|---|
| November 2005 | 20.0 | 16.3 | 18.1 |
| December | 21.3 | 18.8 | 20.0 |
| January 2006 | 21.3 | 18.8 | 20.0 |
| February | 21.8 | 17.5 | 19.6 |
| March | 24.3 | 16.8 | 20.5 |
| April | 30.0 | 18.0 | 24.0 |
| May | 26.5 | 19.0 | 22.8 |
| June | 27.8 | 19.3 | 23.5 |
| Mean | 24.1 | 18.0 | 21.1 |

### 5.1.4 Experiment 1

**Pollen-free of *Liporrhopalum tentacularis* (Grandi)**

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 would have had the opportunity to fill their pollen baskets.

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. If the syconium became soft, it meant the figs start to be receptive. In a few hours later, they would be pollinated. Therefore, the hard fig would be selected.

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 (Figure 5.2).

a. b.

**Figure 5.2** The female entering; *a.* some parts of wings and antenna left, *b.* the fig with entering female pollinator was bagged with the fine mesh bag

The treatment and control figs were located on the same plants. The bags were replaced after wasp's entry to prevent further pollinators entering, and also to prevent attacks by parasitic wasps. The abortion rates of the figs were monitored, and the remaining ripe figs were harvested and their contents were recorded. The features of measuring are pollinator progeny, male and female flowers and bladders (unoccupied, empty but galled female flowers).

**Results**

There were fifty percent of harvest figs in the pollen carrying or control experiment (30/60) whereas in the pollen-free or treatment experiment was twenty percent (12/60) left. Means ±SD of exit galls was 56.27 ±14.99 in control and 27.75 ±16.31 in treatment. The bladder number in the control was 30.47 ±15.33 while in the treatment was 52.50 ±23.16 (Table 5.2, 5.3).

Duration of fig development was diverse from 30 days to 71 days, however both experiments took average 41 to 46 days for reproductive stage. In pollen carrying experiments, the abortion figs commonly represented in two weeks after pollinated, whereas it took longer (2-4 weeks) in pollen free carrying experiment (Table 5.2, 5.3).

The exit galls were the galls contained progeny pollinators that divided to male and female wasps. In pollen-free experiment, the ratio of male and female was 5.92: 21.83 (Table 5.2) or about 1:4, whereas in control was 8.70: 47.90 (Table 5.3) or about 1:5.5.

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 ($\chi2 = 11.87$, $P<0.01$, df = 1; Figure 5.3, Table 5.4). The figs that survived through to maturity (treatment No. 9) included one that produced no fig wasps, but contained numerous bladders. Normally, abortion figs had bright yellow color and fig turned soft.

**Table 5.2** The feature components of *F. montana* in pollen-free experiment (treatment)

| No. | exited gall | blad ders | unpollinated flowers | total flowers | male flowers | male wasp | female wasp | duration (days) |
|---|---|---|---|---|---|---|---|---|
| 1 | 17 | 52 | 9 | 78 | 18 | 2 | 15 | 46 |
| 2 | 37 | 37 | 8 | 82 | 15 | 4 | 33 | 39 |
| 3 | 45 | 19 | 43 | 107 | 17 | 10 | 35 | 46 |
| 4 | 53 | 37 | 28 | 118 | 13 | 5 | 48 | 46 |
| 5 | 18 | 62 | 3 | 83 | 15 | 1 | 17 | 44 |
| 6 | 52 | 62 | 2 | 116 | 7 | 4 | 48 | 43 |
| 7 | 23 | 71 | 0 | 94 | 16 | 2 | 21 | 43 |
| 8 | 22 | 29 | 30 | 81 | 11 | 4 | 18 | 42 |
| 9 | 0 | 94 | 4 | 98 | 16 | 0 | 0 | 44 |
| 10 | 13 | 53 | 18 | 84 | 11 | 6 | 7 | 40 |
| 11 | 21 | 84 | 7 | 112 | 17 | 2 | 19 | 40 |
| 12 | 32 | 30 | 5 | 67 | 12 | 31 | 1 | 30 |
| Sum | 333 | 630 | 157 | 1120 | 168 | 71 | 262 | 503 |
| Mean | 27.75 | 52.50 | 13.08 | 172.31 | 14.00 | 5.92 | 21.83 | 41.92 |
| SD | 16.31 | 23.16 | 13.66 | 285.19 | 3.25 | 8.33 | 16.21 | 4.46 |

**Table 5.3** The feature components of *F. montana* figs in pollen-free experiment (control)

| No. | exited gall | bladders | unpollinated flower | total flowers | male flowers | male wasp | female wasp | duration (days) |
|---|---|---|---|---|---|---|---|---|
| 1 | 50 | 22 | 0 | 74 | 12 | 8 | 42 | 52 |
| 2 | 71 | 37 | 6 | 114 | 16 | 5 | 66 | 71 |
| 3 | 69 | 45 | 5 | 119 | 14 | 8 | 61 | 52 |
| 4 | 63 | 27 | 4 | 94 | 10 | 19 | 54 | 69 |
| 5 | 64 | 24 | 1 | 99 | 27 | 5 | 59 | 54 |
| 6 | 60 | 15 | 12 | 87 | 14 | 10 | 50 | 63 |
| 7 | 58 | 28 | 5 | 91 | 12 | 8 | 50 | 52 |
| 8 | 62 | 29 | 24 | 115 | 16 | 10 | 52 | 53 |
| 9 | 65 | 30 | 12 | 107 | 14 | 11 | 54 | 52 |
| 10 | 52 | 42 | 4 | 98 | 10 | 10 | 42 | 51 |
| 11 | 64 | 40 | 8 | 112 | 12 | 12 | 52 | 42 |
| 12 | 52 | 43 | 7 | 102 | 20 | 6 | 46 | 45 |
| 13 | 73 | 11 | 2 | 86 | 12 | 6 | 67 | 43 |
| 14 | 60 | 21 | 7 | 88 | 17 | 7 | 53 | 42 |
| 15 | 14 | 68 | 3 | 85 | 17 | 1 | 13 | 44 |
| 16 | 79 | 4 | 9 | 92 | 14 | 3 | 76 | 41 |
| 17 | 45 | 58 | 0 | 103 | 21 | 8 | 37 | 40 |
| 18 | 27 | 26 | 27 | 80 | 9 | 3 | 24 | 41 |
| 19 | 62 | 36 | 0 | 98 | 16 | 4 | 58 | 44 |
| 20 | 59 | 34 | 5 | 98 | 11 | 3 | 56 | 41 |
| 21 | 53 | 39 | 4 | 96 | 16 | 8 | 45 | 40 |
| 22 | 41 | 43 | 5 | 89 | 10 | 7 | 34 | 40 |
| 23 | 34 | 56 | 0 | 90 | 12 | 9 | 25 | 40 |
| 24 | 57 | 34 | 22 | 113 | 19 | 3 | 54 | 44 |
| 25 | 88 | 6 | 16 | 110 | 15 | 55 | 33 | 40 |
| 26 | 68 | 19 | 6 | 93 | 16 | 13 | 55 | 36 |
| 27 | 45 | 33 | 12 | 90 | 17 | 4 | 41 | 36 |
| 28 | 45 | 19 | 0 | 64 | 16 | 6 | 39 | 39 |
| 29 | 52 | 11 | 5 | 68 | 10 | 7 | 45 | 39 |

**Table 5.3** The feature components of *F. montana* figs in pollen-free experiment (control) (continued)

| No. | exited gall | blad ders | unpollinated flower | total flowers | male flowers | male wasp | female wasp | duration (days) |
|---|---|---|---|---|---|---|---|---|
| 30 | 56 | 14 | 4 | 74 | 20 | 2 | 54 | 39 |
| Sum | 1688 | 914 | 217 | 2829 | 445 | 261 | 1437 | 1385 |
| Mean | 56.27 | 30.47 | 7.23 | 94.30 | 14.88 | 8.70 | 47.90 | 46.17 |
| SD | 14.99 | 15.33 | 7.08 | 13.98 | 4.00 | 9.52 | 13.49 | 9.06 |

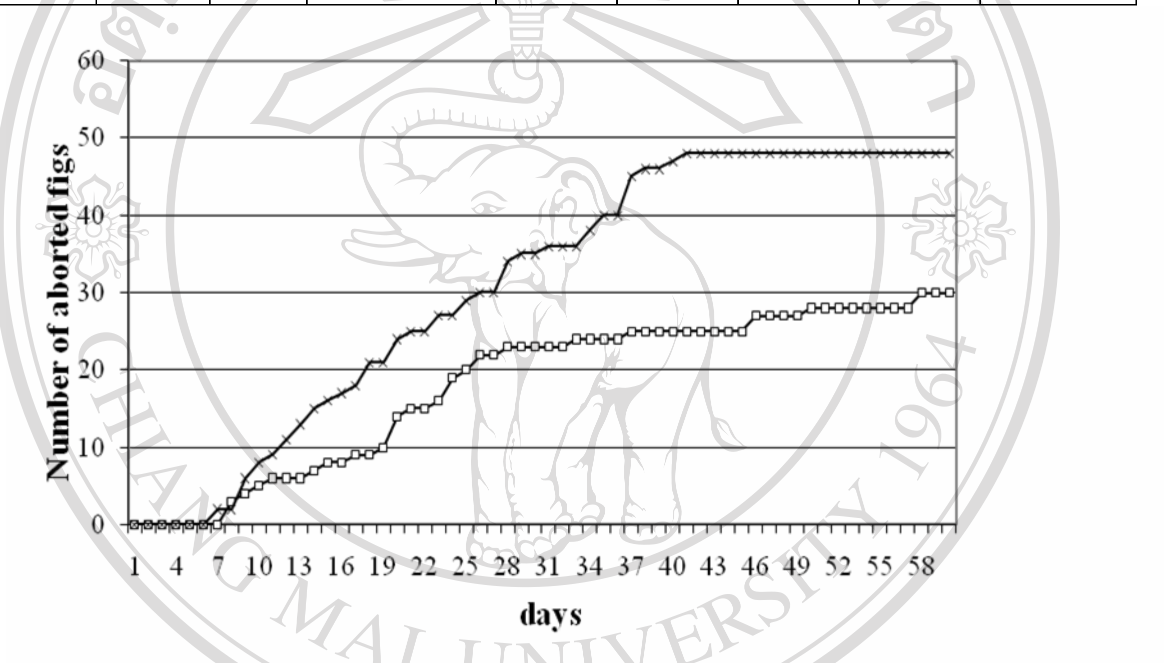
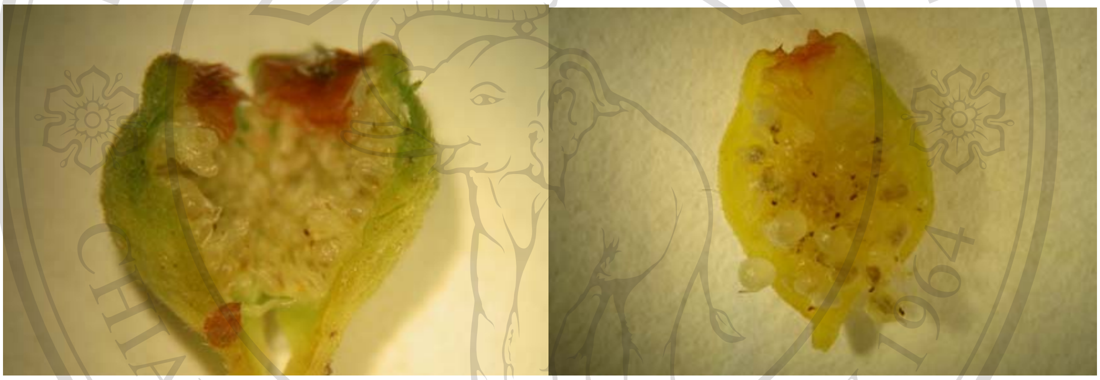



**Figure 5.3** The cumulative numbers of *Ficus montana* male figs aborting after entry by single pollen-free (x–line, n = 60) or pollen-carrying foundresses (□ –line, n = 60).

**Table 5.4** chi-square test of abortion *F. montana* figs in pollen free experiment.

| **observed values** | **abortion** | **harvest** | **Total** |
|---|---|---|---|
| control | 30 | 30 | 60 |
| treatment | 48 | 12 | 60 |
| **total** | 78 | 42 | 120 |
| **expected vales** | | | |
| | 39.00 | 21.00 | |
| | 39.00 | 21.00 | |
| **P value** | 0.00 | df | 1 |
| **Chi value** | 11.87 | | |

Abortion figs that failed in a couple weeks after pollination contain mostly non pollinated flowers. There were small flowers packed in small fig (Figure 5.4 a.). The abortion figs which failed after two weeks of pollination were large with galling size containing some developed flowers (Figure 5.4 b.). However, in this case the wasp larvae always died before reaching to develop successfully that the gall flowers were called ‘bladders’. The color of bladders was represented in both white and brown galls with brown stigma.



a. b.

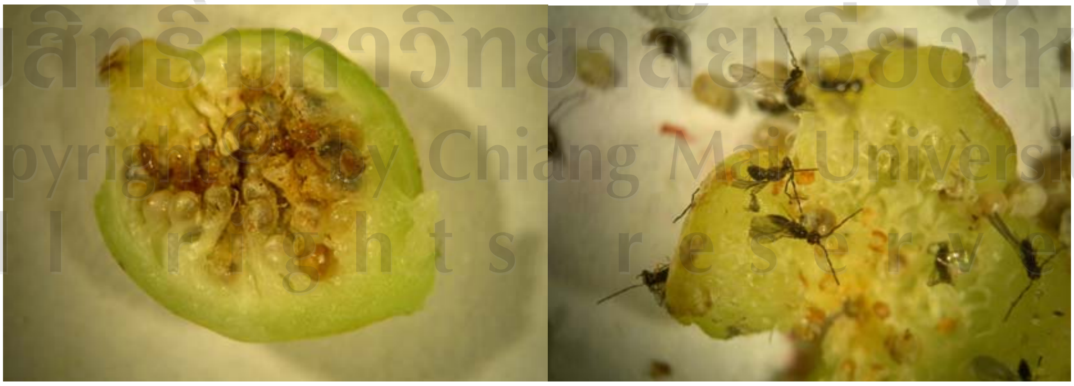
**Figure 5.4** The dissection of abortion *F. montana* figs; *a.* The fig failed within two weeks after female wasp entering, and *b.* The fig failed during three or four weeks after entering.

Overall, the figs entered by pollen-carrying wasps that reached maturity produced over twice as many pollinator progeny as those that did not (Table 5.4, Z test, $z_{(30, 12)} = 5.27$, $\rho < 0.001$). The sex ratios amongst the progeny did not differ significantly (Z test, $z_{(30, 12)} = 0.932$, $\rho > 0.05$). This difference in the numbers of progeny produced was reflected in the presence of far more bladders in those figs that lacked pollen. The numbers of undeveloped flowers were similar in the two groups of figs, suggesting that foundress activity had been unaffected (Table 5.5).

**Table 5.5** The contents of *F. 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 | - | 21.33 | - |
| 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 |

In order to get the correct number of wasp progeny ratios, the fig was harvested in the early stage of D phase (Figure 5.5 a.). If the fig was collected late, the female pollinators would emerge when the ostiole became looser (Figure 5.5 b.). Hence, at that time, male and female galls would not be distinguishable. In this study, the former emerging wasp was male. After emerge, they walked around in the fig and mated the female from outside their galls. One male wasp could mate with more than one female.



a. b.

**Figure 5.5** Dissection of male fig; a. early maturity phase (D-phase), b. female pollinators emerge

In the experiment of pollen-free carrying, information that received was the male fig that immerged with non-pollen foundress could not produce any seeds. It was shown that female flowers in male fig (gall) of *F. montana* could not develop themselves to be fruits and seeds without pollination (Table 5.5).

### 5.1.5 Experiment II

#### Oviposition prevention

Male figs of *F. montana* 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 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. The fine bags were removed in a week later (Figure 5.6a.). The treatment and control figs were located on the same plants. The figs were monitored similar to the experiment I and their contents at maturity recorded (Figure 5.6b.).

a. b.

**Figure 5.6** Fig with oviposition experiment; *a.* control experiment that fig was pollinated and oviposition, *b.* dissected fig for measuring.

**Results**

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 (Figure 5.7) and when the figs were opened they were found to contain only undeveloped male and female flowers (Figure 5.8).

Most figs without oviposition failed in a few weeks. Some of them could survive longer but they turned abort. The longer survival figs showed the develop galls inside, it seemed to be gall flowers developed when they received the pollen. However, no fig reached maturity. The result showed that the male fig of *F. montana* could not produce seeds without oviposition.

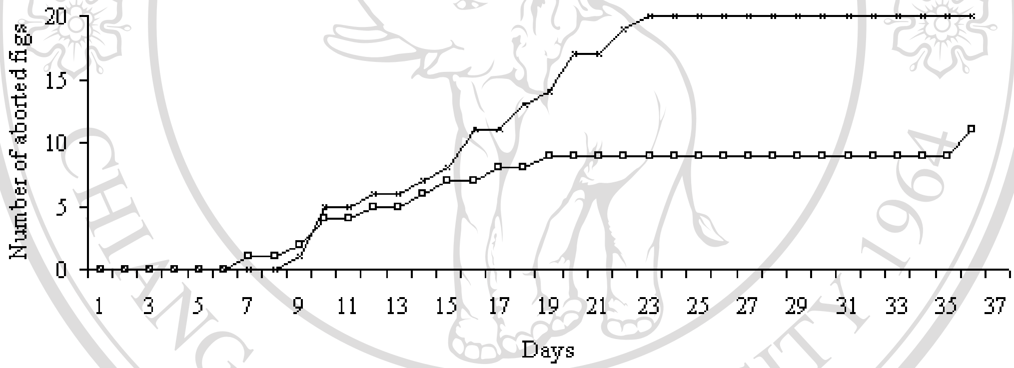



**Figure 5.7** Cumulative abortions amongst *Ficus montana* male figs after entry by single foundresses with (□–line, n = 20) and without intact ovipositors (x–line, n = 20).


**Figure 5.8** Fig without oviposition; *a.* the falling figs in 7-10 days (ovipositor removed), *b.* falling fig after 10 days.

The falling figs within 7-10 days after the wasp entered showed that female flowers did not develop to gall. Meanwhile, the longer survival figs showed galled inside.

Eleven of the control figs also aborted, but the rate of abortion was significantly lower in these figs ($\chi 2 = 11.61$, $P<0.01$, df = 1) (Table 5.6).

**Table 5.6** The chi-square test of abortion figs in the prevented oviposition experiment.

| **observed values** | **abortion** | **harvest** | **total** |
|---|---|---|---|
| control | 11 | 9 | 20 |
| treatment | 20 | 0 | 20 |
| **total** | 31 | 9 | 40 |
| **expected vales** | | | |
| | 15.50 | 4.50 | |
| **P value** | 0.00 | df | 1 |
| **Chi value** | 11.61 | | |

**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; Suleman and 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 suggested that flowers were being galled, but a smaller proportion of their larvae survived. 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.

## 5.2 MONOECIOUS FIG

**The pollination study of *Ficus racemosa* L.**

### 5.2.1 Introduction

The interaction between *Ficus* and their pollinators is an important model for studying obligatory mutualism. Between monoecious and dioecious figs the mechanisms are different. Monoecious figs contain three kinds of flowers in the same fig and same tree, whereas dioecious female and male trees were separated (Kjellberg *et al*., 2001).

*F. racemosa* is a member of subgenus *Sycomorus*, which is one of a few monoecious species in this group (Berg, 1989). Its distribution range is from India to Australia (Corner, 1965). In Thailand, it was found in all part of the country, especially in moist areas and along rivers (Gardner *et al*., 2000). The fig tree is a large tree that can reach up to 30 m high with a great number of cauliflorous syconia bearing and producing a large number of figs in each crop year-round (Wang *et al*., 2005). Inside a syconium, there are three kinds of flowers: male, female and gall flowers. The large number of tiny flowers with those flowers packed on the inner surface (Wiebes, 1979). Female flowers in monoecious figs produce both seeds and wasp offspring at the same time. The flowers with short style (gall flower) produce insects, while the type of long style flowers produce seeds (Berg and Wiebes, 1992). The proportion of flowers inside the fig is various in different species, therefore the trade off between them and this system sustaining is the interesting point to study (Wang *et al*., 2005).

Many publication reported that people used their fruits and leave as vegetable. The leave can be used for vegetable and medicinal plant. Mandal *et al*. (2000) studied an anti-inflammatory property of *F. racemosa* leaf extract and reported that 200 and 400 mg/kg extracts was found to possess significant anti-flammatory activity. Khan and Sultana (2005) found that the powder extract from this fig species can also be a potent chemopreventive agent and suppresses Fe-NTA-induced renal carcinogenesis and oxidative damage response in Wistar rats. Rahuman *et al*. (2008) reported the extracts of leaf and bark of *F. racemosa* can against the early fourth-instar larvae of *Culex quinquefasciatus* (Diptera: Culicidae).

### 5.2.2 Literature review

The pollination process starts with the pollen-carried female wasps emerged from the natal fig then looked for the new receptive fig for laying eggs. In dioecious figs, she is able to succeed in laying eggs only in male fig and produce seed in female figs. While she can success in both laying eggs and pollination in the same syconium of monoecious figs (Kjellberg *et al.*, 2005). Hence, inside the monoecious fig, the female flowers are shared for seeds and wasps production. The factors affecting the system maintenance of their production were very interesting for investigation (Corlett, *et al*., 1990).

*F. racemosa* is pollinated by specific obligated wasp, *Ceratosolen fusciceps* Mayr (Berg and Wiebes, 1992). Its fig is 3-5 cm in diameter when mature with pear shape. The color of fig is green or purplish green in the early stage then turn to reddish orange when mature.

This fig species has an active pollination mode, which means pollinators need to have specialized structure that called "pollen pocket" to collect pollen. Before leaving their natal fig, female pollinators will pick some pollen with their forelegs and introduce it into pollen pocket. The behavior was the activity of pollination and oviposition which was done at the same time (Kjellberg *et al*., 2001).

At receptive phase, inside monoecious fig flowers were packed in several layers but the entire stigmas placed in the same height (Ganeshaiah and Kathuria, 1999). As well as other monoecious, *F. racemosa* has a large number of female flowers densely packed inside syconium. The stigma arranged in the same layer continuously (Berg and Wiebes, 1992). The female pollinator went into the fig cavity then started pollinating and ovipositing by walking around on the layer of stigma. Afterwards, seeds and wasp larvae develop inside female flowers and the fig cavity was filled with liquid until the maturity. It was normally found in *Sycomorus* and may be the function of larva and pollinative protection (Berg and Corner, 2005).

The periods of flowering and fruiting are also various in each fig species. Wang *et al*. (2005) reported that the period of flowering to fruiting of *F. racemosa* needed no more than 2 months in the rainy season and 2 or 3 months in dry season to complete. It showed that the season and climate are important effects on their growth and development periods. Several monoecious figs were studied and the periods of development were devided to six phases (Table 5.7).

In the wild, *F. racemosa* fruits are food for squirrels, monkey, fruit-eating bats, and birds. Particularly, during fruit ripening, insects are also beneficial to insectivorous birds.

Normally, these trees grow along river blanks and function to be an erosion protective plant. In Chiang Mai, *F. racemosa* is widely distributed everywhere both the wild and urban areas. Hence, a study of *F. racemosa* pollination would not only serve the knowledge of plant and insect co-evolution, but also support the future studies such as the benefit, germination, dispersal of this plant.

### 5.2.3 Materials and methods

**Studied species**

***Ficus racemosa* L.** (Figure 5.9)

**Trees**, 25-30 m tall, monoecious; bark grayish brown, smooth; branchlets, stipules ovate-lanceolate, 1.5-2 cm. **Leaf**: ovate-lanceolate, 5-8 x 10-15 cm, glabrous; base cuneate; margin entire; apex acute; young leaf blades and figs with bent hairs. Petiole 2-6 cm. **Figs**: paired or cluster on leafy branchlets, reddish orange when mature, pear-shaped, 2-5 cm in diam., basally attenuate into a stalk; peduncle ca. 1 cm; involucral bracts triangular-ovate. Male, gall, and female flowers within same fig. **Male flowers**: near apical pore, sessile; calyx lobes 3 or 4, spatulate; stamens 2. **Gall flower**: both short and long pedicel present; calyx lobes broadly with 3- or 4-toothed; style lateral. **Female flowers**: short pedicel; calyx lobes broadly, apex 3- or 4-toothed; style lateral and long; stigma clavate. **Fruit**: achene, subglobse. **Flowering** and **Fruiting**: year-round.

**Table 5.7** Six phases of fig development in monoecious fig species (Galil and Eisikowitch, 1968; Wiebes, 1979; Yang *et al*., 2002).

| **Fig development** | **Characters** |
|---|---|
| Phase A or prefloral | The initiation of fruiting occurrence |
| Phase B or female phase | The female flowers mature several weeks before male flowers. It becomes "receptive" and the figs release some species-specific olfactory compounds which attract the pollinator wasp. The female flowers in the monoecious figs show a variation in style lengths. Wasps must oviposit in the ovaries of flowers having styles shorter than their ovipositor. The flowers with styles longer than the ovipositor are not oviposited into and if pollinated these flowers bear seeds. |
| Phase C | The wasp larvae develop in the flowers where eggs were laid while the rest of pollinated female flowers develop into seeds. The unpollinated flowers remain vacant while some flowers develop into bladders, which are flowers that appear galled, but contain unsuccessful developed larvae. |
| Phase D or male phase | The next generation of wasps emerge. The male wasps come out first and chew to open the ovaries containing female wasps after mating. Male wasps then make the hole through out for female leaving and they usually die at the natal fig. At this time, the male flowers in the fig dehisce and release pollen. The female wasps emerge and often shovel pollen from the anthers into their meso-thoraic pollen pockets. These female wasps then leave the natal fig through the tunnel dug by the male and fly out in search of other receptive figs. |
| Phase E or post floral phase | The wasps leave the fig. It swells and ripens and is dispersed by animals such as fruit eating mammals and birds. |
| The emergent wasp phase | The newly emerged adult female wasps look for the receptive fig in the other plants to lay her eggs and also pollinate. The lifespan of them depend on species. Normally wasps cannot survive more than two or three days. |

a. b.

**Figure 5.9** Studied species; *Ficus racemosa* L., *a.* tree, *b.* figs on the branches

**Field observation**

Phenological censuses of *F. racemosa* were made in urban areas of Chiang Mai at elevation from 313-372 m above sea level (Table 5.8). Five fig trees were monitored during September 2006-September 2007. The census was made each crop of all the sampling tree as follows;

1. Fig bearing; count the number of figs in the early D-phase in each crop
2. Leaf duration; check the periods of bearing and falling leaves.
3. Style length measuring in the B-C phase; collect 10 figs from 5 trees in the rainy and the dry season to study in the laboratory.
4. Observe behavior of pollinators during fig development and record; the number of foundress, the behavior of male and female pollinators, the sequence of wasps emerging, etc.

**Table 5.8** The locations of the studied fig *F. racemosa* in urban areas of Chiang Mai.

| No. | Sampling tree | Altitude (m asl.) | Latitude | Longitude |
|---|---|---|---|---|
| 1 | T1 | 329.4 | 18º 47´ 97.3" N | 98º 57´ 16.3" E |
| 2 | T2 | 368 | 17º 48´ 13.1" N | 98º 57´ 04.7" E |
| 3 | T3 | 335.5 | 17º 48´ 26.6" N | 98º 57´ 23.7" E |
| 4 | T4 | 371.3 | 17º 47´ 68.7" N | 98º 56´ 79.7" E |
| 5 | T5 | 313.8 | 17º 48´ 64.9" N | 98º 58´ 74.0" E |

**Laboratory study**

1. Plant specimens, such as leaves, shoots, and syconia were taken to the laboratory for morphological fig wasps studying.
2. Style measuring; figs in the B-C phase were collected to dissect longitudinally from ostiole to pedicel. Fifty sampling female flowers in the each fig of 5 fig trees were measured under a stereomicroscope.
3. Twenty figs in early D phase were collected and kept in small containers that covered by fine cloth. All of wasps emerged were then dried for counting. The figs were taken to dissect in search of some remaining wasps and to sample their seed production.
4. Wasps were identified inside the syconia and the number of male, female pollinators, non pollinators and seeds were counted.
5. Both plant and insect specimens were preserved in 70 % alcohol solution for future study.
6. Morphological characters of the figs and their pollinators were described by description and they were photographed.



**Data analysis**

T-test was used to analyze quantitative measurements such as number of figs/tree, seeds/fig, male, female and total wasps/figs, etc.

The hypothesis was “Is it different in the number of seed and wasp production between dry and rainy seasons?”

**Climate data (2006-2007)**

The dry season in Chiang Mai is composed of dry and cold (winter) and dry and hot (summer) periods. The average temperature was about 21-22 º C during November and January. The relative humidity was between 70-77 % RH (Figures 5.10 and 5.11). In summer, during March and mid of May, the average temperature was 26-29 º C and the relative humidity was between 51-67 % RH (Figures 5.10 and 5.11). The rainfall was highest in May at 400 mm and the annual rainfall was 1,251.9 mm (Figure 5.12).

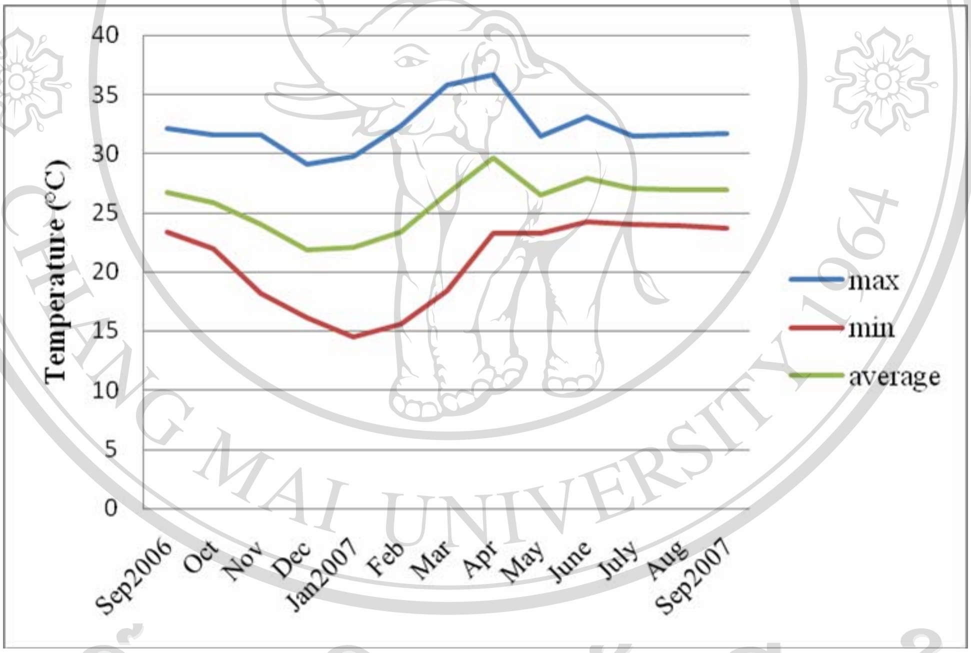



**Figure 5.10** Temperature (C°) of Chiang Mai city from September 2006-September 2007 (Department of Meteorology, Chiang Mai, 2007).

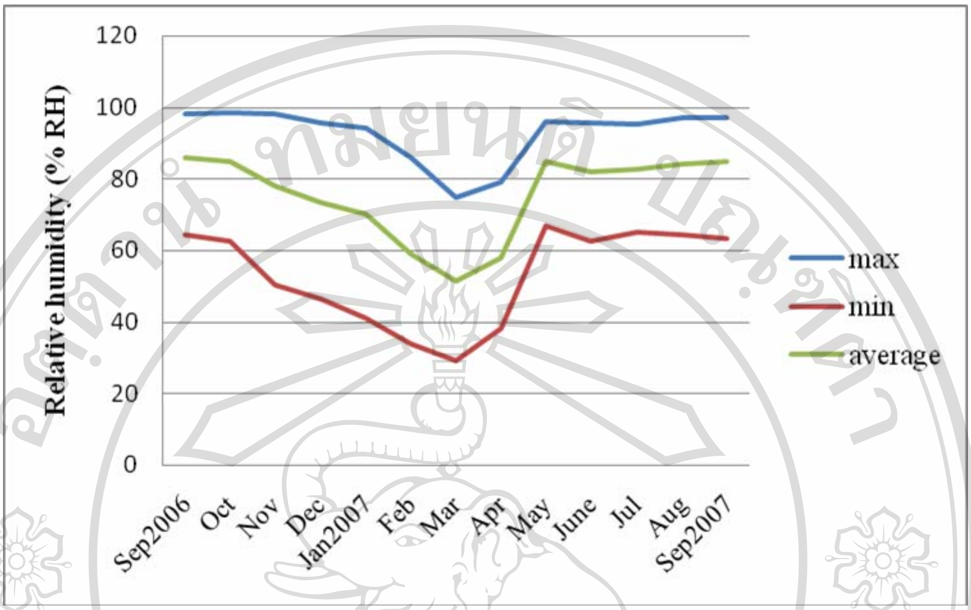



**Figure 5.11** Relative humidity (% RH) of Chiang Mai city from September 2006- September 2007 (Department of Meteorology Chiang Mai, 2007).

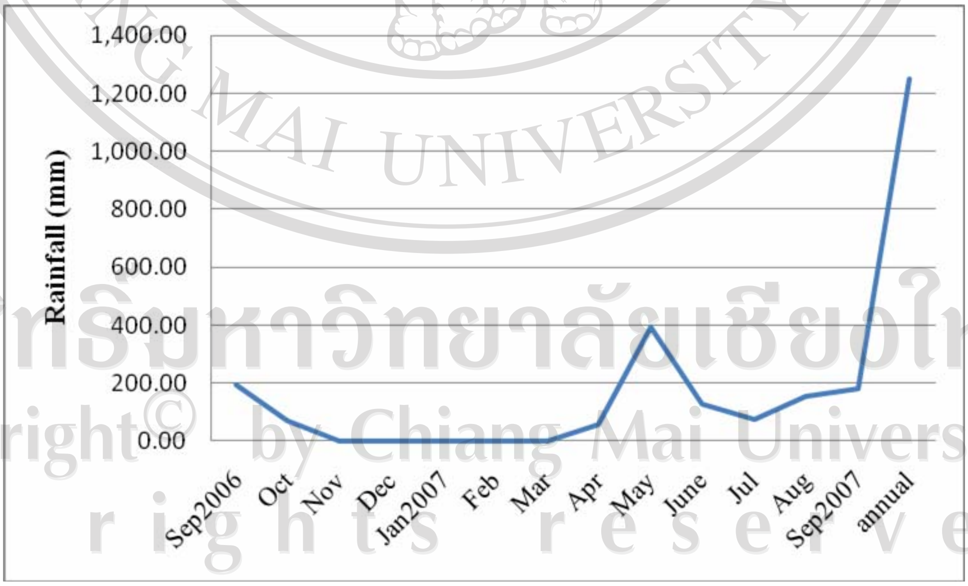



**Figure 5.12** Rainfall (mm) of Chiang Mai city from September 2006-September 2007 (Department of Meteorology, Chiang Mai, 2007).

**5.2.4 Results**

There were 8 crops of *F. racemosa* in 13 months from September 2006-September 2007, the crops were divided to 4 crops in the dry season (November, January, February and April) and 4 crops in the rainy season (May, July, August and September). The number of figs (Means±SD) per tree was 3,166.8±855.7 in the dry season and 3,544.7±1029.9 in the rainy season, which was not significantly different ($\rho > 0.05$) (Table 5.9). The average number of seeds was significantly different ($\rho < 0.05$) between the dry and the rainy seasons; in the dry season was 1,528.3±171.6 seeds and in the rainy season was 2,130.2 ± 230.3 seeds per fig (Table 5.9).

The average of male offspring production in the dry season was 319.5±100.1 and in the rainy season was 192.1±62.2 (not significant, $\rho > 0.05$), whereas female pollinators the average number was differ significantly ($\rho < 0.05$); 597.1±90.3 in the dry season and 398.3±78.2 in the rainy season. However, the number of total pollinators was not significant ($\rho > 0.05$); 835.5±297 in dry season and 671.5±110.9 in rainy season (Table 5.9).

The number in female progeny increased in the dry season, which was about twice as much as in the rainy season. This may be the result of high wasp progeny production in the same period. Male and female progeny ratio was about 1:2 in both seasons. Therefore, an ability of laying eggs in the female wasps was not different, even the number of foundress in the dry season was higher than in the rainy season (Table 5.9). Hence, their eggs were laid in the same quantity but the development of larva seemed different in each season.

The number of flowering and fruiting periods varied in each fig tree, it could be 7-8 times annually. In the present study, *F. racemosa* needed 5-6 weeks in the rainy season and 5-8 weeks in the dry season to complete their maturity. Therefore in each tree, there were about 6-7 crops in a year (Figure 5.13).

*F. racemosa* mostly has 2 times of deciduousness in a year during dry and cold season (January-February) and rainy season (August-September).

**Table 5.9** The number of figs, seeds and pollinator wasps in the dry and rainy seasons between September 2006 and September 2007 in Chiang Mai city.

| Number | Season | Number of Trees | Cropping time | Mean | SD | ρ-value | Significance |
|---|---|---|---|---|---|---|---|
| Figs | Dry | 5 | 4 | 3,166.8 | 855.7 | 0.546 | NS |
| | Rain | 5 | 4 | 3,544.7 | 1029.9 | | |
| Seeds | Dry | 5 | 4 | 1,528.3 | 171.6 | 0.002 | S |
| | Rain | 5 | 4 | 2,103.2 | 230.3 | | |
| Male wasps | Dry | 5 | 4 | 319.5 | 100.1 | 0.074 | NS |
| | Rain | 5 | 4 | 192.1 | 62.2 | | |
| Female wasps | Dry | 5 | 4 | 597.1 | 90.3 | 0.016 | S |
| | Rain | 5 | 4 | 398.3 | 78.2 | | |
| Total wasps | Dry | 5 | 4 | 835.5 | 297 | 0.341 | NS |
| | Rain | 5 | 4 | 671.5 | 110.9 | | |
| Foundress | Dry | 5 | 4 | 11 | 7.1 | 0.015 | S |
| | Rain | 5 | 4 | 6.2 | 4.3 | | |

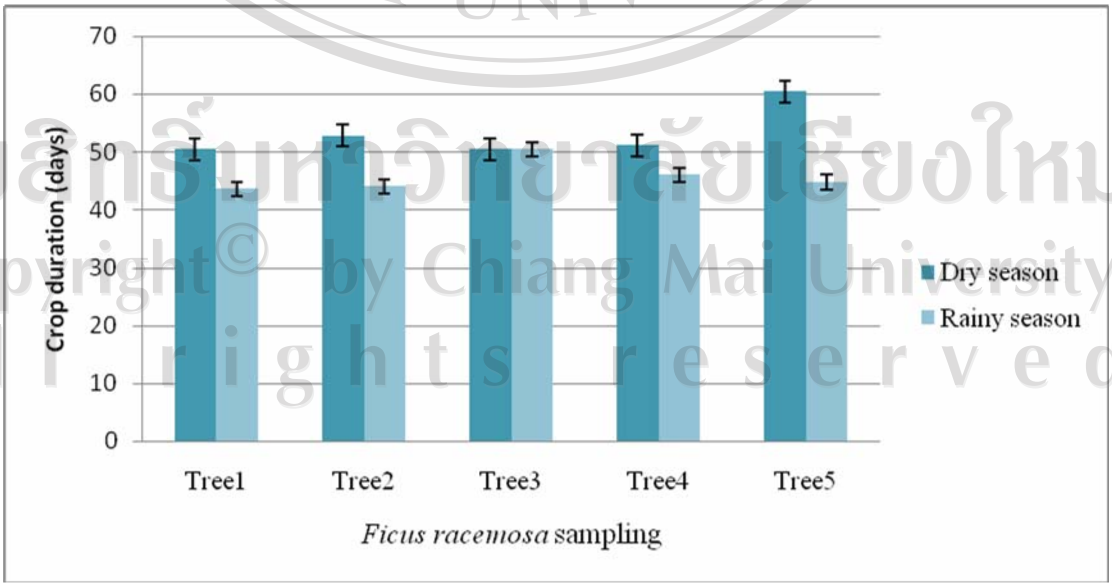


**Figure 5.13** Crop duration of 5 fig trees, *F. racemosa* between the dry and the rainy season.

One of the factors that believed to be an important reason for different ratio of seeds and progeny production was "style length". In *F. racemosa*, there are various style lengths in female flowers. Female flowers in monoecious figs can produce both seeds and wasp offspring. Hence, the style length in different crops between the dry season, and the rainy season were observed and measured (Figures 5.14 and 5.15).

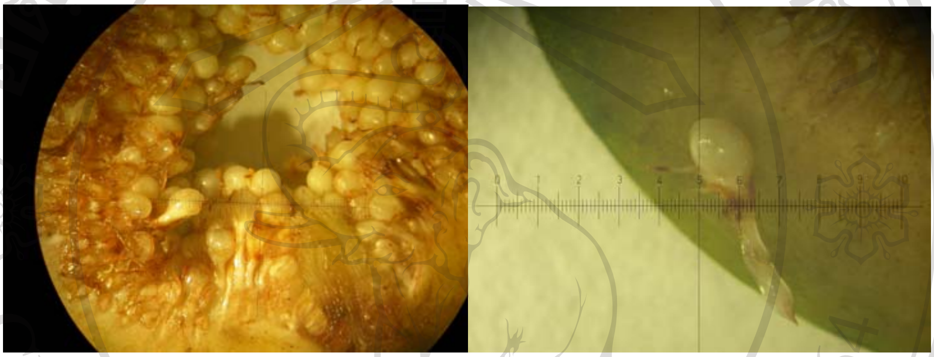


**Figure 5.14** Style lengths measuring of a female flower fig

In the rainy season, the average lengths of style (50 style sampling/fig) in most of the studied figs was between 1.73 and 2.16 mm, whereas in the dry season it was between 1.70 and 1.95 (Figure 5.15). The means of style length in different seasons revealed the opposite way as the number of seeds and female wasp production.

The lengths of pollinator ovipositor were between 1.8 and 2.0 mm and not different in the dry and the rainy seasons. Therefore, in the rainy season most style lengths were longer than female ovipositors (Figure 5.15). The results were similar to seed production, which was high in the same period (Figure 5.16).

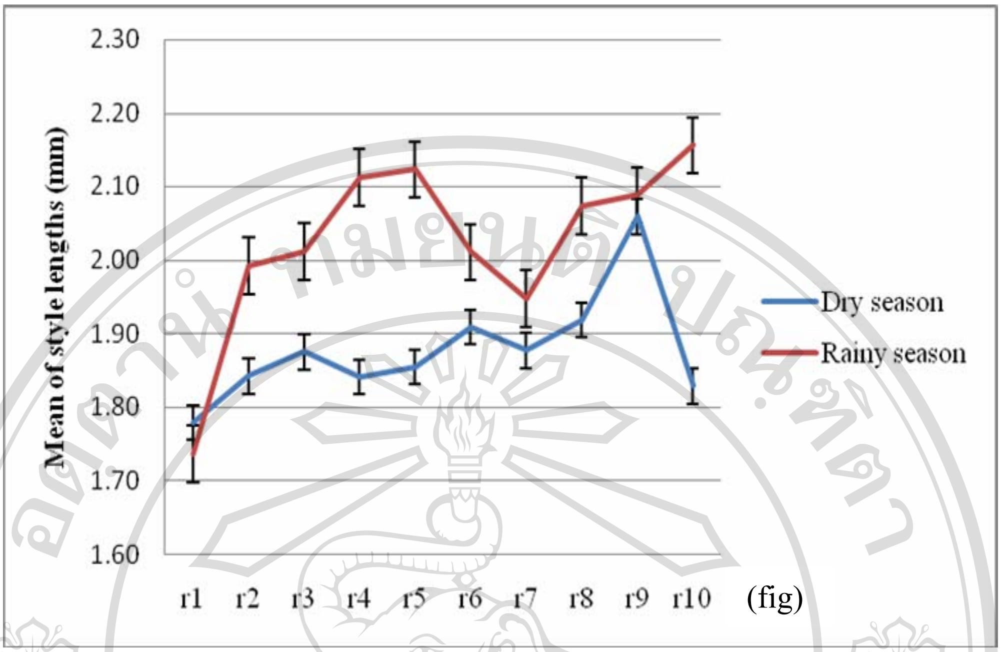



**Figure 5.15** Mean of style length (mm) in the dry and the rainy seasons, r1-r10 were the average of style lengths sampling in each fig.

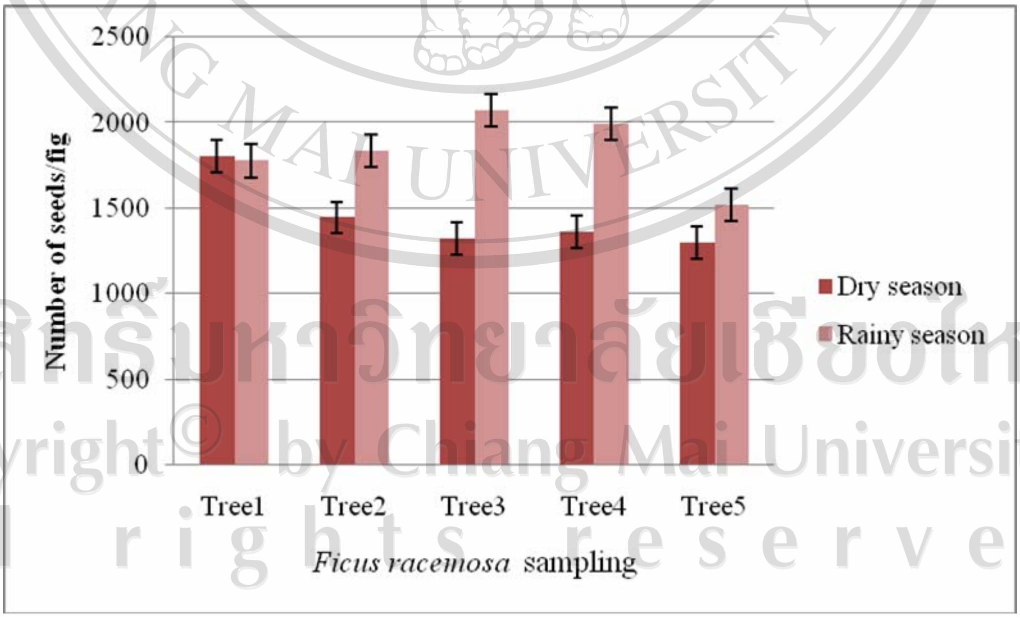



**Figure 5.16** The number of seeds fig in each sample tree, *F. racemosa*

**Fig and wasp interaction**

The syconium developed from bud to receptive stage when it was 2.4-3.2 cm in diameter. Before the receptive phase, both male and female flowers were very tiny in size and ostiolar bracts filled most of the space of fig cavity. About two weeks later, the period of receptive fig, it was the period that fig ready to accept pollinators. At this stage, the ostiolar bracts turned looser and the tiny hole wider. The pollinators immerge with various numbers, about 1-30 per fig. The female pollinator that carried pollen went into the syconium was called foundress. The average number of foundress was 11±7.1 in the dry season and 6.2 ±4.3 in the rainy season, which was significantly different ($\rho < 0.5$) (Table 5.9).

The sequences of emerging in *F. racemosa* syconium was started by male pollinators making a small hole for emerging, then female pollinators and female non pollinators both come out almost at the same time (Figure 5.17). Early period of emerging, most male pollinators first appeared at the tiny hole of receptacle. Some figs, two or three holes were made, and the prior emerged group was also male pollinator wasps.

**Figure 5.17** Sequences of emerging in the fig of *F. racemosa*

Not only female pollinators used the benefit of fig trees, but also female non pollinators. They could lay their eggs from the outside of the syconium (Figure 5.18), sometimes made galls and sometimes function as parasitoid.

The dominant character of female non pollinator was the long ovipositor that essential for eggs laying. The number of non pollinators was also tendency to the production of pollinators.

Five species of non pollinator wasps were found which consisted of two species of *Apocrypta* and three species of *Platyneura (Apocryptophagus).* Number of non pollinator production was also high in dry season and the dominant species were *P. testacea* and *P. mayri* (Figure 5.19). The highest number of *P. testacea* per fig was 34.6 in November (Figure 5.19). The male of non pollinators emerged together with male pollinators inside fig, but their size was very small and difficult to classify.

**Figure 5.18** Oviposition of *Apocrypta* sp. from out side the fig, and gall making by the female non pollinators.

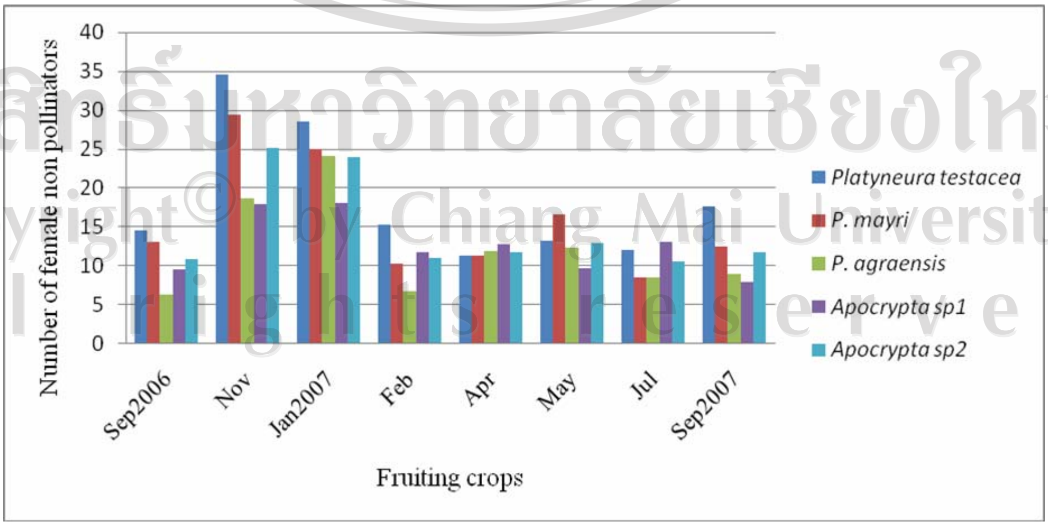


**Figure 5.19** Number of female non-pollinator offspring in each crop from September 2006-September 2007.

The active period of female pollinator wasps was about 2-3 hours after emerging when they were left in the small container at room temperature. Next two or three hours, they could survive, but had less activity. On the other hand, non pollinators could leave longer, it took more than 24 hours at the same condition.

### 5.2.5 Discussion

The periods of fig maturity were lesser than the same species in Southern China which it took more than 2 months (8 weeks) in the rainy season and 2 or 3 months (8 or 12 weeks) in the dry season (Wang *et al*., 2005). And also the number of seeds and offspring were lesser than the previous study in China.

Its non pollinators are three species of *Apocryptophagus* (Sycophaginae) and two species of *Apocrypta* (Sycoryctinae). The former genus is a competitor with pollinators, while the latter is a parasite of other wasps. (Kerdelhue *et al*., 2000 and Weiblen, 2002). The community of non pollinators has more or less impact on fig and pollinator interaction, however the information of non pollinators fig wasp is poorly known (Kjellberg *et al*., 2005).

Wang *et al*. (2005) compared the proportion of seeds and gall flowers in each fruit of *F. racemosa* between the rainy (May to October) and the dry season (November to following May) in south of China, it was showed that gall production in the dry season (January and November) was higher than rainy season, whereas seeds production was rather stable. This study showed the results in the same pattern but in Chiang Mai, the crops of *F. racemosa* were higher in number, seed production rather high in the rainy season, whereas the number of offspring was lesser than in south of China. In order to predict the population and distribution of *F. racemosa*, the factors such as non pollinator wasps and individual variation of fig tree that thrive in different micro climate should be considered in the future study.