Ficus deltoidea and its associated fig wasps in Peninsular Malaysia

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ABSTRACT

The interaction of fig trees (*Ficus*) and their fig wasp pollinators (Agaonidae) is an obligate mutualism where the fig trees need the pollinators in order to reproduce while the pollinators require the figs as their brood sites. *Ficus deltoidea* is a common dioecious fig tree in Malaysian oil palm plantations and may contribute figs and other resources for birds and other animals that would otherwise not be able to survive in the plantations. *F. deltoidea* is a true epiphyte – a growth form that is very rare in *Ficus*. It has numerous named varieties and the taxonomic and biological status of these varieties is unclear, as is the extent to which different varieties support different *Blastophaga* pollinators. A period of 18 months practical study was carried out in Malaysian oil palm plantations and the *F. deltoidea* germplasm collection in Universiti Sultan Zainal Abidin. *F. deltoidea* was found to be one of ten fig tree species in oil palm plantations in Peninsular Malaysia, but the only true epiphyte. The figs of different varieties of *F. deltoidea* showed a high degree of variation in terms of flower numbers, tepal numbers and size of the figs. Male and female *F. deltoidea* var. *angustifolia* produced new leaves and figs all year around. There was no existence of non-pollinating fig wasps in this species. A *Blastophaga* sp. foundress can enter several figs to lay their eggs and pollinate, but many figs on female plants were not entered and many figs aborted. The pollinators liked to enter figs on their natal male plants if the figs were available. Experiments found pollinators preferred to enter figs of their own variety. The high specificity of the pollinators suggests that many varieties might be distinct species. The limited dispersal of *Blastophaga* sp. helps them to reproduce but results in the pollinator shortage that is prominent in the female figs. The pollinator can be said to be ahead of the trees. Greater understanding of how they interact may help explain how the mutualism can persist. This is important because the mutualism between *F. deltoidea* and its pollinators can increase biodiversity in oil palm plantations. This study can also increase our understanding of coevolution between plants and insects.

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cm	Centimetre
com.	Communication
Fd (a)	*Ficus deltoidea* var. *angustifolia*
Fd (d)	*Ficus deltoidea* var. *deltoidea*
Fd (t)	*Ficus deltoidea* var. *trengganuensis*
GLM	Generalized Linear Model
KESEDAR	Lembaga Kemajuan Kelantan Selatan
KLIA	Kuala Lumpur International Airport
km	Kilometre
Lab.	Laboratory
mm	Millimetre
NPK	Nitrogen, phosphorus, kalium
Pers.	Personal
SD	Standard deviation
SE	Standard error
sqrt	Square root
TGC	Terengganu Germplasm Collection
UKM	Universiti Kebangsaan Malaysia
UniSZA	Universiti Sultan Zainal Abidin
var.	Varieties

Chapter 1

General Introduction

1.1 Introduction

1.1.1 Plant and insect interactions and pollination in general

Plants routinely interact with insects. These interactions be either beneficial or detrimental to one or both parties. Interactions between plants and the herbivores that eat them are often highly species-specific. Many plant- feeding insects are also specific in the parts and developmental stages of the plants that they feed on (Bernays and Chapman, 1994). Plant-feeding insects can be divided into three categories, those which are monophagous (feeds on a single host plant), oligophagous (feeds on a number of related plants in the same family) or polyphagous (feeds on a wide range of host plants from different families) (Bernays and Chapman, 1994). The specificity of this relationship varies between feeding guilds – groups of insects that feed on the same parts of a plant in similar ways (Novotny et al., 2010). Leaf miners are often more specific than leaf chewing species while seed feeders and gall-formers are typically highly specific. Insect herbivores can feed by chewing, sap sucking, epidermis scraping, flower feeding, root or shoot mining, boring, gall induction and seed predation (Gossner et al., 2014). The interactions involving plant-feeders are usually antagonistic and harmful to the plants. However, in some cases the plants benefit from being eaten. An example of this is pollination. Insects are the animal group most widely utilised by plants for pollination.

Pollination is an unusual type of herbivory where the plants provide rewards for pollinators and the pollinator behaviour and structures evolve to help collect the rewards. As such, it is a mutualistic interaction where both participants can benefit. The rewards provided to pollinators by plants include pollen, nectar, oils, wax, resin and edible trichomes (Pansarin and Maciel, 2017). Pollinators, like other herbivores, can be either generalist or specialist. Generalists take rewards from many plants while specialists get their rewards for pollination services from one or a few species of plants.

Generalists are often more abundant than specialists because the pollinators can switch to other hosts if one host is absent (Bosch et al., 2009). However, pollination by generalists has some disadvantages for the plants because it can lead to interspecific pollen transfer and pollen deposition (Morales and Traveset, 2008). Heterospecific pollen deposition causes low fertilization and seed production. On the other hand, specialists visit a limited range of flowers and the risk of deposition of heterospecific pollen and loss of pollen through transfer to other plant species is low (Maldonado et al., 2013).

The ability to remove and deposit pollen on the stigmas of conspecifics varies between pollinators and between flowers. Host plant specialists tend to be more effective at removing the pollen from host anthers and depositing them onto host stigmas (Parker et al., 2016). For example, pollinator effectiveness in removing pollen from the herb *Knautia arvensis* (Dipsacaceae) that is visited by both generalist and specialist pollinators was examined by (Larsson, 2005). He showed that a more specialist pollinator, a solitary bee *Andrena hattorfiana* (Andrenidae) moved more pollen in a single visit compared with generalist visitors (bumblebees and flies).

Pollinators provide a service to plants in order to get a reward and the plants need to make sure that when visitors collect their rewards that pollination is taking place. In order to achieve this, plants have evolved morphological specializations to attract their most effective pollinators (Sakai, 2002). Similarly, the morphological adaptations of specialist pollinators mean they are well adapted to exploit efficiently the flowers they visit (Kahnt et al., 2017). Two fly families in Africa, Nemestrinidae and Tabanidae have a proboscis up to three time longer than their body length in order to gain the rewards provided by their long tubed host flowers (Goldblatt and Manning, 2000). The plants develop long tubes to prevent generalist pollinators getting access to the rewards and thereby enhance the chance of successful pollination when visited by the flies adapted to pollinate them.

Another example where specialist pollinators have evolved their morphology in order to maximize getting the rewards from their host plants can be seen in South African *Rediviva* bees (Melittidae) where females have evolved extremely elongated forelegs in order to get to the oil rewards from the long paired floral spurs of *Diascia* species (Scrophulariaceae). They also have highly modified structures to damage oil-producing cells and to collect the oil. Phylogenetic studies have shown that their ancestors had short legs and *Diascia* was their host plant (Kahnt et al., 2017).

In order to achieve pollination, plants need to attract the pollinators by giving appropriate cues to help them be recognised and located. Host-plant selection by pollinators is usually linked with floral traits like colour, size, volatiles (Burger et al., 2010), flower surface texture, symmetry and shape (Glover, 2011). Floral traits can lead to specific interactions between a plant and pollinator and this can cause reproductive isolation and facilitate speciation (Klahre et al., 2011). A study by (Bradshaw and Schemske, 2003) discovered that a mutation in a single gene can have a major influence on pollinator preferences between closely-related species that leads to host shifts. Klahre et al. (2011) compared the significance of two floral traits (colour and scent) in attracting a pollinator hawkmoth (Sphingidae) and showed they were equally weighted. On the other hand, a study by (Hirota et al., 2012) suggested that colour traits had a more significant role than scent in shifting pollinators from visiting daylily *Hemerocallis fulva* L. var. *aurantiaca* (Baker) M. Hotta (Asphodelaceae) to nightlily *Hemerocallis citrina* var. *vespertina* (H. Hara) M. Hotta (Asphodelaceae).

To find flowers, floral colour (visual cues) together with olfactory cues (floral scents) appear to be the most critical for many pollinators (Burger et al., 2010). As with most other insects associated with plants, pollinators must be able to recognize suitable hosts from a distance away and upon arriving on a potential host they must make sure that they are choosing one with the correct good quality rewards (Bernays and Chapman, 1994). Angiosperms associated with specialized pollinators usually emit highly specific volatiles to allow their pollinators to discriminate and recognize them (Sakai, 2002; Milet-Pinheiro et al., 2013).

Of all the cues, floral volatiles emitted from receptive flowers (those at the developmental stage when their pollen is mature and ready to be collected and their stigmas can utilise pollen) are a key factor in pollination mutualisms (Hossaert-McKey et al., 2016). Volatiles can also allow the pollinators to distinguish between rewarding and non-rewarding plants of the same species via the nectar’s odours (Howell and Alarcon, 2007). Some pollinators depend on the wind to bring floral scents to them and the insects then perform positive anemotaxis (orientate up-wind) to locate the host, and zigzagging flight behaviour helps sustain contact with the scent (Borges, 2016). Some insects can orient into the wind from the ground. When they perceive the odour, they will fly for a short distance and land on the ground again. They will take off again when they continue to respond to the odours until they reached the target (Bernays and Chapman, 1994). Some small insects depend on the wind and this gives them the opportunity to fly for greater distances, but at the cost of not being able to control their direction of flight (Compton, 2002). Once landed on a suitable host, pollinators depend on short-range cues to decide whether the flower will provide the rewards they want or not. Short range cues include morphological features and the surface texture of the flowers (van Noort, 2003).

1.1.2 Nursery pollination

The rewards that are most usually associated with pollination are nectar and pollen. These rewards are provided to insects such as most bees, moths and flies and are often associated with quite generalist plant-pollinator interactions. A less common, and often highly specific pollinator reward is involved with nursery pollination where the reward for pollination services is mating sites and sites where insects offspring can develop (Waser and Ollerton, 2006). This interaction, is also known as brood-place pollination (Hossaert-McKey et al., 2016). In this type of pollination, the insects need to pollinate the flowers to secure a place for their offspring to live and feed, and it is the resources provided to their offspring that represents the reward (Duthie and Nason, 2016). The larvae of the pollinators typically feed on the seeds, ovules or other female tissues within the flowers of their host plants (Bronstein, 2001; Hembry and Althoff, 2016). This kind of interaction is usually obligate and there is a high degree of specificity between each host plant and its pollinators (Borges, 2016).

Pollination in a nursery pollination is started when (1) the plants release a specific volatile compound when they are receptive and need to attract pollinators (Hossaert-McKey et al., 2016) (2) the volatile diffuse and are transported mostly by the wind to where pollinators occur (3) the pollinators detect the chemical cues with their antennae (van Noort, 2003) (4) the sensory systems of the pollinators interpret the cue (Kjellberg et al., 2005b) and (5) lastly, the pollinators move to locate their reward's location (Borges, 2016). Most insects associated with nursery pollination are short-lived as adults and thus a learning process is not important in locating their hosts (Borges, 2016). The scent emitted by the host must be very effective and specific to ensure the success of finding the host by their obligate partner (Hossaert-McKey et al., 2010). The high specificity in this form of mutualism requires synchronization between the development times of the fruit and the development of their pollinators (Sakai, 2002).

The availability of receptive flowers at the same time as the release of pollinators helps maintain the populations of the pollinators (Peng et al., 2010).

Sakai (2002), divided nursery pollination into three major groups based on how the pollinators breed in the flowers they pollinate; (1) the brood are ovule/seed parasites (2) the brood are pollen parasites and (3) the brood feed on the decomposing flowers after pollination has taken place. The receptive flowers may also act as the sites for mating for the pollinators as well as the brood sites for the pollinator's progeny (Borges, 2016). Nursery pollinators mostly come from the insect Orders Hymenoptera, Lepidoptera, Coleoptera, Diptera and Thysanoptera (Sakai, 2002).

Nursery pollination provides a model for studying the evolution of cooperation and diversification among populations and species (Hembry and Althoff, 2016). The speciation of one party caused another party to diversify. Examples of nursery pollination are provided by yuccas (*Yucca*) (Asparagaceae) and yucca moths (*Tegeticula*) (Prodoxidae), leafflowers (*Glochidion*) (Phyllanthaceae) and leafflower moths (*Epicephala*) (Gracillariidae) (Borges, 2016), saxifrages (Saxifragaceae) and *Greya* moth (Prodoxidae), *Silene* (Caryophyllaceae) species and *Hadena* moths (Noctuidae), senita cacti (Cactaceae) and senita moths (Crambidae) and fig trees *Ficus* (Moraceae) and fig-wasps (Agaonidae) (Hembry and Althoff, 2016). Of all the organisms involved in nursery pollination mutualisms, the fig tree-fig wasp mutualism is the most widely studied and involves the largest number of plant and insect species.

1.1.3 *Ficus* trees

Fig trees belong to the genus *Ficus* and family Moraceae. Their distribution is worldwide in warmer areas, with almost 900 described species making them one of the largest genera of land plants (Weiblen, 2002) and the only woody genus found in almost all lowland tropical forests, though often at low densities (Ghazoul and Sheil, 2010). Fig trees occupy a very wide-ranging spectrum of habitats, from wet lands to deserts and have coevolved with their highly specific fig wasp pollen carriers (Kjellberg et al., 2001). *Ficus* is likely to have originated from western Asia Minor but can now be found globally in tropical, sub -tropical and some warm temperate areas (Mat et al., 2012). The genus is possibly the most important source of food to frugivores in lowland tropical rainforests (Harrison, 2005) and more generally (Shanahan et al., 2001).

Trees of the genus *Ficus* produce large quantities of white latex in their inflorescences, branches and leaves, have stipules covering the new buds at the twig tips, ring scars on their twigs left by the stipules that have fallen off, and urn-shaped inflorescences with numerous florets (Burrows and Burrows, 2003). Fig trees exhibit a wide range of growth forms from epiphytes, hemiepiphytes, climbers, shrubs and as trees (Herre et al., 2008). They also can be bushy with leaves organised in spiral and ascending twigs (Mat et al., 2012). All fig trees bear an enclosed urn-shaped inflorescence that is called a fig that is lined with many tiny florets on its inner surface (Cook and Rasplus, 2003). This inflorescence is closed by apical bracts around an ostiole (Kjellberg et al., 2005b). The ostiole is the path that allows the entry of the pollinators. Ecologically, a fig is a compound fruit but as it is both the site of pollination and pollen production and the unit of dispersal, it acts as an inflorescence and a fruit (Gonzaga, 2012). The sizes of figs are different depending on the species. They can be from pea to peach sized or larger and the colour of ripe figs also differs from red, yellow, and green or purple (Borges et al., 2008). Different fig trees appear to produce different volatile compounds that their associated fig wasps recognize (Herre et al., 2008). Later, the figs ripen to form ‘pseudo-fruits’ containing the seeds, each of which is technically a fruit (Ghazoul and Sheil, 2010).

Fig development can be divided into five phases (1) pre-female flowers – young figs and before the ostiole is open, (2) female flowers – receptive for pollination and female wasp start entering their ostioles, (3) interfloral – right after the ostiole is closed the ovaries occupied by wasps larvae will develop into galls, (4) male flower - the next generation of wasps are released from the galled ovules where they developed and (5) a postfloral phase – after the wasps have emerged and the figs ripen (Galil and Eisikowitch, 1968). These developmental phases are widely used in studies of fig development, though not all the stages are necessarily present in all the figs of each species.

Figs also support a diverse community of non-pollinating fig wasps, either parasitoids, or ovule gallers. Similar to any mutual relationship, the presence of a third party always creates constraints and weakens the strength of rewards in the mutualism. Seed gallers will compete with the figs’ pollinators for food resources, and parasitoids attack pollinator larvae in figs (Weiblen and Bush, 2002) and compete with the pollinating wasps for oviposition sites (Kerdelhue et al., 2000). Typical seed-eating fig wasps are rare (Wang et al., 2014). The development of non-pollinating fig wasps does not benefit the plant and they have been referred to as ‘parasites’ of the mutualism

(Compton and van Noort, 1992). The specificity of the non-pollinating fig wasp was less specific (van noort, 2003).

Fig trees are believed to act as keystone species in tropical forests (Yang et al., 2002) and specially in Malaysia (Lambert, 1991), India (Kannan and James, 1999), South Africa (Bleher et al., 2003), Panama (Korine et al., 2000). However, due to relatively low densities of figs in the Afrotopical region (continental Africa and the Malagasy region), Gabon (Gautier-Hion and Michaloud, 1989) and Uganda (Chapman et al., 2005), some authors did not consider them as keystone species there (Goodman and Ganzhorn, 1997). Their keystone status elsewhere is because they sustain populations of vertebrate frugivores, which often comprise a high proportion of the total vertebrate biomass in tropical rainforests (Thornton et al., 1996).

The year-round production of figs and their abundance attracts large numbers of frugivores like hornbills, bulbuls, monkeys, squirrels, fruit pigeons, bats and non-volant mammals to consume them (Shanahan et al., 2001). The number of frugivores locally usually peaks at the time the figs ripen (Mackay et al., 2018). A study done by (Lambert, 1991) revealed that at least 60 species of bird and 17 species of mammals consumed figs in Peninsular Malaysia. Fig trees also support numerous other species that live inside the figs, including microorganisms, mites (Compton et al., 1994), nematodes (Jauharlina et al., 2012) and flies (Lanchaise et al., 1988; Macgowan and Compton, 2018). In addition to animals, fig trees provide habitats that can host saprophytes, epiphytes, parasitic plants and also shade-enduring flowering plants (Yang et al., 2002).

1.1.4 *Ficus* pollination

The pollination between fig trees and fig wasps is one of the classic examples of obligate mutualism (Deng et al., 2016). For obligate mutualists, the loss of one species will have critical effects on the other species, possibly leading to cascading effects across trophic levels because of the high degree of dependency and coevolution between the species (Cook and Rasplus, 2003). The mutualism between fig trees and fig wasps involves the pollination by the fig wasps and as a reward, the fig trees provide sites for mating, development and reproduction sites of the progeny together with the nutrition for the brood to complete their life cycle (Wachi et al., 2016). Fig trees depend on the fig wasps for transmission of their pollen and therefore for viable seed production (Herre et al., 2008). With a few rare exceptions, the fig wasps only reproduce within the figs of one fig tree species and in return, the fig trees are often pollinated exclusively by one species of wasp (Yang et al., 2002; van Noort, 2003). Examples where a fig tree is pollinated by more than one species of fig wasp are nonetheless increasing (Yang et al., 2015).

The pollination in *Ficus* starts when specific volatile compounds are released from receptive figs to attract the specific fig wasps associated with each species of fig tree (Hossaert-McKey et al., 2016). The female fig wasp (known as a foundress when she enters a receptive fig), detects the volatiles and flies towards the receptive figs (Molbo et al., 2003). The foundresses can be seen hovering around receptive figs (Ware and Compton, 1992) before penetrating through a narrow (usually) bract-lined tunnel ostiole (Liu et al., 2013), which closes soon afterwards, sealing the foundress wasps in a ‘tomb blossom’ (Weiblen, 2002). This ostiole become loose during the receptivity period (Yang et al., 2002; Liu et al., 2013). On entry into a receptive fig, they lose their wings and parts of their antennae, so they cannot fly away to subsequent figs (Suleman et al., 2013b).

Once inside they pollinate the flowers (Nefdt and Compton, 1996) using the pollen from their natal fig (Herre et al., 2008) and at the same time lay eggs in some of the flowers (Kjellberg et al., 2005b). Only one egg is laid per flower (Ghana et al., 2012). Once the flowers have been probed, she and other fig wasp foundresses will avoid ovipositing their eggs in those flowers (Jousselin et al., 2001). A single ovule then provides the site for larval development and turns into a seed-sized gall (Kjellberg et al., 2005b). The larva feed on the endosperm (Deng et al., 2016) that developed from either double fertilisation or parthenogenesis (Borges and Kjellberg, 2014).

The wingless adult male fig wasps hatch first from their galls and search for galls containing females (Yang et al., 2002). The males then bite holes into the female galls and insert their genitalia in order to mate (Kjellberg et al., 2005b). Individual females may mate once or several times. Because sex ratios are skewed towards females, each male can mate with numerous females. Adult female fig wasps then emerge into the fig cavity and at this time the male fig flowers are mature and liberating pollen. The time from oviposition to adult emergence can take as little as one month (Harrison, 2005). The adult fig wasps have a short life span that rarely exceeds 48 hours (Harrison and Yamamura, 2003; Dunn et al., 2008). Within this limited time, they do not feed but need to find a receptive inflorescence of the correct fig tree species

(Harrison and Yamamura, 2003). Ahmed et al. (2009) found that a pollinator of an African fig tree *Ficus sycomorous* L. is able to fly up to 160 kilometres with the assistance of the wind.

Ficus species exhibit two breeding systems namely dioecy and monoecy. Roughly half of all fig species are monoecious, with individual inflorescences providing both female (seed production and dispersal) and male (pollen production and dispersal) reproductive functions (Herre et al., 2008). The remaining *Ficus* species are functionally dioecious. In these species, there are two types of trees; female trees that produce only seed-bearing figs; and male trees with figs that produce only pollen and pollinator wasp progeny to transport the pollen (Patel and Hossaert-McKey, 2000).

In a monoecious fig, both wasp larvae and seeds develop within the same figs. Pollinated ovules that do not receive an egg will develop into a seed (Bain et al., 2014). Monoecious fig trees contain many female flowers compared to male flowers with variable style length and each mature fig on these trees contains a mixture of pollen, wasps and seeds (Galil and Eisikowitch, 1968). Usually, in this type of breeding system, only 40-50% of the flowers will develop into viable seeds that signify a large portion of the fig tree's investment in female function (Kerdelhue and Rasplus, 1996) while the flowers that support the development of the pollen carrier female wasps represent the fig tree's investment in male reproductive function. In monoecious species of *Ficus*, the separation in time between male and female developmental phases (protogyny), both at the level of inflorescences and often the individual tree (because of within-tree fruiting synchrony), are much longer than in many other dichogamous plants (Hossaert-McKey and Bronstein, 2001). In monoecious species, normal pattern is that shorter styled flowers tend to support the development of offspring, longer styled flowers are more likely to be seeds (Nefdt and Compton, 1996). Male eggs are often laid first, and the shortest styled flowers tend to be the ones where eggs are laid first (Li et al., 2016).

In a dioecious fig tree, the female fig wasps enter the female figs and pollinate flowers which produce only seeds (Patel, 1996). The long styles of the flowers in the female figs and the structure of their stigmas prevent the wasps from laying their eggs but the insects continue to pollinate (Berg, 1989). In *Ficus*, the structure of the stigma varies significantly between sexes and taxonomic groups (Berg and Corner, 2005). The foundress can attempt to probe the styles but even if she succeeds to insert her ovipositor the long styles of the flowers usually prevents the tip of the fig wasp ovipositor from reaching the fig ovule (Kjellberg et al., 2005b; Ghana et al., 2017). Entering the female fig has a fatal effect as no offspring are produced and the loss of her wings means that she cannot fly to another tree (Hossaert-McKey et al., 2016).

On male trees, the female flowers are modified for receiving a pollinator egg and only wasp larvae develop there. One to several months after pollination, the male fig wasp adult offspring emerge and mate with the gall-enclosed females (Yang et al., 2002). Due to protogyny, the female flowers become receptive simultaneously, several weeks before the male flowers mature (Patel, 1996). The short styled flowers in male figs are consumed by pollinators larvae (Ghazoul and Sheil, 2010). The persistence of dioecy in many species of figs is enigmatic because it represents an intense conflict between the interest of the pollinating wasps and those of the plant (Weiblen and Bush, 2002).

Most plants are pollinated passively, but the pollination in *Ficus* can be done actively or passively (Ahmed et al., 2009). The frequency of active pollination differs between lineages and consequently between regions (Kjellberg et al., 2001). Actively pollinated fig trees have pollinators with special behaviour and morphological adaptations (Kjellberg et al., 2014). Actively pollinated fig wasps are equipped with special sternal structure called pollen pockets that function in storing the pollen (Kjellberg et al., 2005b). The fig wasps actively collect pollen into their thoracic pollen pockets and later deposit it deliberately onto flower styles (Jousselin et al., 2003). They also engage in complex behaviour before leaving their natal figs, when they will search for the anthers and collect the pollen using their forelegs lined with fined coxal combs to aid the collection (Deng et al., 2016).

In the receptive figs, they remove the pollen from the pockets and transfer them to the stigmas while depositing their eggs (Kjellberg et al., 2014). The presence of these morphological structures and its associated behaviour results in efficient pollen transfer and reduces the amount of pollen needed, so actively-pollinated figs have a reduced number of anthers (Kjellberg et al., 2001). Passively pollinating fig wasps do not have either special morphological structures or complex pollination behaviour. Dehiscent anthers on their natal figs release pollen that becomes trapped on their bodies before they leave their natal figs (Waser and Ollerton, 2006). Subsequently, the pollen is brushed off onto the stigmas in the receptive figs, thus allowing pollination to occur.

1.1.5 Fig wasps

Figs act as brood sites for their pollinators - chalcid wasps from the family Agaonidae. They are the only routine pollinators of *Ficus* species and fig wasps are only able to reproduce within the figs they pollinate (Hossaert-McKey and Bronstein, 2001). The intriguing association between *Ficus* and these agaonid wasps has been known for a long time (Hill, 1967) but many species are still un-described (Quicke, 2012). Even at the start of this century when around 900 species of figs were known, only 350 pollinators had been described (Weiblen, 2002).

The body length of an adult fig wasp ranges from less than one millimetre up to two millimetres (van Noort, 2003). The fig wasp exhibit sexual dimorphism (Weiblen, 2002). Males differ from female fig wasps in that the wings are absent, they have reduced eyes, reduced middle legs and shortened antennae (Kjellberg et al., 2005b) (Figure 1.1). The male fig wasp has well developed mandibles and a telescopic gaster (Yang et al., 2002). Their morphology is well adapted to conditions in the lumen of the figs where they may spend their entire life (Compton and McLaren, 1989).

The female fig wasp has wings and their morphology is adapted to locate figs and to penetrate the ostiole. They bear a flattened head to help them enter the ostiole, mandibular appendages and short and strong spiny legs to push them through the ostiole (Liu et al., 2013). They also bear very sensitive antennae to locate receptive figs by smell, and the organs of smell (sensilla) are often elongate. The antennae break off during their entry into through the fig ostiole (Kjellberg et al., 2005b). Together with the loss of wings this helps the plant because it prevents the pollinators from flying to other trees. It may also make the wasps be less likely to get trapped in the ostiole.

The morphology of female fig wasps, especially their heads are correlated with the morphology of the ostiole length and fig wall thickness, further ensuring that pollination is very species specific (van Noort and Compton, 1996). These morphological traits act as the filter and help maintain the specificity of the relationship between fig wasp and its host species (Souto-Vilaros et al., 2018). The length of the ovipositor is also strongly correlated with the mean style length of the host flowers (van Noort, 2003).

Figure 1.1 Adult female and male fig wasps (*Blastophaga* sp. from *Ficus deltoidea* var. *angustifolia*).

1.1.6 Host specificity in *Ficus*

Among plants in general the specificity of the relationship is maintained before and after pollination. Pre-pollination barriers include the production and detection of cues produced by the host plant (Hossaert-McKey et al., 2010), morphological compatibilities and the behaviour of the pollinators (Sedeek et al., 2014). The post-pollination filters potentially include pollen competition, gametic mismatches, negative fitness and hybrid sterility (Coyne and Orr, 2004). In nursery pollination mutualisms, the specificity between plants and pollinators are often very high, with one plant species regularly pollinated by one or a few insect species (Rodriguez et al., 2017). The obligate mutualism between fig and fig wasps can be used as a model of evolution and speciation (Wei et al., 2014). Entering atypical hosts can lead to hybrids (Ghana et al., 2017). This hybridization may contribute to speciation and the diversification that has occurred in the long history of the association between fig trees and fig wasps (Kusumi et al., 2012).

In the fig and fig wasp mutualism, the most obvious filters that maintain the specificity between the obligate partners are the volatile organic compounds emitted from receptive figs (Proffit et al., 2009). In addition, surface features of the ostiole together with its size and shape (Nefdt and Compton, 1996), the size of the fig wasp (Liu et al., 2013), the compatibility of fig wasp ovipositor and the style length in the female flowers (Ghana et al., 2017) and the ability of the pollinators to induce galls (Ghana et al., 2015a) all contribute to the specificity of their interaction.

It used to be believed that only a single fig wasp species pollinated each fig tree species and vice versa (Deng et al., 2016) and many *Ficus* species still have only one recorded pollinator species (Herre et al., 1996; Machado et al., 2001). However, more sampling and molecular approaches have showed increasing numbers of fig trees with two or more pollinators (Kerdelhue et al., 2000; Machado et al., 2005; Su et al., 2008; Cornille et al., 2012; Rodriguez et al., 2017). In some cases fig wasps may also be shared across closely related fig species (Cook and Segar, 2010) but this seems rare.

A breakdown of specificity in the fig and fig wasp mutualism occurs when a fig wasp responds to the volatile chemicals emitted by the non-usual host receptive figs and successfully gains access in the fig cavity via the ostiole. It may pollinate and may be able to reproduce inside (van Noort, 2003). Generally, fig trees emit volatile compounds that attract only their pollinators but sometimes fig wasps do enter figs produced by species other than its regular host (Cook and Segar, 2010; Souto-Vilaros et al., 2018). There are a few cases where closely related *Ficus* species share the same pollinators. This might occur due to host shifts between sister species (Cook and Segar, 2010). Closely related *Ficus* may produce rather similar volatile compounds when receptive and some fig wasps may not be able to differentiate between the volatiles (Cornille et al., 2012) thus making gene flow between taxa possible. A study by (Wang et al., 2016) found that sharing of pollinators within the same section of *Sycomourus* was five times greater compared to section Hemicardia. A study by (Ware and Compton, 1992) also showed the successful reproduction of pollinators in figs of *Ficus lutea* Vahl. (subgenus *Urostigma*) that normally reproduce in *Ficus thonningii* Blume (also subgenus *Urostigma*) but that pollinators of *Ficus sur* Forssk. (subgenus Sycomourus) that entered the figs did not reproduce. Even so, gene flow between distantly related fig tree species is possible. Distantly related fig species (*Ficus auriculata* Lour. and *Ficus tsiangiI* Merr. ex Corner) in subgenus *Sycidium* occasionally share pollinators (Wang et al., 2016).

Exceptions to the strict species-specific relationship in the fig tree and fig wasp mutualism occurs among fig trees with both breeding systems (Moe , 2011), though there may be with more cases where breakdown occurs in monoecious figs (Cook and Segar, 2010; Moe et al., 2011). Yang et al. (2015) examined the cause of pollinator sharing in monoecious species and concluded that it is due to host shifting and duplication (where the two pollinators of a fig tree are sister species) whereas the presence co-pollinators in dioecious figs was due to only duplication. Yang et al. (2015) also proposed that the higher breakdown of specificity in monoecious figs was due to the cost for entering the wrong host was more serious in dioecious figs. Since the life span of the pollinator fig wasp is really short (Compton, 1993), they have such a short time to live that they cannot afford to be too choosy and rushing to enter the first receptive fig they found (Ashman, 2009). The dispersal ability of the pollinators might also cause the monoecious figs tree to have more breakdowns. A study by Borges (2016) discovered that the pollinators of dioecious fig trees tend to have shorter ranges of dispersal compared to the long range dispersal by pollinators in monoecious fig trees. Being able to fly further may increase the chance of a pollinator being attracted to another host apart from their normal host. All of these reasons make pollinator sharing in dioecious figs less likely (Kobmoo et al., 2010).

It is clear that a single *Ficus* species with more than one pollinator is not very rare. Multiple pollinators usually do not overlap in distribution (are parapatric). Surveys of pollinators therefore need to extend across the distribution of the host trees to establish how many pollinator species are present on that tree. Examples where two or more *Ficus* species routinely share the same pollinator appear to be much rarer, but confirmation of pollinator specificity is difficult because it requires surveys across a range of fig tree species, with each fig tree sampled in multiple locations. Two *Ficus* taxa with two different pollinators throughout their ranges are likely to represent two distinct biological species, because pollinator behaviour determines plant gene flow. Plant species can be subdivided into varieties and sub-varieties on the basis of morphological or other differences (Hahnke et al., 2016). If these varieties consistently have different pollinators, then pollen flow between them will be absent, and they represent distinct biological entities. Conversely, varieties that share the same pollinators can inter-breed, so long as other barriers such as their geographical locations do not prevent this. One *Ficus* species that has a lot of described varieties is *Ficus deltoidea*. Their ecology is also poorly understood, and the specificity or otherwise of the relationship between different varieties and their fig wasp pollinators is unknown.

1.1.7 *Ficus deltoidea*

Ficus deltoidea Jack also known as the mistletoe fig, is one of the more frequently found fig trees in South East Asia. Its natural distribution includes Thailand, Indonesia and Malaysia (Lansky and Paavilainen, 2011). Some varieties are also commonly grown as ornamentals, usually as pot plants. They are locally known as “Mas Cotek” by Malaysians because of having gold (in Malay gold means emas) coloured fine spots on the surface of the leaves (Mat et al., 2012). They also are known as sempit-sempit in Sabah, Sarawak and Kalimantan and tabat barito in Indonesia (Desaku, 2005).

Traditionally, *F. deltoidea* is widely used to cure a variety of diseases such as rheumatism, diabetes, toothache, headache, cold, and to cure sore throats using the powdered roots and leaves (Bunawan et al., 2014). More recently it has been shown that *F. deltoidea* has anti-hyperglycaemic (Adam et al., 2012), anti-diabetic (Sirisha et al., 2010), antioxidant (Hakiman and Maziah, 2009) and wound-healing properties (Abdulla et al., 2010). Adam et al. (2007) stated that all parts of *F. deltoidea* have potential as medicinal properties that include the roots, barks, leaves and fruit. *F. deltoidea* is a well-known house plant, and is also significant as a source of traditional pharmaceutical products (Bunawan et al., 2014). Their leaf also has been used commercially to make a herbal tea (personal observation).

F. deltoidea commonly occurs as a true epiphyte or terrestrial bush in coastal areas, heath land and mountainous sites below 3200 m altitude. Plants usually range between 0.3 m to 7.0 m in height and are often found as a shrub rather than a tree (Starr et al., 2003). *F. deltoidea* produces picturesque aerial roots under warm and humid conditions (Riffle, 1998). Species in the *F. deltoidea* group can be epiphytic, epilithic (germinating on rocks as ‘rock-splitters’) or terrestrial shrubs and treelets. As terrestrial plants they are often found on nutrient-poor soils (Berg and Corner, 2005). These authors linked the slow growth of *F. deltoidea* and its production of figs sequentially and asynchronously to their occurrence in nutrient poor habitats. Traits developed in response to poor soils, including the plants exceptionally large seeds may represent a pre-adaptation that has allowed them to survive as epiphytes, rather than as hemiepiphytes (stranglers).

F. deltoidea is dioecious, with separate male and female plants that either support fig wasp pollinators or produce seeds, respectively. *F. deltoidea* exhibits several characteristic that differ from many other *Ficus* such as its large seeds, small number of flowers and different juvenile and adult leaf forms (Corner, 1969). Female figs of this group are exceptional insofar as they only contain small numbers of flowers. This allows individual seeds to be far larger than is normal for fig trees (Kraft et al., 2015a) and is a presumed adaptation for an epiphytic life style. Birds and Orang Utans are the only confirmed feeders on the figs (Shanahan et al., 2001).

Whether the varieties of *F. deltoidea* are actually distinct species is unclear. Morphological studies of *F. deltoidea* show that all characters portray a high variability among the varieties (Awang et al., 2013). This variation between and within species may be due to cross-pollination and sexual recombination between the species (Corner, 1997). Genetic studies showed the variation between the varieties in *F. deltoidea* reflected morphological variation rather than the geographical origin of the plants (Zimisuhara et al., 2015).

Leaf morphology and anatomy are often used for identification of *F. deltoidea* varities because they are particularly discriminative (Nur Fatihah et al., 2014). However, F *deltoidea* often exhibit different states of leaf characters between the young and mature plants that has often led to misleading identification of the varieties. The leaf forms of *F. deltoidea* often exhibit great variation, from lanceolate, elliptic, obavate, obdeltoid, spathulate or show a combination of two or three of such forms (Awang et al., 2013). The leaf lengths are between 4 cm and 8 cm. They are bright-green coloured on the upper surface and rust-red to olive-brown on the bottom surface of the leaf (Mat et al., 2012). The figs vary from 6 mm to 18 mm long and 5 mm to 22 mm wide with yellow, orange, red or purple in colour when ripe (Awang et al., 2013). The shape of the figs can be either globose, oblong, fusiform, conical or obconical (Laman and Weiblen, 1998).

Corner (1969) classified 13 varieties in *F. deltoidea*, with seven of them found in Peninsular Malaysia. Subsequent treatments have been based on this work, and the taxonomy of the group has remained essentially unchanged since 1969, except that many of his varieties were merged by Berg and Corner (2005). This study was concordant with a study by Mat et al. (2012) and Nur Fatihah et al. (2014) which found those seven varieties were native varieties in peninsular Malaysia. The varieties are var. *angustifolia* (Miq.), var. *deltoidea* Corner, var. *trengganuensis* Corner, var. *kunstleri* King, var. *bilobata* Corner, var. *motleyana* (Miq.) and var. *intermedia* Corner.

Information regarding the pollinators of *F. deltoidea* is very scarce. The only pollinator recorded for *F. deltoidea* is *Blastophaga quadrupes* Mayr, which probably pollinates var. *lutescens* (Corner, 1969) as it was collected in Java and Sumatra (Wiebes, 1993). The common characteristics in the genus *Blastophaga* is the absence of coxal and sternal corbiculae (Ramirez and Malavasi, 1997). Female fig wasp from genus *Blastophaga* does not have pollen pocket that makes them passively pollinate the host fig tree (Wiebes, 1994). Wiebes (1993) found that there was some variation in the females of *Blastophaga quadrupes* among different varieties of *F. deltoidea*, but the male seems uniform. According to Weibes, (1994) *Blastophaga quadrupes* pollinate several varieties of *Ficus deltoidea*. However, Corner (1969), based on floral and inflorescence differences, suggested that different pollinators were likely, and different varieties of *F. deltoidea* in Brunei have been found to support distinct species of pollinator (F. Kjellberg, personal comm. to S. G. Compton). If this is the case elsewhere, then *F. deltoidea* may represent a complex of closely-related but biologically distinct species.

A study by Zimisuhara et al. (2015) showed the gene flow between varieties is occurring in 30 accessions of *F. deltoidea* from five varieties (var. *angustifolia*, var. *bilobata*, var. *deltoidea*, var *kunstleri* and var. *trengganuensis*) collected in Peninsular Malaysia but also that there was a significance difference in eight morphological characteristics between the varieties. This result supports (Corner, 1965) who could not differentiate all the varieties and placed them in a single species. Tsukaya (2005) concluded that many plants have different type of leaves within the same species (and could be regarded as varieties) due to having adapted to different environmental conditions such as light intensity. However, Tnah et al. (2016) found four sympatric varieties of *Ficus deltoidea* living in the same environmental conditions, and the molecular results detected great inter-varietal variation. The four varieties of *Ficus deltoidea* were divided into two groups, namely a small leaves group that consisted of var. *angustifolia* and var. *deltoidea* and a large leaf group that consisted of var. *kunstleri* and var. *lutescens*. A similar result was found by Zimisuhara et al. (2015) who separated var. *angustifolia*, var. *deltoidea* and var. *bilobata* as one group while var. *kunstleri* and var. *trengganuensis* were placed as another group. Based on their morphological characters, all the varieties in the first group have small leaves while the second group have big leaves. Varieties in both groups can grow as facultative epiphytes.

1.1.8 Epiphytes and Hemiepiphytes (stranglers)

Epiphytes are non-parasitic plants that live on the external surface of living plants where they take nothing from the host except a safe anchorage (Thomas and Packham, 2007). They can be vascular plants (pteridophytes, gymnosperms and angiosperms) or non-vascular plants like algae, lichens or bryophytes (Nadkarni et al., 2001). Epiphytes exploit sunlight by using other plants without giving any harm to the host tree (Zotz, 2013). Epiphytes obtain nutrients and water from the air, fog, water present in the humus and compost that lies on tree branches (Petruzzello, 2017). Despite not taking nutrients from hosts, the epiphytes may compete for sunlight with the host tree. Epiphytes have the ability to maintain positive carbon balance and retain their leaves especially during dry season (Putz et al., 1995). Epiphytes can be differentiated into hemiepiphytes and non-hemiepiphytes. Non-hemiepiphytes are true epiphytes that spend their entire life without contact with the ground (Benzing, 1990).

A hemiepiphyte is a plants that spend at least a part of their life cycle as epiphytes on their host and the other part rooted to the ground (Williams-Linera and Lawton, 1995). Hemiepiphytes can be divided into two categories which are primary hemiepiphytes or secondary hemiepiphytes. Primary hemiepiphytes start their life as epiphytes before spreading their roots to the ground while secondary hemiepiphytes germinate in the soil and grow up the sides of large trees utilizing adhesive roots and finally cut their connection with ground (Nadkarni et al., 2001). In primary hemiepiphytes, their earlier epiphytic stage traits resemble the true epiphytes (Zotz and Winter, 1994).

Hemiepiphytes can be found abundantly in tropical forests (Hao et al., 2016). There are several factors that contribute to the distribution of hemi-epiphytes in the canopy. These incude their seed dispersers, the growth of hosts after formation of hemi-epiphytes, and host microsite quality (Harrison and Yamamura, 2003). Hemiepiphytes are very abundant in lowland tropical rainforests, and in lower montane and mid-montane cloud forests (Putz and Holbrook, 1986; Williams-Linera and Lawton, 1995). They play an important role in canopy dynamics by competing with their host tree species, and stabilize mats of epiphytic organic soil that affect rainfall interception and ecosystem hydrology (Veneklaas et al., 1990; Williams-Linera and Lawton, 1995). The flexibility of their physiology is important as the drastic changes between the two phases in hemiepihytes life cycle include radical changes in their rooting environments (Hao et al., 2010). When hemiepiphytes reach the ground they encounter an intense change in rooting volume and characteristics of the rooting zone (Holbrook and Putz, 1996b).

Two important hemiepiphytic genera are *Ficus* (Moraceae) and *Clusia* (Clusiaceae) (Hao et al., 2016). The physiology and ecology of these plants, is poorly known despite approximately 500 *Ficus* species recorded as being hemi-epiphytes (Putz and Holbrook, 1986). Epiphytic and hemiephytic *Ficus* species often colonise palm trees. The morphology of the erect trunk with a surface protected by persistent leaf bases makes them as an ideal habitat (Nadarajah and Nawawi, 1993). This includes planted palms and palms in plantations, such as oil palms. Some varieties of *F. deltoidea* are among the epiphytes that routinely take advantage of the trees growing in oil palm plantations.

1.1.9 Oil palm

Elaeis guineensis (Arecaceae), commonly known as oil palm, is planted mainly for their oil. This species originated from Africa where they live naturally in Sierra Leone, Liberia, Ivory Coast, Ghana, Cameroon and Republic of Congo and Zaire (Prokurat, 2013). In Soth East Asia,it was first planted in Sumatra in 1911 before spreading to Malaysia in 1917 (Kushairi et al., 2017). Today, Malaysia has become the second largest producer of oil palm after Indonesia (Dayang Norwana et al., 2011). Oil palm is planted frequently in Malaysia due to its climate suitability which received adequate rainfall, sufficient light and its good soil conditions that ideal for the growth of the oil palm (Kushairi et al., 2017). The main contributor to Malaysian gross national profit comes from oil palm production with 16.8 billion USD and this industry provides more than 600 000 working opportunities (Oettli et al., 2018).

Oil palm cultivation is expanding due to rising global demand for palm oil and this trend is forecast to increase until 2050 where approximately 109 hectares of natural habitats will be transformed into agriculture (Tilman et al., 2001). Animal taxa are known to decrease in species diversity and species richness due to conversion of natural forest to oil palm (Foster et al., 2011). The fast-growing conversion of natural forest into agricultural land has urged Malaysian government to diversify the mitigation and maintains a biodiversity programme through the establishment of Roundtable on Sustainable Palm Oil (RSPO) that includes big-range cultivators to small-range farmers (Kushairi et al., 2017). A statistic from (Lee, 2011) showed 60% of overall oil palm plantations in Malaysia are managed by big companies, 28% from smallholders and 12% from independent smallholders.

Until now, various methods were proposed by RSPO to implement environmental-friendly practices and thus mitigate the effect of conversion of natural forest to oil palm plantation. These include planting beneficial plants alongside the plantations to serve as food for beneficial insects, maintaining above-ground weeds that provide nectar for pollinators and herbivores (Siti Khairiyah et al., 2012). Maintaining epiphyte communities on the palm trunks is another option as all taxa showed a significant decrease due to conversion of natural habitat to oil palm plantation (Foster et al., 2011). Most published studies are on the impact of conversion of the forest into oil palm plantations on mammals, birds and reptiles and insects, but the role of epiphytes is rarely considered. The ecology of oil palm epiphytes together with their contribution to the insect populations in oil palm little understood (Suzanti et al., 2016).

An oil palm plantation that is ‘well maintained’ is a virtual monoculture crop. Palms nonetheless offer opportunities for colonisation by epiphytes, even if they are not like a natural forest and epiphytes can be abundant if they are not cleared by plantation workers (Suzanti et al., 2016). Epiphyte communities in oil palm plantations include lichens, algae, mosses, ferns and angiosperms (Nadarajah and Nawawi, 1993). The epiphyte community serves as buffer to species loss caused by the conversion of natural habitat to the plantation (Fayle et al., 2008). In Malaysia, epiphytes can be found more abundantly in small plantations managed by independent smallholders and farmers (personal observations). These smallholders have more autonomy to decide their own practices and how to manage them. They are not linked to any bodies and receive limited funds, have low technical expertise and lack knowledge on best practices and new technologies (Nagiah and Azmi, 2012). The 'poor' management by these small holders often lets the epiphytes remain on the trunks rather than cutting them or spraying them. This is in contrast to a big company that runs big plantations, where the management tend to clear out everything as they think the epiphytes might give negative effects to yields, or they have staff at certain times of year with nothing else to do. The intense management by big companies aims to remove competing plant species that compete with crops for water, light and nutrients. These competing vegetation includes herbaceous understory and epiphytes that live on the trunks of the oil palm (Prescott et al., 2015).

In contrast to currently common assumptions by oil palm companies, maintaining the epiphytes does not have any significant effects on profits and at the same time reduces the biodiversity losses (Zotz, 2016). A study conducted by Prescott et. al. (2015) in three oil palm estates in Sabah, Malaysia found 58 species of epiphytes present comprised of ferns and angiosperms and also mosses. These epiphytes serve as microhabitats for smaller organisms such as ants and other insects (Fayle et al., 2008). A study by Suzanti et al., (2016) showed that plantations that maintain their epiphytes recorded a greater number and diversity of insects compared to plantations that had removed the epiphytes completely or reduced them by half. Another study by (Koh, 2008) found that an increase of species richness of 1.5 birds was correlated with the epiphytes in Malaysian Borneo oil palm plantations. These epiphytes and their associated insects act as a source of food for the birds. A study has concluded that oil palm plantations can support more bird species than other non-forest land-uses such as soy bean plantations (Lees et al., 2015). These birds not only eat the fruits of the angiosperm epiphytes but also act as seed dispersers for the angiosperms. Apart from birds, animals found in oil palm plantations might also act as seed dispersers include squirrels and monkeys.

Oil palm matters for *F. deltoidea* as it can be suitable habitat for this significant true epiphyte that does not give any harm to the oil palm trees. This fig tree species later can provide a continuous food source for frugivores. *F. deltoidea* also has an economic value, so having more of this species is valuable, and oil palms can support high densities of this fig tree.

1.2 Research aims and objectives

The research described in this thesis aims to explore the biology of epiphytic *Ficus deltoidea* and its pollinator in selected oil palm plantations in Peninsular Malaysia. The general *Ficus* community present as epiphytes were also studied. The specificity of pollination of varieties of *F. deltoidea* is unknown. This is the first study to the determine the biology and the host specificity of the pollinators for several varieties in *F. deltoidea* in peninsular Malaysia. The phenology of epiphytic *F. deltoidea* in oil palm plantations was also studied. The objectives of this research were to:

i. Assess the epiphyte communities in certain oil palm plantations in Peninsular Malaysia and to determine which *Ficus* species are most abundant and whether they occupy different heights on the oil palm trunks.
ii. Characterise the morphological characteristic of fig from seven native varieties of *F. deltoidea* and their *Blastophaga* sp. fig wasps from three different varieties.
iii. Determine the host specificity of the *Blastophaga* sp. Pollinator from var. *angustifolia*
iv. Describe the leafing and fruiting phenology of epiphytic *F. deltoidea* var. *angustifolia* in oil palm and the pollination limitation.
v. Describe the pollination biology of *Blastophaga* sp. that pollinates *F. deltoidea* var. *angustifolia.*

1.3 Thesis outline

The contents of this thesis are organised as follows: Chapter 1 provides the background of this research and describes the research aims and objectives. Chapter 2 describes general methods and equipment and where the studies were conducted. This includes climate data, the age of plantations, what type of soil they were planted on and the management of each plantation. The general methods chapter also describes the fig developmental phases used in several chapters in this study. Further details of methods are elaborated in each chapter where appropriate (Chapters 3-7). Chapter 3 discusses the epiphytes present in five oil palm plantations in Peninsular Malaysia and describes differences in their height preferences. Host preferences of two different varieties of *Ficus deltoidea* that live sympatrically are also described. Chapter 4 provides characterisation of male and female figs of all seven native varieties of *F. deltoidea* in Peninsular Malaysia., The morphologies of three

Blastophaga sp. pollinators from three varieties are also compared. Chapter 5 describes experiments aiming to determine the host specificity of the *Blastophaga* sp. that pollinates *F. deltoidea* var. *angustifolia*. An apparent preference for male over female figs is also described. Chapter 6 describes the phenology of epiphytic *F. deltoidea* var. *angustifolia* in an oil palm plantation near Kuala Lumpur. Chapter 7 describes the pollination biology of this variety of *F. deltoidea*. Chapter 8 provides a general discussion and includes suggestions for future work.

Chapter 2

General methodology

2.1 Study sites

The study was conducted from June 2016 until August 2017 at five oil palm plantations in Peninsular Malaysia (Figure 2.1). The main study site was located in an oil palm plantation in Banting. The other plantations were at Dengkil, Batu Pahat, Bagan Serai and Tembila. Four of the plantations are managed by small-sale farmers, while Tembila is owned by a private company. Further studies were carried out in a *Ficus deltoidea* germplasm collection owned by Universiti Sultan Zainal Abidin (UnisZA) and inside the university area of UniSZA located in Besut,Terengganu.

The study sites were chosen because they had *F. deltoidea* growing as epiphytes on oil palm trunks. Before the selection of study sites, a number of other sites were visited to check for the presence of this species. Other criteria included personal safety, permission from the owner to conduct the study, the numbers of *F. deltoidea* at the site and travel distances (for the main study site).

2.1.1 Banting oil palm plantation

This 2.10 hectares plantation was the main study site. It is located in Selangor, about 50 km from Kuala Lumpur city centre. The coordinates of this plantation are 2^{o}50.094” N and 101^{o}35.074” E. It is managed by small-scale farmer who has lived on the site since the palms were planted in 2001. The plantation has a peat soil. There are about 285 oil palm trees in the plantation and 86 of the trunks serve as hosts for 113 epiphytic individuals of *F. deltoidea* var. *angustifolia* (Chapter 3). These include some big male trees of var. *angustifolia* that are convenient for experimental purposes (Chapter 5). The phenology of the fig trees is described in Chapter 6.

Climate data for Banting and the other sites was obtained from the Malaysia Meteorological Department. The closest station to Banting is the KLIA Sepang station located about 19 kilometres away. Banting has a tropical climate with an average annual temperature of 27.8 degrees celsius that varies little throughout the year. Along the sampling duration (June 2016 until August 2017), the average annual precipitation throughout the year was 144.3 mm. The precipitation ranged between 30.2 mm and 345.8 mm. The average daily minimum and maximum temperatures were relatively stable (Figure 2.2). The average daily minimum temperatures ranged from 24.0 to 25.2 ˚C, while average daily maximum temperatures were between 30.9 and 32.5 ˚C. Rainfall was lowest during August 2017 at only 26.5 mm the rainfall was the second lowest in the same month a year before with 64.8 mm. The rainfall recoded In April 2017 was the highest at 236.0 mm.

Figure 2.1 The six study sites in Peninsular Malaysia.

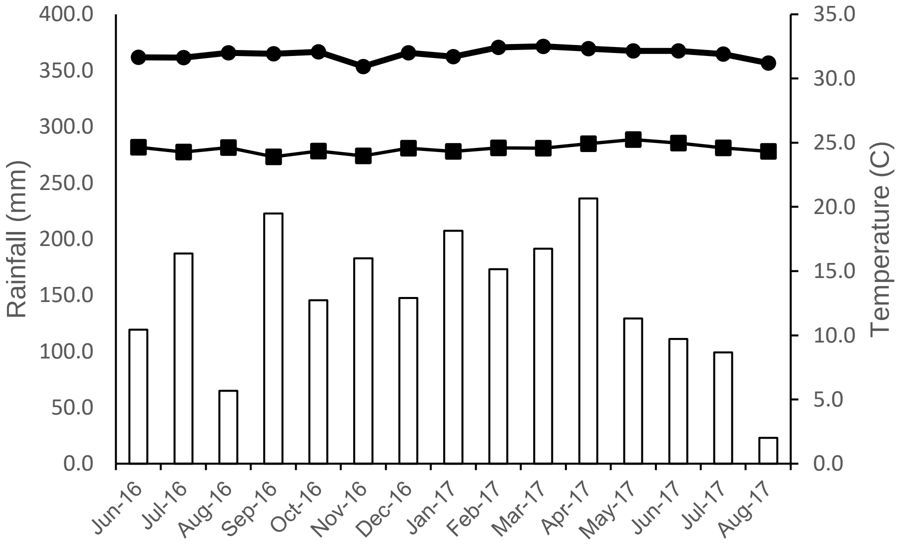


Figure 2.2 Climate data for Banting oil palm plantation, Selangor. Monthly percentage of rainfall (open bars), relative humidity (■) and monthly average temperatures (•) for June 2016-August 2017 as measured by KLIA Sepang (19 km from the research site).

2.1.2 Dengkil oil palm plantation

The Dengkil, plantation is also in Selangor, about 10 km away from Banting, but has a silt soil. The coordinates for this plantation are 2^{o}51.125” N and 101^{o}39.424” E. This 40.53 -acre plantation is managed by small farmers and the oil palms were planted in 1995. There are about 427 oil palms in this plantation, which host numerous epiphytes (Chapter 3). KLIA Sepang was again the nearest meteorological station to Dengkil.

2.1.3 Batu Pahat oil palm plantation

Batu Pahat is located in Johor state, the southern part of Peninsular Malaysia, about 239 km from Kuala Lumpur. Its coordinates are 5^{o}07.070” N and 100^{o}39.445” E. This plantation is about 2.50 hectares and the palm trees were planted in 1991. This peat soil plantation is a private residence of a *F. deltoidea* trader, Mr Ismail. He grows examples from the *F. deltoidea* complex including var. *angustifolia*, var. *deltoidea*, var. *kunstleri* and var. *trengganuensis* and sells their leaves for research and pharmaceutical purposes. Apart from the planted trees, two varieties of *F. deltoidea* namely var. *angustifolia* and var. *deltoidea* grow as epiphytes in the old oil palm plantation within the residential area. Both varieties show evidence of being pollinated and can be found in abundance on the trunks. The planted examples of var. *angustifolia* and var. *deltoidea* were made from cuttings taken from the wild epiphytes. Plants from the other two varieties were brought from KESEDAR in Kelantan in 2006 and planted at Batu Pahat. The climate data for this plantation were taken from Batu Pahat station, 24.7 km from the plantation. The range of temperatures during the period of this study (June 2016 until August 2017) were stable and ranged from 27.0°C to 28.9°C while the rainfall ranged from 82.2 mm to 292.2 mm (Figure 2.3).

2.1.4 Bagan Serai oil palm plantation

Bagan Serai, is located on peat soil in Perak state about 305 km north of Kuala Lumpur. The coordinates for this plantation are 5^{o}07.070” N and 100^{o}39.445” E. This old plantation is about 0.9 hectares in area and the palms were planted in 1989. The trees are hosts for epiphytic *F. deltoidea* var. *angustifolia*. Climate data were obtained from Chersonese Est. Kuala Kurau, about 36.7 km away. The mean temperatures were stable within the range of 26.4 to 28.2°C (Figure 2.4). The highest rainfall during the study period was in September 2016 with 428.4 mm, while the driest period was July 2017 when only 20.1 mm of rainfall recorded.

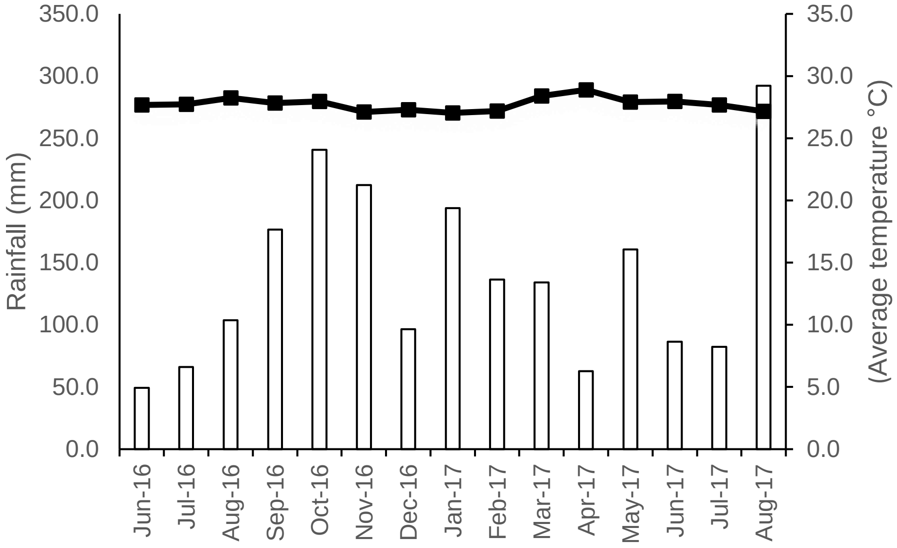


Figure 2.3 Climate data for Batu Pahat oil palm plantation, Johor. Monthly mean rainfall (open bars) and average temperature (■) for June 2016-August 2017 as measured by Batu Pahat station.

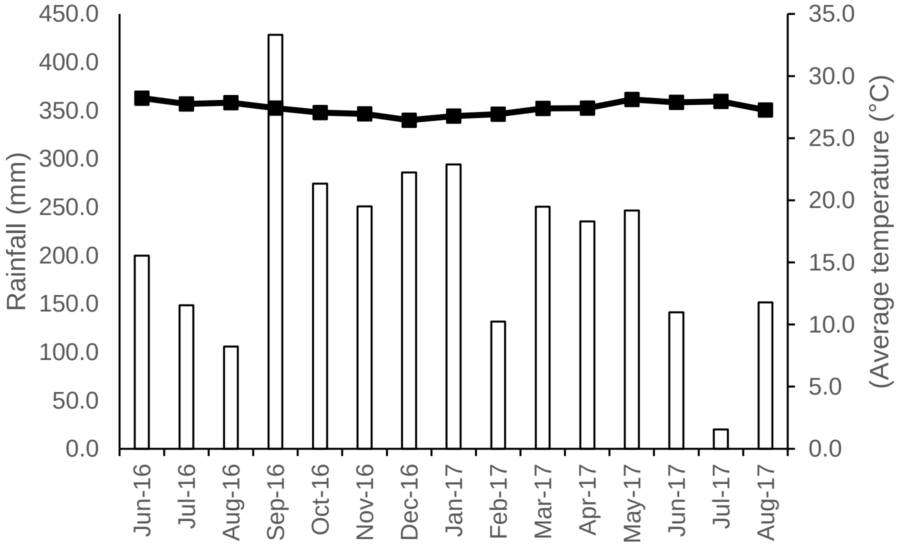


Figure 2.4 Climate data for Bagan Serai oil palm plantation, Johor. Monthly mean rainfall (open bars) and average temperature (■) for June 2016-August 2017 as measured by Chersonese Est. Kuala Kurau station.

2.1.5 Tembila oil palm plantation

Tembila oil palm plantation is located on sandy soil close to the coast in Tembila, Terengganu, a south eastern coastal state. The coordinates for the plantation are 5^{o}43.402” N and 102^{o}39.445” E. It is managed by a company not small farmers. The overall plantation is about 129.5 hectares and the oil palms were planted in 2007. The climate data for rainfall and temperatures were taken from Institut Pertanian Besut station about 15 km away. Temperatures between June 2016 until August 2017 ranged from 25.0 to 28.8 °C (Figure 2.5). The driest month was April 2017 when only 10.9 mm rainfall was recorded while the wettest month was December 2016 which received a total of 632.5 mm rainfall. December is known locally as the month when floods are likely in Terengganu.

2.1.6 Terengganu germplasm collection

The germplasm collection is owned by Universiti Sultan Zainal Abidin (UniSZA) located in Terengganu. The collection includes varieties of *F. deltoidea* from Peninsular Malaysia together with others from Borneo. This collection was established in 2006 and contains 165 medium-large individuals and many smaller plants. It contains eight varieties: var. *angustifolia*, var. *deltoidea*, var. *trengganuensis*, var. *kunstleri*, var. *bilobata*, var. *motleyana*, var. *intermedia* and var. *borneensis*. Of these varieties, only var. *borneensis* is not native in Peninsular Malaysia. The trees originate mainly from cuttings taken from several places in Peninsular Malaysia namely Johor, Kelantan, Melaka and Terengganu. The var. *borneensis* were received from the Sarawak Biodiversity Centre. The cuttings vary from one to 10 years old. The plants are grown in clay pots with either a 39 cm or 49 cm diameter. The media was a combination of top soil, pome, rice husk and peat soil. All of the collection were given NPK Green organic fertilizer and were watered regularly.

The university area of UniSZA is about one kilometre from the germplasm collection. The nearest available weather data is from the Institut Pertanian Besut station, as given for the Tembila plantation.

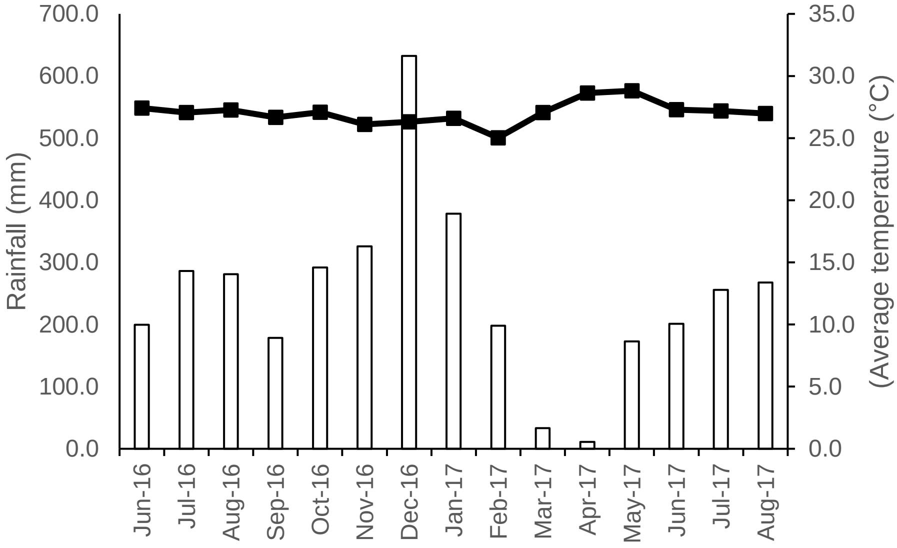


Figure 2.5 Climate data for Tembila oil palm plantation, Johor. Monthly mean rainfall (open bars) and average temperature (■) for June 2016-August 2017 as measured by Institut Pertanian Besut station.

2.2 Fig development phases

An assessment was made to look for possible ways in which the developmental phases of *F. deltoidea* var. *angustifolia* could be assessed without removing the figs. This was necessary because relatively small crop sizes and asynchronous fruiting made extensive destructive sampling problematic. Initially, about 30 figs from male and female figs were collected from the Banting plantation. The figs covered the whole range of sizes. After that, the figs were examined individually and differences in their size, thickness, colours and contents were recorded. Based on these results a visual assessment of fig developmental stages was possible and used during phenological studies and to obtain suitable figs for experiments.

The assignments to fig developmental phases were based on the scheme of Galil and Eisikowitch (1968) where there are five phases in the fig developmental cycle. During the A phase in male figs, the ostiole is still closed and the size is about 0.2 cm (Figure 2.6). During this phase, the red tepals are about the same size as the female flowers. The styles of the female flowers are white in colour. Up to B phase, the female flowers, tepals and male flowers are growing. In terms of diameter, both phases are soft and green in colour and around 0.4 cm. Both phases were squeezable.

C phase male figs usually look yellowish green from the outside and the texture is hard Figure 2.6). During this phase, squeezing the figs is not possible. This is because fig wasp offspring development has started and the galled ovules have expanded. During this phase, the tepals change colour from red to brown and stop growing. At D phase, the next generation of fig wasp offspring emerge from their galls. Usually this phase has a softish feel when squeezed, though no clearly defined central cavity develops. The colour at D phase is usually yellow green. The anthers of the male flowers start to dehisce. The size of D phase figs varies but is usually between 0. 45cm to 0.55 cm.

At E phase the figs are attractive to frugivores that can become seed dispersers. The texture of this phase is also softish with a yellowish green to mild red colour. In this phase, the pollinator exit hole can be seen through the ostiole and the anthers are dehiscing. The tepals are brown colour and shrunken. The fig diameter is usually more than 0.56 cm.

An additional ‘phase’ was recognised, to record figs that had failed to develop because they had not been entered by pollinators. This condition is not included in the Galil and Eisikowitch (1968) scheme. In aborting figs the tepals are brown and the size is variable but usually does not exceed 0.6 cm. Their colour is often yellow to orange and if squeezed, a hollow texture can be felt.

The structure of female figs is simpler than that of male figs because they only contain female flowers and their associated tepals. The female figs also display fewer phases as compared to male phase, because D phase is absent (Figure 2.7). The size of figs on female trees is often bigger than for male figs at the same developmental stage. As in male figs, phase A is immature figs where the ostiole is still closed. Usually in this phase, the size is between 0.1 cm - 0.3 cm and the texture is soft with a green colour. The tepals appear more rounded in female than male figs. The mean style length is also much longer in female figs.

In B phase, the tepals and seed are growing and usually the tepals are bigger than the ovules. The texture is still soft, with green colour and the size reaches about 0.4 cm. Meanwhile, in C phase, there is not so much difference in colour, texture and size between female and male figs. The texture is hard, yellow green and within a range of 0.5 to 0.6 cm. In this phase, the seeds grow and the colour of the seed change from green to yellow, while the size of the tepals remains the same.

At E phase, the texture of the seed is soft with a slight red colour and the figs are ready to be eaten by frugivorous. The colour of the tepal has faded from bright red to mild red. The female figs lack a D phase because no pollinator offspring develop in the figs. The ripe are often more than 0.7 cm in diameter.

Aborting female figs develop when no pollinator enters the figs. The ovules and tepals stop growing and turn yellow. The style colour becomes brown. The colour is always yellow and their hollow structure can be felt when squeezed. As in male figs, they remain for extended periods on the trees before falling to the ground.

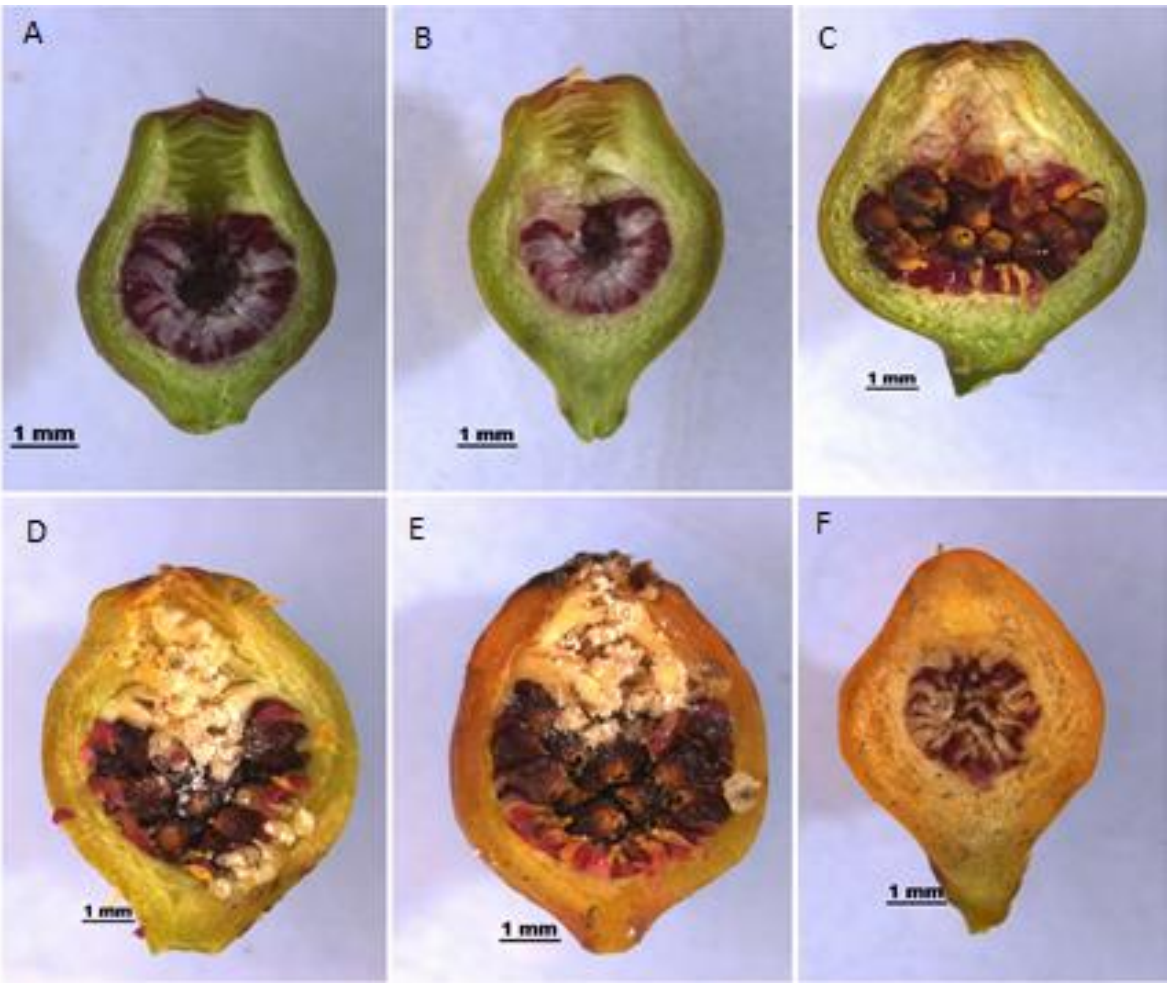


Figure 2.6 Fig developmental stages in male *F. deltoidea* var. *angustifolia*. (A) Young fig. (B) The fig is receptive to pollinators. (C) Development of fig wasp offspring. (D) The next generation of wasps are released. (E) After the wasps have emerged. (F) An aborting, un-entered fig.

Figure 2.7 Fig developmental stages on female *F. deltoidea* var. *angustifolia* fig trees. (A) Young fig. (B) The fig is receptive to pollinators. (C) Development of the seed. (D) Late Development of the seed. (E) Mature seeds ready to be dispersed (F) Aborting fig.

Chapter 3

Epiphytes in Malaysian oil palm plantations

3.1 Introduction

3.1.1 Epiphytes

Epiphytes are a group of autotrophic organisms that live on the external surfaces of other plants without causing any harm to the host plants. They are widely distributed, especially is widely tropical and subtropical areas (Thomas and Pachams, 2007; Zotz, 2016). Epiphytes most commonly inhabit humid climates where they receive less nutrients, more light, less water and larger daily temperature amplitudes than terrestrial plants (Benzing, 1990; Holbrook and Putz, 1996b). Epiphytes are significant for primary production and help retain water and nutrients (Veneklaas et al., 1990). Communities of epiphytes increase the canopy complexity and provide important resources for other organisms especially insects (Suzanti et al., 2016). Individual trees can have more than 80% epiphyte cover (Kreft et al., 2004) so they are therefore important for maintaining biodiversity in tropical areas (Ellwood and Foster, 2004).

Epiphytes include vascular and non-vascular plants. Vascular groups consist of ferns (pteridophytes) and higher plants (gymnosperms and angiosperms). A recent survey by Zotz (2013) recorded 27 614 species of vascular epiphytes from 913 genera and 73 families. Vascular epiphytes are usually associated with lower latitudes (Nadkarni et al., 2001) and are favoured by a continuous water supply from rainfall and deep organic layers on their hosts (Hemp, 2006). Non-vascular epiphytes like algae, lichens and bryophytes are also found abundantly in the tropics, where they prefer to inhabit the upper canopy (Nadkarni et al., 2001). Compared to vascular epiphytes, this group of epiphytes remains understudied due to their small size (Affeld et al., 2008).

About 2 700 species of ferns and their allies, in 121 genera, are known to develop as epiphytes (Zotz, 2013). Assessments in five oil palm plantations in North Sumatra found three fern species *Nephrolepis biserrata* (Sw.) Schott (Nephrolepidaceae), *Goniophlebium verrucosum* (Wall. ex Hook.) (Polypodiaceae), *Vittaria elongate* Sw. (Pteridaceae) and one angiosperm species *Asystasia gangetica* L. (Acanthaceae) were the dominant epiphytes (Suzanti et al., 2016). Similarly, in Malaysian oil palm plantations, Nadarajah and Nawawi (1993) recorded that ferns were the dominant group of epiphytes.

General characterisations of epiphytes often include having small leaves, slow growth and large seeds (Hao et al., 2016). Being detached from the soil causes the epiphytes to experience a microclimate different from ground-living rooted plants (Zotz, 2016). The morphology of epiphytes is adapted to extreme conditions, especially water and nutrient scarcity (Nadkarni et al., 2001). Living in a water limited environment is the biggest challenge faced by epiphytes and this problem is more critical in seasonally dry climates (Benzing, 1990). Having small leaves with a higher leaf mass per unit area, and a thicker epidermis helps them to reduce water loss (Hao et al., 2010). They also have the ability to retain carbon in the leaves during times of water scarcity (Putz et al., 1995).

Seed size reflects a compromise between the number of seeds produced and the investment in each seed (Westoby et al., 1992). The seeds of some epiphytes are large compared to related taxa. For example Rockwood (1985) found that Neotropical epiphytes had seeds of similar weight or averaged slightly larger than their relatives with other growth forms. Larger seeds can provide more nutrients in the early stage of epiphyte establishment and may be important due to the slow growth typically shown by epiphytes (Kraft et al., 2015b). Some epiphytic plants like orchids nonetheless have tiny wind-blown seeds while others have larger seeds structurally adapted for wind dispersion or depend on animals for dispersal (Zotz, 2016). This suggests that different taxonomic groups adapt differently to the selection generated by an epiphytic life style (Arditti and Ghani, 2013).

Host plant characteristics influence the epiphyte communities growing on them. Bark traits like thickness, rugosity, shedding rate, density and water holding capacity are all significant (Wyse and Burns, 2011; Wagner et al., 2015). Bark shedding prevents colonisation of tree trunks (Steward and Beveridge, 2010). According to Mondragon and Calvo-Irabien (2006), the germination of epiphyte seeds does not depend on tree height and the age of the host tree. However, Zotz and Vollrath (2003) note that the age of a tree does affect the abundance of epiphytes it supports, because older trees have a larger surface area to support epiphytes and as the age of the tree increases so does the chance of repeated colonisation.

Epiphytes can be hemiepiphytes or non-hemiepiphytes (true epiphytes). Hemiepiphytes are plants that spend parts of their life as epiphytes whereas non-hemiepiphytes spend their whole life as an epiphyte (Quaresma et al., 2017). Hemiepiphytes can be primary hemiepiphytes where they start their life by being epiphytes on a tree before they develop aerial roots reaching the ground (Putz and Holbrook 1986; Zotz and Volrath. 2003). Secondary hemiepiphytes start their life by germinating on the ground and seedlings search for a host to ascend (Balcázar-Vargas et al., 2015). Once they reach the tree crown, as with true epiphytes, their longevity depends on the survival of the host plant (Suzanti et al., 2016).

3.1.2 *Ficus* as epiphytes

There are about 800 described *Ficus* (Moraceae) species. About 500 of them are hemiepiphytes while 300 are non-hemiepiphytes that include shrubs, free-standing trees and climbers (Berg and Corner, 2005). Most hemiepiphytic *Ficus* species are strangler figs that start their life after germination in the canopy or trunk of a host tree and after a period as epiphytes they spread their roots to the ground. They are primary hemiepiphytes in the sense of (Nadkarni et al., 2001). There are also creepers and scandent species that germinate on the ground and climb up and over other plants they come into contact.

Within the genus *Ficus* the habit of hemiepiphytism has evolved perhaps four times (Harrison, 2005). Hemipepiphytic fig trees get their mechanical support from the host while competing with it for light, nutrients and space (Male and Roberts, 2005). Hemiepiphytes can have the ability to strangle the host before becoming independent fig trees (Hao et al., 2016). Stranglers can benefit by having low risks of fire, floods, terrestrial herbivores and damage caused by falling debris (Hao et al., 2016). Conservative water use and better drought tolerance with strong control on stomatal conductance are important adaptations of hemiepiphytes (Hoolbrook and Putz, 1996a; Hoolbrook and Putz, 1996b). Strangler figs germinate from tiny epiphytic seedlings into terrestrial rooted trees that exhibit root fusion and larger species can surround and eventually kill their hosts (Holbrook and Putz, 1996a). Some host trees have chemical and morphological features that make colonisation by hemiepiphytic figs less likely. These include a lack of deep branch crotches and hollows and a smooth bark (Compton and Musgrave, 1993; Wyse and Burns, 2011). These features are all absent in many palms, which is why they are frequently colonised by hemiepiphytic and other fig trees.

3.1.3 Epiphytes in oil palm plantations

Epiphyte establishment is associated with host traits such as plant morphology (Male and Roberts, 2005), bark stability (Wagner et al., 2015), water retention capacity (Wagner et al., 2015) and the presence of allelopathic components (Benzing, 1990). Palm trees have a prominent crown and a trunk that favours high detritus accumulation that allows epiphyte seeds to take hold and germinate (Kramer, 2011). The rough bark and leaf bases of oil palms provides a strong foothold that lets epiphytes avoid being washed away (Wagner et al., 2015) and allows a thicker layer of ‘canopy soil’ to develop (Williams and Sillett, 2007). The bases of the large leaf petioles store the humus and other organic materials that form canopy soil and helps keep them damp (Sofiyanti, 2013). Once the epiphytes successfully germinate they can remain growing until the surfaces they are attached to are shed, perhaps due to their weight (Zotz, 1998).

Thanks to their dramatic increase in popularity as a crop, oil palm plantations are now a major host for tropical epiphytes. The biotic and abiotic factors that determine the abundance of epiphytes in oil palm plantations have been identified as climate, especially water availability (from the rain), host plant size and the type of soil (Ding et al., 2016). Luskin and Potts (2011) concluded that the age of oil palm trees affects the abundance of their epiphytes (Teuscher et al., 2015). As plantation age increases, the canopy closes and increases the structural complexity if the epiphyte communities remain undisturbed (Luskin and Potts, 2011).

Each oil palm plantation has a different management system (Teuscher et al., 2015). They differ in terms of the intensity and frequency of use of fertilizers and herbicides to remove ground flora weeds and epiphytes on the trees (Roll et al., 2015). With smallholders, the clearing of oil palm trunks does not occur systematically or routinely as they are self-financed, self-managed, and free to decide their own practices (Dislich et al., 2015). ‘Poor’ management in smallholdings allows epiphyte communities to become well established on the oil palm trunks and maintain their complexity (Foster et al., 2011). This contrasts with the actions typical of bigger companies (Azhar et al., 2015). In big and managed plantations, removing the epiphytes is conducted routinely as they are seen as obstructing the collection of mature palm fruits (Prescott et al., 2015).

A few decades ago, epiphytes were regularly removed from nearly 40% of mature plantations in West Malaysia to reduce the competition between the epiphytes and the oil palm trees (Piggott, 1980). However, removing the epiphytes leads to loss of many species that depend on epiphytes as their habitat. The growth of epiphytes creates a soil, litter and foliage environment on oil palms that can act as resource for herbivorous insects (Schmidt and Zotz, 2001). Among the dominant insects on epiphytes in oil palm plantations are weevils and cockroaches which themselves attract insect predators and parasites such as ants and braconid wasps (Suzanti et al., 2016). Epiphytes can also support pollinator populations (Wittman, 2000). Bryophytes, pteridophytes and angiosperms all commonly inhabit oil palm plantations. Fig trees are among the common angiosperms and their figs may provide food for birds in oil palm plantations.

3.1.4 Frugivores

Frugivores have shaped the evolution of fruit traits in *Ficus* (Lomascolo et al., 2010) and influenced the diversity of fig sizes and positions seen today (Compton et al., 2010). Smaller fruits, containing smaller seeds, have a broader spectrum of seed dispersal agents than larger-seeded fruits (Corlett, 2017). *Ficus* species usually produce smaller seeds than many angiosperms and often produce large quantities of seeds (Mumford, 2008). Many species also produce fruits at times of year when other trees are not fruiting (Mackay et al., 2018). More species of animals are recorded as feeding on figs than any other fruits (Shanahan et al., 2001). Among the known seed dispersers for *Ficus* are birds (Kerdkaew, 2018), bats (Lambert, 1991), apes (Mumford, 2008), monkeys (Chaves et al., 2018), squirrels and tree shrews (Peh and Fong, 2003) and others. Some hemiepiphytes undergo two-phased seed dispersal first by vertebrates followed by secondary dispersal by ants (Kaufmann et al., 1991). Primary hemiepiphytes and true epiphytes require their seeds to be deposited on host trees, whereas this is not important for secondary hemiepiphytes such as fig trees that grow as creepers. Terrestrial mammals that feed close to the ground can disperse these plants to suitable germination sites, but not the others.

Conversion of natural forest and fragmentation of habitats reduces fruit availability for birds and mammals (Chaves et al., 2018). Oil palm plantations support much less biodiversity than forests, but epiphytes growing on the palms can partially compensate (Turner and Foster, 2009; Vijay et al., 2016). Fig trees are often seen as being difficult to identify, and consequently they are not as well studied as some other groups of epiphytes. If they are allowed to mature, their figs can nonetheless support frugivores. In Peninsular Malaysia, some varieties of *Ficus deltoidea* are true epiphyte and are a widespread in oil palm plantations.

3.2 Objectives

We addressed the following questions about fig trees growing as epiphytes and hemiepiphytes on oil palms in Peninsular Malaysia (1) How common is *F. deltoidea* as an epiphyte in oil palm plantations around Kuala Lumpur? (2) Which groups of epiphytes are present in oil palm plantations and do they grow on the same individual palms as the fig trees? (3) Which *Ficus* species are present on the oil palms as epiphytes, what are their growth forms and do they ever manage to produce fruit? (4) What proportion of palms support the epiphytes and what densities? (5) Do different *Ficus* species become established on different parts and at different heights on the palms? And (6) which species of frugivores visit the *F. deltoidea* epiphytes.

3.3 Methods

3.3.1 Study sites

A survey was carried out to assess the presence of *F. deltoidea* var. *angustifolia* in oil palm plantations in an area near Kuala Lumpur. All plantations along the roadsides between the Banting and Dengkil study sites (which are approximately 10 kms apart) were assessed. The numbers of var. *angustifolia* were determined by naked eye counts of how many plants could be seen growing as epiphytes on oil palms within 20 m of the road. Accurate assessments could not be made for distances further into the plantations. No other varieties were seen.

Five plantations with high densities of *F. deltoidea* were used to assess their epiphyte communities. The plantations were at Banting and Dengkil in the state of Selangor, near Kuala Lumpur; Batu Pahat, to the South in the state of Johor, Bagan Serai in Perak state to the north of Kuala Lumpur and Tembila plantation on the east coast of Malaysia, in the state of Terengganu. Details of their locations, soils and climates were described in Chapter 2.

3.3.2 Epiphyte assessments

All the oil palms in the available sections of each plantation were assessed for the abundance and diversity of their epiphytes. Their trunks were scored based on their percentage cover of bryophytes, pteridophytes, *Ficus deltoidea*, other *Ficus* species and other angiosperms. The remaining bare areas of trunk were also recorded.

The heights of the oil palm trunks were measured using an adjustable pole and measuring tape and the percentage cover of each plant group was then estimated by eye. If there was overlap of different plant groups, the microepiphytes cover was omitted. For *Ficus* species, the heights where they appeared to have originated were recorded and herbarium samples were made for identification purposes. The identifications of *Ficus* species were made from herbarium specimens presented to botanists from Universiti Kebangsaan Malaysia (UKM). The growth forms of the *Ficus* species (creeper, strangler or true epiphyte) were also recorded. No attempt was made to further identify the bryophytes, pteridophytes and other angiosperms.

3.3.3 *Ficus deltoidea* assessment

For each individual of *F. deltoidea*, its variety was recorded as well as the sex if figs were present. The heights and diameters of thickest stems of the fig trees were also noted. Some *F. deltoidea* were inaccessible and their details could not be recorded. The diameter of the thickest stem was measured using Vernier calliper. For plant apparent sex ratio assessment, the data were collected on 11th March 2017.

3.3.4 Frugivore observations

In Batu Pahat plantation, six camera traps (Bushnell 24MP Trophy Cam HD Aggressor) were installed opposite large female *F. deltoidea* individuals that had high numbers of figs. This was to record any frugivores present on the *F. deltoidea*. The cameras were installed at heights that provided coverage of the whole crowns of the plants. The traps were left in place for two weeks from 2nd July 2017 until 15th July 2017. Any frugivorous birds and mammals were noted.

3.3.5 Statistical methods

A normality test using Kolmogorov-Smirnov was conducted for each analysis to see whether the distribution was normal or not. ANOVA was later used if the distribution was nomal while Kruskal-Wallis for non-normal distribution. Tukey and Mann-Whitney test were later conducted for normal and non-normal respectively to see the diffrences occurred between groups.

Differences in the proportions of the trunks covered by *Ficus* and *Ficus deltoidea* groups were analysed using non-parametric Kruskal-Wallis tests. The differences in height of origin of the *Ficus* species on the trunks were analysed Kruskal-Wallis again. For this analysis, the creeper *Ficus sagitatta* was excluded because it comes into contact with the trunks near or at ground level.

Differences in the numbers of individuals of two varieties and the frequency of their sexes at Batu Pahat were compared using Chi square tests. For each variety, two way contingency Chi square tests were performed to test against a 50:50 sex ratio. The height preferences of the two varieties of *F. deltoidea* and the male and female trees were analysed using ANOVA if the distribution was normal and Mann-Whitney tests if the distribution was not normal. A more detailed study was conducted of *F. deltoidea* var. *angustifolia* at the Banting plantation. The heights of male and female plants on the oil palms were compared using ANOVA. All test were all performed in SPSS Statistics 20.

3.4 Results

3.4.1 *Ficus deltoidea* var. *angustifolia* in plantations near Kuala Lumpur

There are 26 other oil palm plantations along the 10 km of road between Banting and Dengkil, and one residential area that is surrounded by some oil palms within their compound. From all the plantations observed, only 4 showed the existence of *F. deltoidea* var. *angustifolia* within 20 m from the main road. These were the start and end points of Banting (23 trees) and Dengkil (22 trees) plus the houses (5 trees) and Plantation 26 (18 trees) (Table 3.1). The rest of the plantations did not show any signs of *F. deltoidea* presence on the trunks of the oil palms when viewed from the road.

Table 3.1 The number of *Ficus deltoidea* var. *angustifolia* sighted within 20 m from the road.

Plantation	Distance from previous plantation (m)	*F. deltoidea* var. *angustifolia*
Banting plantation	0	23
Plantation 1	500	0
Plantation 2	47	0
Plantation 3	110	0
Plantation 4	290	0
Plantation 5	200	0
Plantation 6	650	0
Plantation 7	170	0
Houses	130	5
Plantation 8	1300	0
Plantation 9	300	0
Plantation 10	280	0
Plantation 11	200	0
Plantation 12	170	0
Plantation 13	400	0
Plantation 14	650	0
Plantation 15	200	0
Plantation 16	500	0
Plantation 17	400	0
Plantation 18	600	0

Plantation 19	270	0
Plantation 20	210	0
Plantation 21	290	0
Plantation 22	140	0
Plantation 23	400	0
Plantation 24	200	0
Plantation 25	190	0
Plantation 26	500	18
Dengkil plantation	800	22
Total	10097	68

3.4.2 Epiphyte loads

All of the 1091 oil palm trunks at the five sites that were assessed supported at least two of the groups of epiphytes on their trunks, with a maximum of five groups. Five groups of epiphytes was the most common frequency at most sites. In all study sites some bare trunk was present on almost all the oil palm trees assessed (Table 3.2). Bryophytes and pteridophytes were also present on almost all trunks assessed. The only plantation that did not have other *Ficus* in addition to *F. deltoidea* on the trunks was in Batu Pahat.

The sites had different percentage trunk coverage by the groups of epiphytes (Figure 3.1, calculated by summing trunk coverage across trees). Bare trunks ranged from 3.2 % of the total trunk area (Banting) to 24.34 % (Dengkil). Bryophytes showed a high percentage coverage all sites ranging from 18.70% to maximum 33.24%. Pteridophytes also covered 30% or more of the trunks at sites other than Banting. Non-*Ficus* Angiosperms were less abundant, ranging from 5.42% to 25.83% coverage. *Ficus* other than *F. deltoidea* had the highest coverage at Banting and were absent from Batu Pahat, which had the highest coverage by *F. deltoidea* (Table 3.2. There was a significant difference in percentage coverage by *F. deltoidea* groups (Kruskal-Wallis, $\chi^2 = 97.35$, df = 4, $P < 0.001$) and *Ficus* group (Kruskal-Wallis, $\chi^2 = 324.04$, df = 4, $P < 0.001$) between sites.

Table 3.2. Frequency of bare trunks and epiphyte groups in five different plantations.

Frequency of trees	Study sites				
	Banting	Dengkil	Batu Pahat	Bagan Serai	Tembila
Bare trunk	284	427	101	145	133
Ficus deltoidea	88	128	66	32	53
Other *Ficus*	74	311	0	46	9
Bryophytes	283	427	101	145	133
Pteridophytes	280	426	101	145	134
Other Angiosperms	185	237	87	110	126
Total trunks	284	427	101	145	134

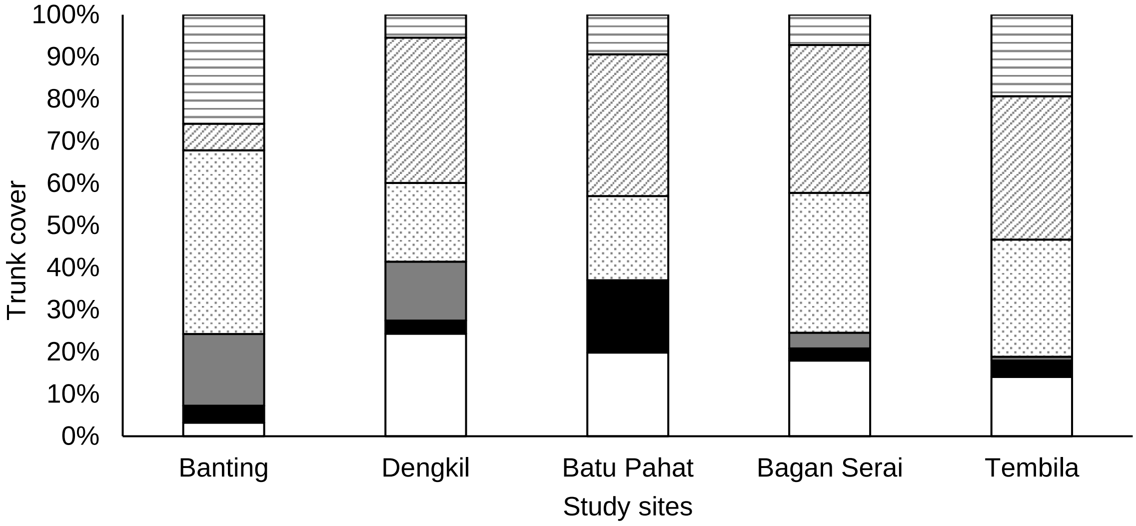


Figure 3.1 The cover of oil palm trunks by different epiphyte groups. Open bars = bare trunks, solid bars = *Ficus deltoidea*, grey bars = other *Ficus*, spotted bars = bryophytes, hashed bars = pteridophytes, horizontal lines = other Angiosperms.

3.4.3 Distribution of *Ficus* species

A total of 11 *Ficus* species were recorded as epiphytes or creepers in the plantations. Eight of the *Ficus* species were hemiepiphytes, one was a true epiphyte (three varieties) and one root climber (Table 3.3). During the study, four species produced figs and *F. deltoidea* was the only species that produced figs all the time (Table 3.3).

Dengkil had the largest number of *Ficus* species while Batu Pahat had the least, with only one species (two varieties) (Figure 3.2 - Figure 3.6). The most common *Ficus* species was *Ficus deltoidea* var. *angustifolia* except for at the Tembila site. At Johor a mean of more than one per host of this plant was recorded and some palms supported both of the *F. deltoidea* varieties. The maximum number of *F. deltoidea* present on one trunk were 9 trees where 5 of them were from var. *angustifolia* while the other 4 were from var. *deltoidea*. In Tembila, *F. deltoidea* var. *trengganuensis* was almost the only *Ficus* present. Here the number of var. *trengganuensis* per palm trunk ranged from 1-4.

F. microcarpa, F. obscura and *F. benjamina* were also quite common *Ficus* species at three study sites. The least overall proportion of *Ficus* present at all study sites was *F. virens*, only found in Tembila and the occurrence ratio was very low. There was a significant difference in the number *F. deltoidea* present per oil palm trunk at different sites (Kruskal-Wallis, $\chi^2 = 80.10$, df = 4, $P < 0.001$) with Batu Pahat having the highest mean Table 3.2) while the numbers of plants were rather similar at Banting, Dengkil and Bagan Serai.

Table 3.3. Characteristics of epiphytic *Ficus* species on the trunk of oil palms.

Ficus species	Based on Berg and Corner, 2005		Produced figs?
	Growth form	Maximum height (m)	
F. deltoidea var. *angustifolia* (Miq.) Corner	True epiphytes	7	Yes
F. deltoidea var. *deltoidea* Corner	True epiphytes	7	Yes
F. deltoidea var. *trengganuensis* Corner	True epiphytes	7	Yes
F. benjamina L.	Hemiepiphytes	35	No
F. caulocarpa (Miq). Miq.	Hemiepiphytes	20	No
F. microcarpa L.f.	Hemiepiphytes	30	No
F. obscura Blume	Hemiepiphytes	8	Yes
F. religiosa L.	Hemiepiphytes	25	No
F. sagittata J. Konig ex Vahl	Root-climber	Depends on host height	No
F. sinuata Thunb.	Hemiepiphytes	6	Yes
F. tinctoria G. Forst.	Hemiepiphytes	15	Yes
F. virens Aiton	Hemiepiphytes	35	No

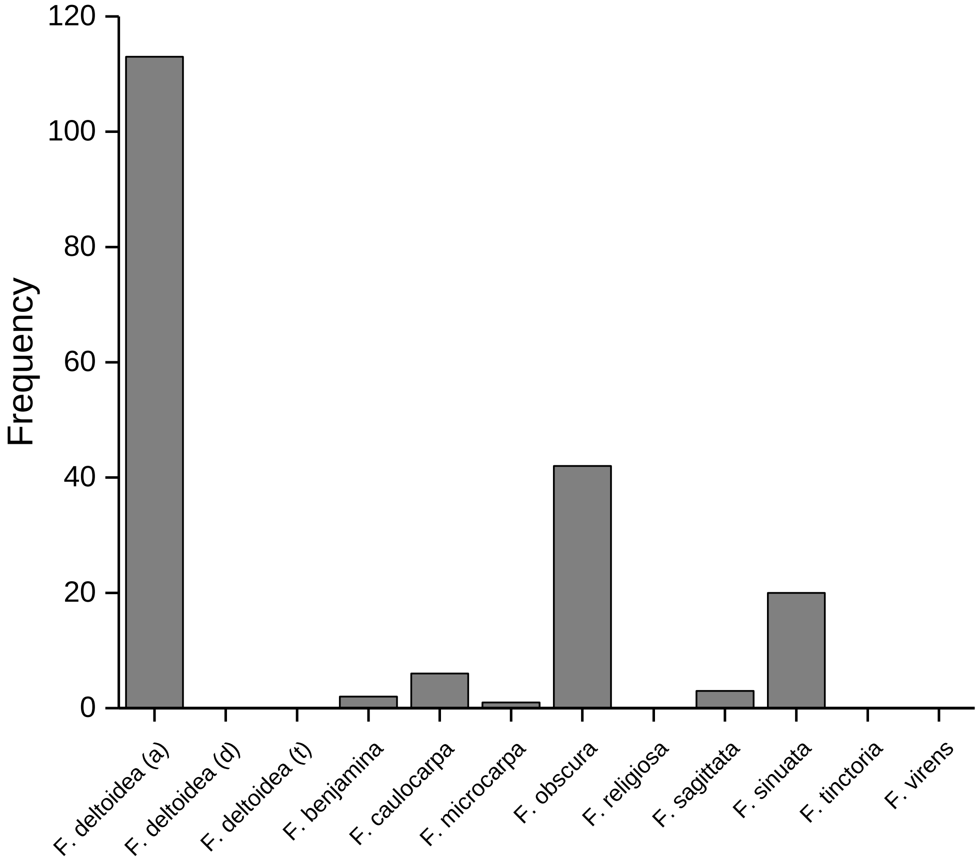


Figure 3.2 Frequencies of *Ficus* species in Banting plantation. *F. deltoidea* (*a*) = var. *angustifolia*, *F. deltoidea* (*d)* = var. *deltoidea*, *F. deltoidea* (*t*) = var. *trengganuensis*.

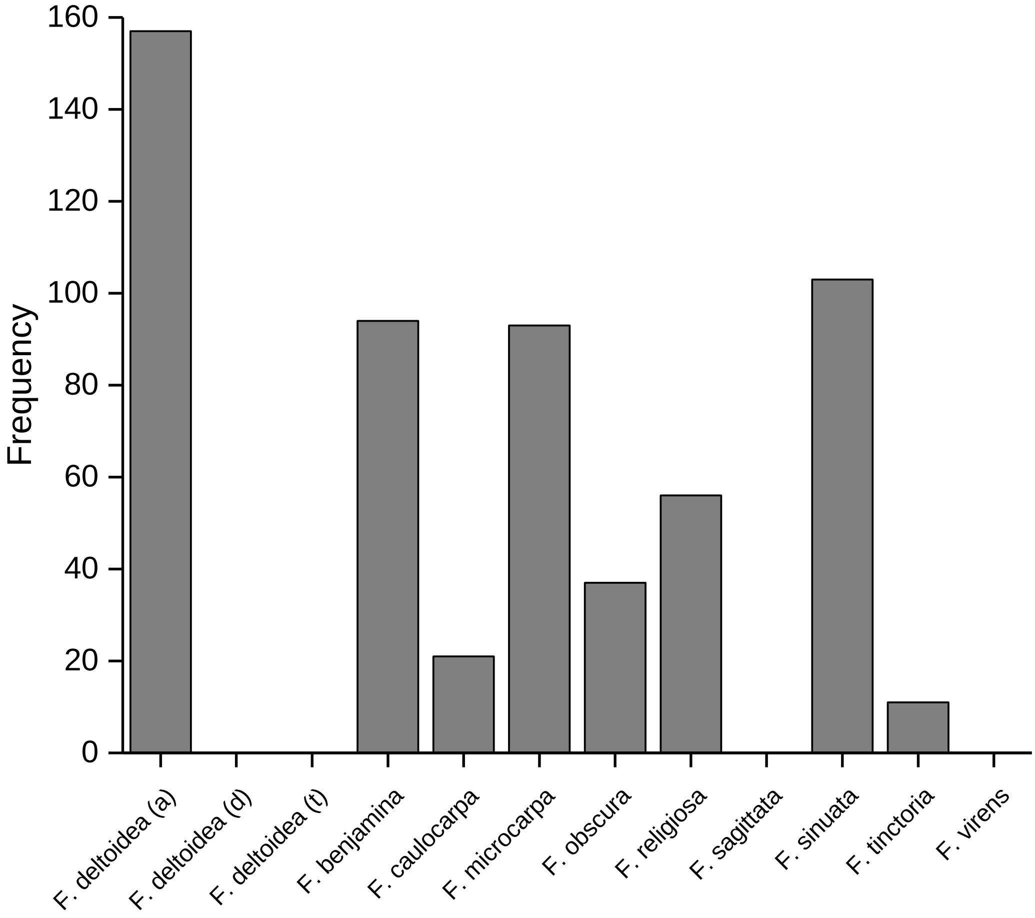


Figure 3.3 Frequencies of *Ficus* species in Dengkil plantation. *F. deltoidea* (*a*) = var. *angustifolia*, *F. deltoidea* (*d*) = var. *deltoidea*, *F. deltoidea* (*t*) = var. *trengganuensis*.

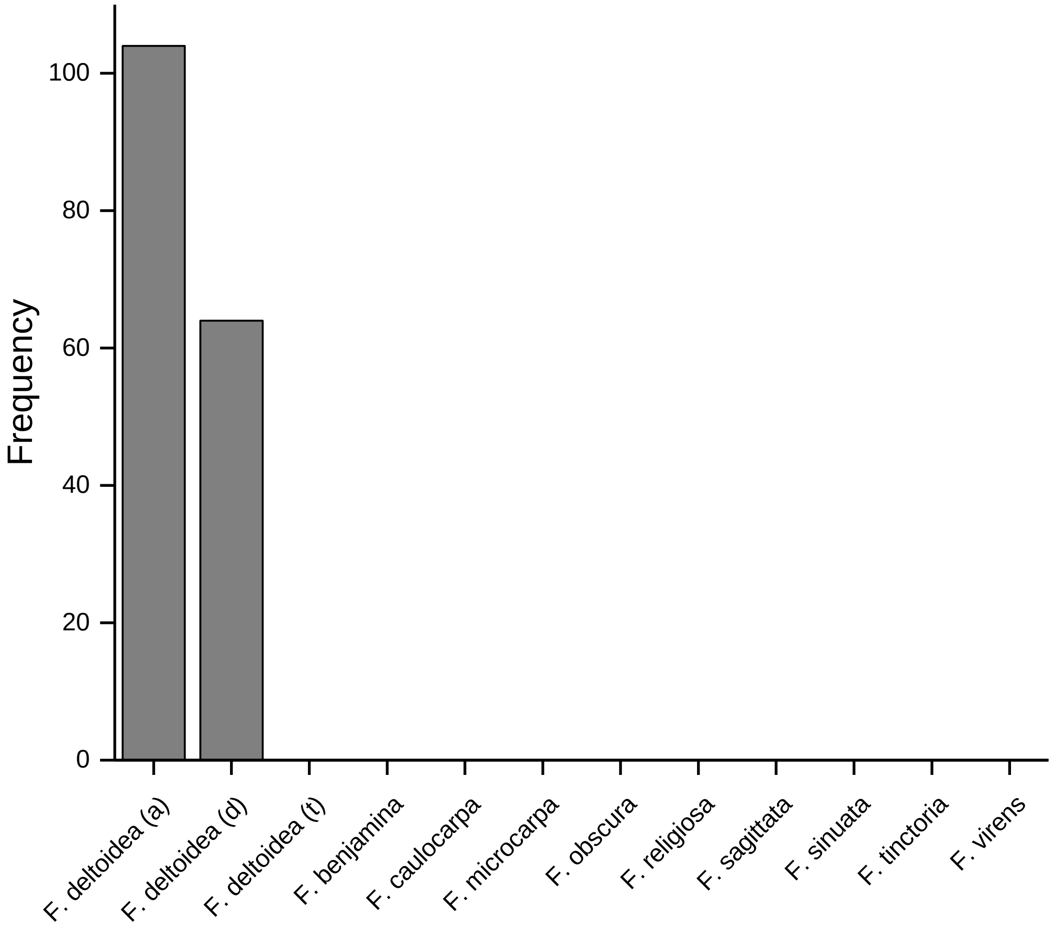


Figure 3.4 Frequencies of *Ficus* species in Batu Pahat plantation. *F. deltoidea* (*a*) = var. *angustifolia*, *F. deltoidea* (*d*) = var. *deltoidea*, *F. deltoidea* (*t*) = var. *trengganuensis*.

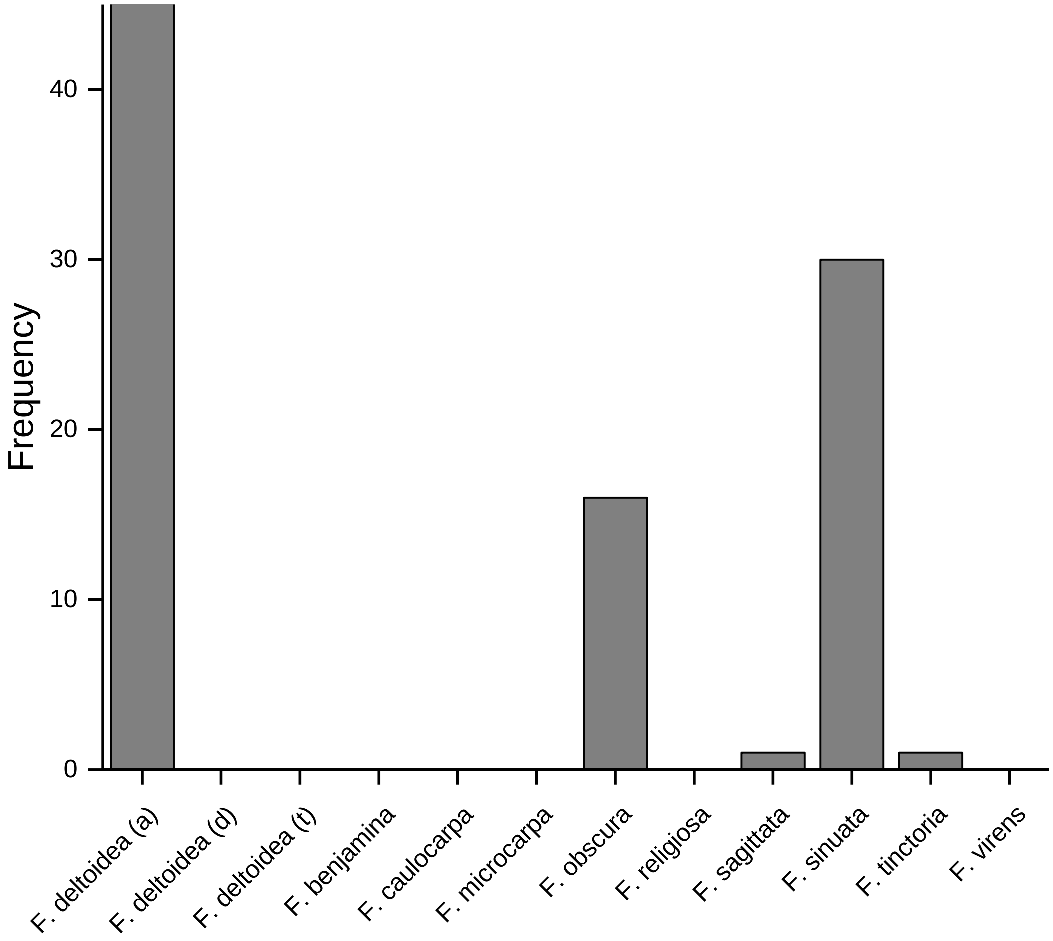


Figure 3.5 Frequencies of *Ficus* species in Bagan Seral plantation. *F. deltoidea* (*a*) = var. *angustifolia*, *F. deltoidea* (*d*) = var. *deltoidea*, *F. deltoidea* (*t*) = var. *trengganuensis*.

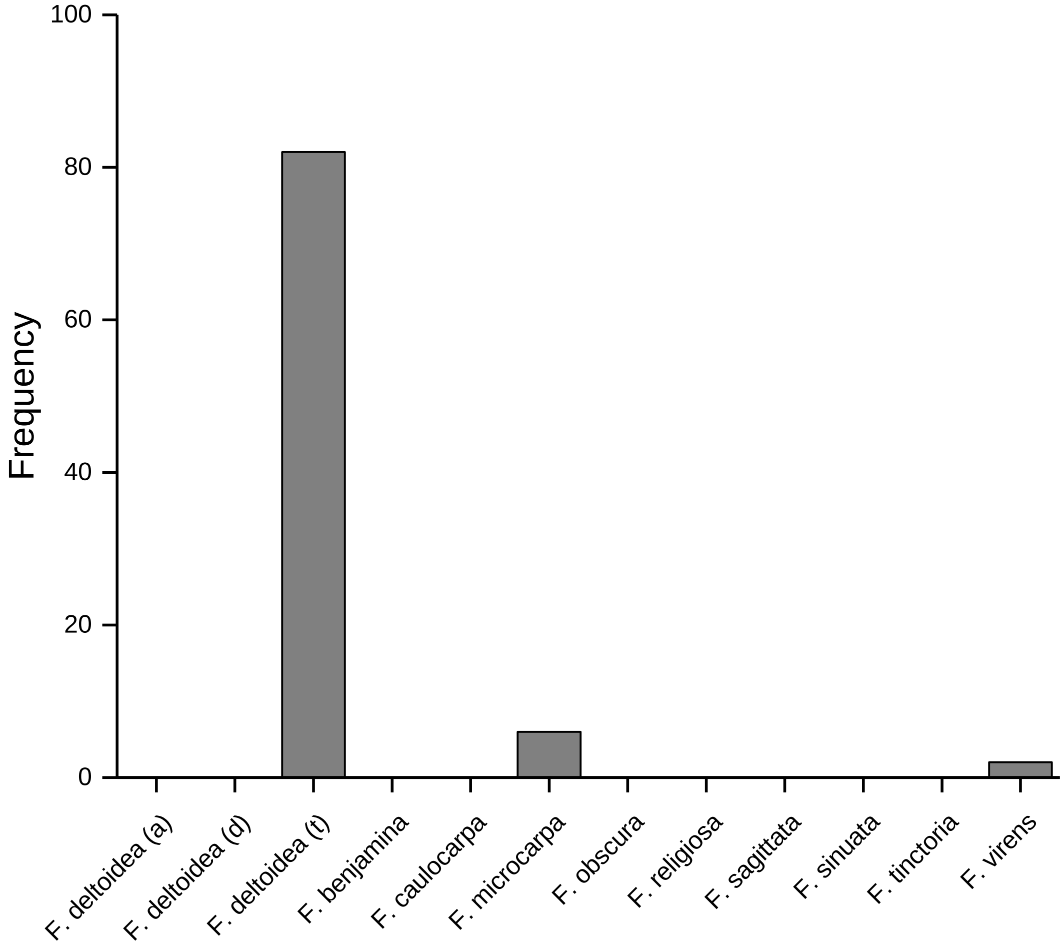


Figure 3.6 Frequencies of *Ficus* species in Tembila plantation. *F. deltoidea* (*a*) = var. *angustifolia*, *F. deltoidea* (*d*) = var. *deltoidea*, *F. deltoidea* (*t*) = var. *trengganuensis*.

3.4.4 Differences in establishment heights of *Ficus* on oil palms

In Banting, the trunks of oil palms ranged from 0.5 m to 8.8 m in height (5.68 ± 1.28 m) (Mean ± SD) (Figure 3.7). Most of the *Ficus* individuals of all species inhabited the middle parts of the trunks with the highest *F. deltoidea* var. *angustifolia*, found at 8.1 m above the ground. The small number of *Ficus caulocarpa* were usually towards the top of the trunks, with none lower than 4.2 m, but there was no significant difference in height occupied by the six *Ficus* taxa in this site (Kruskall-Wallis, $\chi^2 = 7.20$, df = 5, $P > 0.05$).

The oil palms at Dengkil ranged from 0.8 m to 9.1 m in height (6.71 ± 1.36 m) (Figure 3.8). As before most of the *Ficus* inhabited the middle parts of the trunks at around 5 m but there was a significant difference in establishment heights across the eight *Ficus* species (Kruskal-Wallis, $\chi^2 = 57.61$, df = 7, $P < 0.001$). Mann-Whitney tests revealed *that F. religiosa* was present significantly higher up the trunks than some of the other species, but not var. *angustifolia*.

Only two *Ficus* species were present at Batu Pahat (*F. deltoidea* var. *angustifolia* and *F. deltoidea* var. *deltoidea*). The overall trunk heights of the oil palm trees ranged from 0.5 to 9.5 m with a mean of 3.88 ± 1.95 m. The two *F. deltoidea* varieties had a similar range of occupancy from 0.1 to 9.3 m above the ground (Figure 3.9. There was no height preference between the two varieties of *F. deltoidea* (Mann-Whitney, Mdn = 3016.50, df = 1, $P > 0.05$).

The oil palm trunk heights at Bagan Serai ranged between 1.0 and 8.5 m (5.70 ± 1.36 m). Five *Ficus* species were found in this site including a creeper species (*F. sagittata*), with most located between 3 and 4 m above the ground (Figure 3.10). There was no significant difference in heights occupied by the four *Ficus* taxa in this site (ANOVA, $F = 1.82$, df = 3, $P > 0.05$).

At Tembila, the oil palm trunks were shorter than at the other study sites as they ranged from 1.7 m to 4.8 m (mean = 2.77 ± 0.65 m). *F. virens* occupied the lower part of the trunks while most of the *F. microcarpa* were higher (Figure 3.11). There was a significant difference in establishment heights across the three species of *Ficus* using (Kruskal-Wallis, $\chi^2 = 9.0$, df = 2, $P < 0.001$) and Mann-Whitney tests revealed three different groups of *Ficus* were present based on their establishment heights.

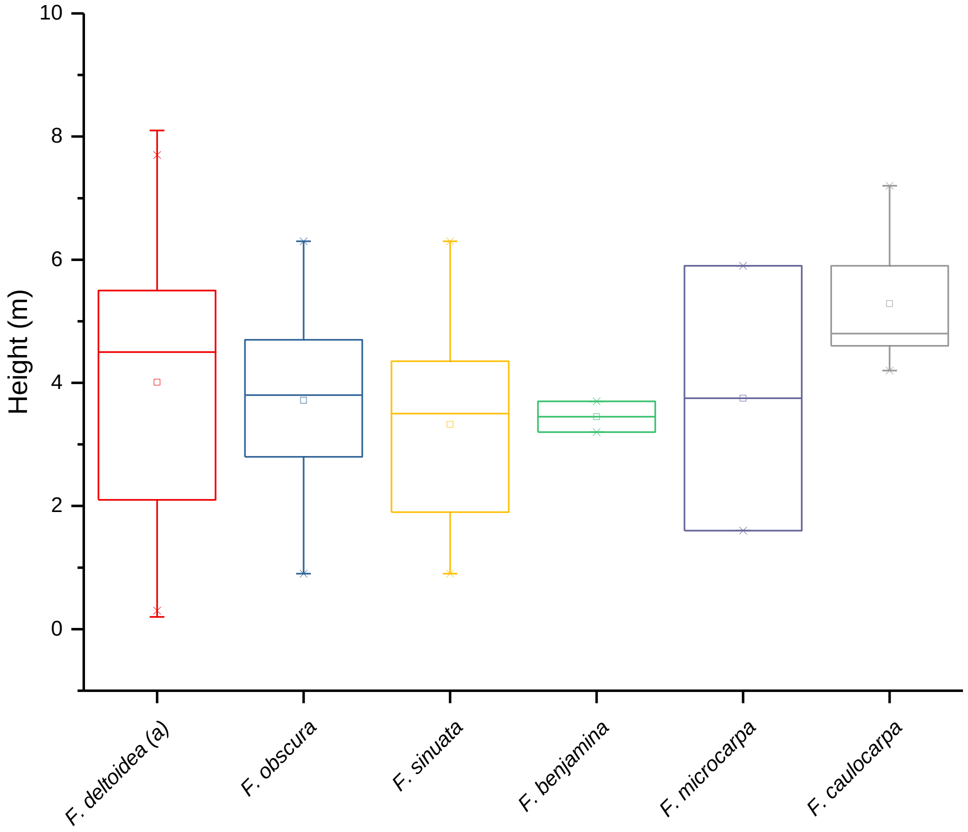


Figure 3.7 The heights of apparent establishment sites of *Ficus* species in Banting plantation. *F. deltoidea* (*a*) = *Ficus deltoidea* var. *angustifolia*. The lines in boxes indicate the median, the boxes indicate upper and lower quartiles with extreme values at the end.

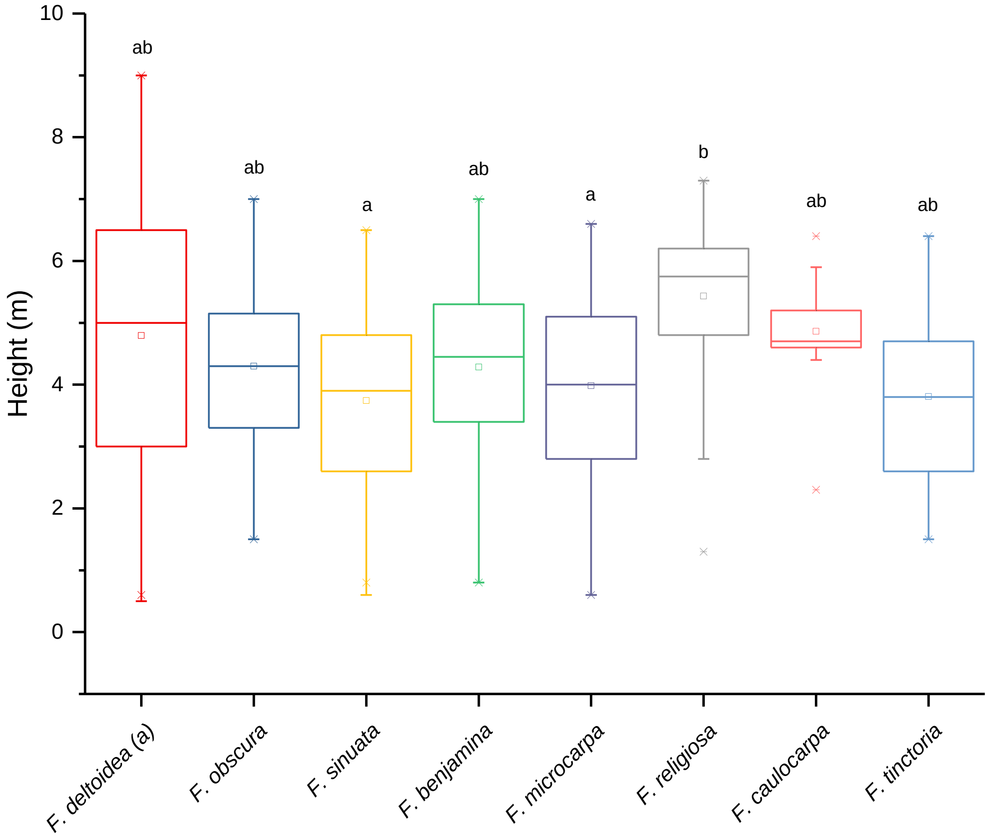


Figure 3.8 The heights of apparent establishment sites of *Ficus* species in Dengkil plantation. *F. deltoidea* (*a*) = *Ficus deltoidea* var. *angustifolia*. The lines in boxes indicate the median, the boxes indicate upper and lower quartiles with extreme values at the end. Values with different superscripts differed significantly in Mann-Whitney tests (P< 0.05).

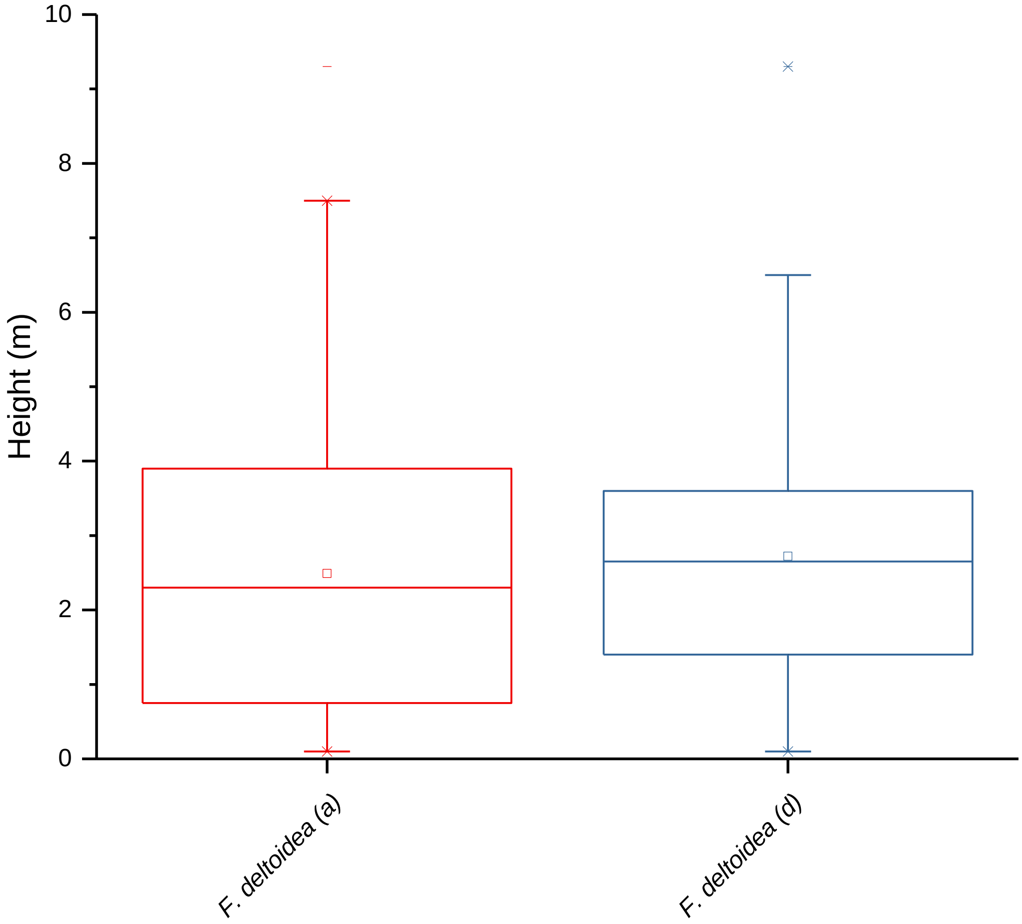


Figure 3.9 The heights of apparent establishment sites of *Ficus* species in Batu Pahat plantation. *F. deltoidea* (*a*) = *Ficus deltoidea* var. *angustifolia*. *F. deltoidea* (*d*) = *Ficus deltoidea* var. *deltoidea*. The lines in boxes indicate the median, the boxes indicate upper and lower quartiles with extreme values at the end.

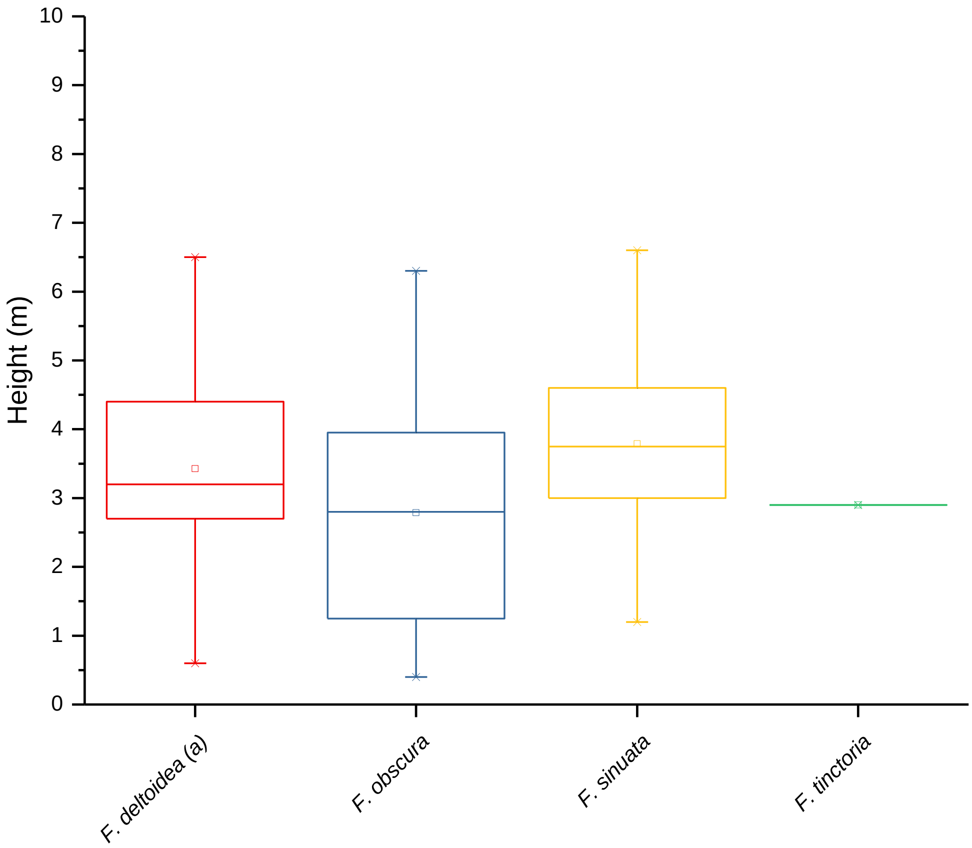


Figure 3.10 The heights of apparent establishment sites of *Ficus* species in Bagan Serai plantation. *F. deltoidea* (*a*) = *Ficus deltoidea* var. *angustifolia*. The lines in boxes indicate the median, the boxes indicate upper and lower quartiles with extreme values at the end.

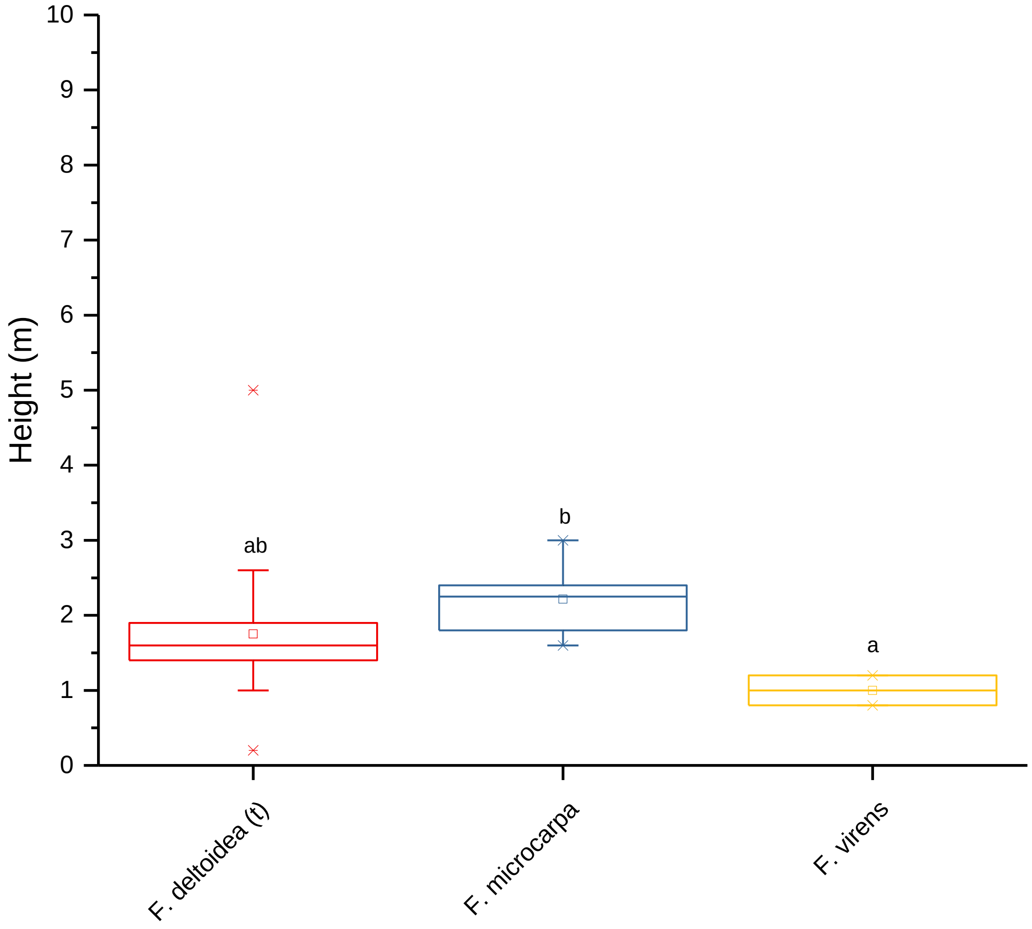


Figure 3.11 The heights of apparent establishment sites of *Ficus* species in Tembila plantation. *F. deltoidea* (*t*) = *Ficus deltoidea* var. *trengganuensis*. The lines in boxes indicate the median, the boxes indicate upper and lower quartiles with extreme values at the end. Values with different superscripts differed significantly in Mann-Whitney tests (P< 0.05).

3.4.5 *Ficus deltoidea* var. *angustifolia* and *Ficus deltoidea* var. *deltoidea*

The only site with two varieties of *F. deltoidea* present was Batu Pahat, where they were both abundant. A total of 101 oil palm trunks supported 104 var. *angustifolia* and 64 var. *deltoidea*. For var. *angustifolia*, 34 males and 11 females were identified while for var. *deltoidea*, 21 males and 14 females were identified. Comparing the sex ratios of the two varieties on 11th March 2017 using a Chi Square test, there was no significant difference in sex ratios between species ($\chi^2 = 2.22$, df = 1, $P > 0.05$). The sex ratios within var. *angustifolia* was biased towards males at this time of year (Chi square, $\chi^2 = 6.81$, df = 1, $P < 0.01$ but for var. *deltoidea* no significant was detected for the sex ratio using Chi square again ($\chi^2 = 0.92$, df = 1, $P > 0.05$).

The two varieties occupied similar heights on the trees, with most located between 2 and 3 m above the ground (Figure 3.12). There was no height preference between the two varieties of *F. deltoidea* (Mann-Whitney, Mdn = 3016.50, df = 1, $P > 0.05$). Male and females from these two varieties also did not prefer different heights (ANOVA in var. *angustifolia* F = 0.26, df = 1,43, $P > 0.05$ and var. *deltoidea* F = 0.11, df = 1,32, $P > 0.05$).

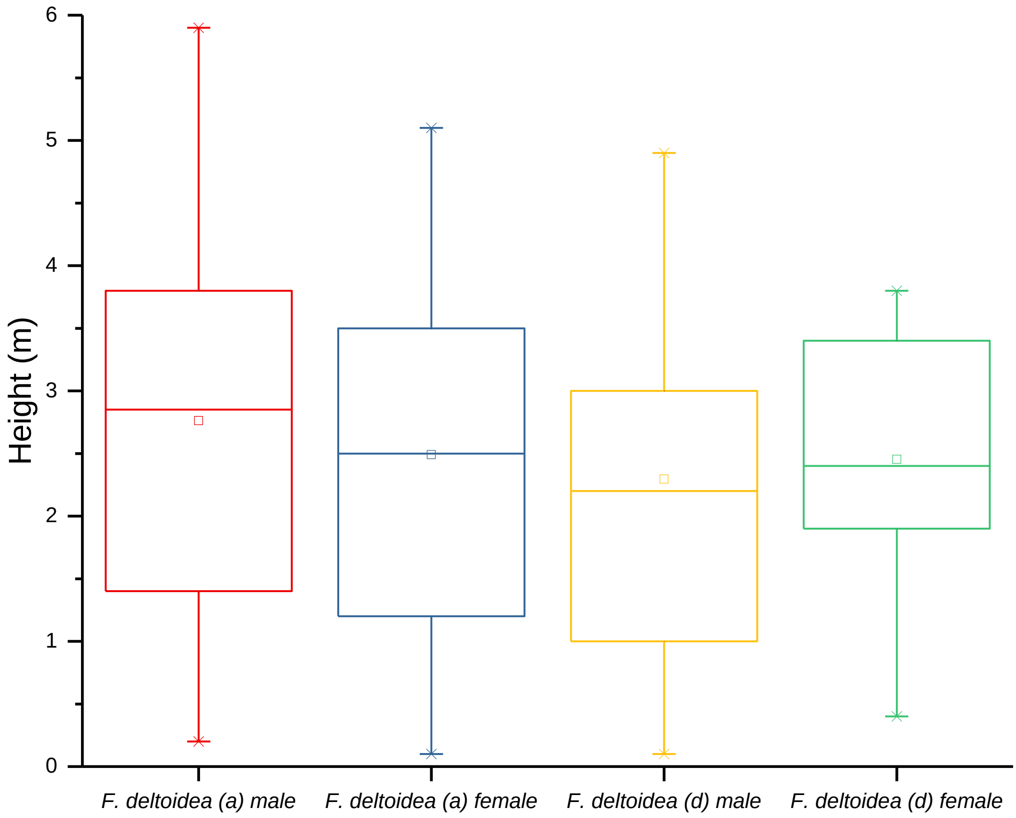


Figure 3.12 Differences in heights on oil palm trunks between sexes of two varieties of *F. deltoidea* in Batu Pahat plantation. *F. deltoidea* (*a*) = *Ficus deltoidea* var. *angustifolia*, *F. deltoidea* (*d*) = *Ficus deltoidea* var. *deltoidea*.

3.4.6 *Ficus deltoidea* var. *angustifolia* in Banting

The only variety of *F. deltoidea* present In Banting was var. *angustifolia*. From a total of 113 plants, 28 were male, 24 were female, 30 did not produce any figs and 31 were too high to be assessed for their sex. Male trees had establishment sites between 0.4 to 7.5 m (3.18 ± 1.87 m) above the ground, while in females the range was from 0.2m to 7.3 m (3.18 ± 2.18 m). The sexes did not differ in the heights they occupied (ANOVA, F = 0.10, df = 1, 50, $P > 0.05$).

3.4.7 Frugivore observations

The observations were made for a two-week period from 2nd July 2017 until 15th July 2017. Only one bird species was recorded, for a total of three times within the female *F. deltoidea* var. *deltoidea* and the same species was found on *F. deltoidea* var. *angustifolia* (five times). At least two individuals were present. The bird was identified by a local ornithologist (Dr Thanate Kerdkaew) as *Pycnonotus goiavier*, the yellow-vented bulbul (Figure 3.13). Both varieties showed synchronous fig phenology where all development phases were present on the tree during the observations. The camera traps also recorded a wild boar (*Sus scrofa*) but no other mammals. Feeding on the figs was not confirmed by the camera trap records.

Figure 3.13 ***Pycnonotus goiavier*** **on a female** ***Ficus deltoidea*** **var.** ***deltoidea*** **(the red arrow).**

3.5 Discussion

3.5.1 Epiphytes

Tropical environments with a high humidity favour a high diversity of epiphytes (Thomas and Packham, 2007). However, most organisms are present at low diversity and abundance in oil palm plantations when compared to natural forests and the expansion of oil palm plantations is a major reason for biodiversity loss in SE Asia (Foster et al., 2011). The presence of epiphytes in these plantations can help to partially mitigate this biodiversity loss without having any impact on productivity (Prescott et al., 2015). Bryophytes, pteridophytes and angiosperms are common epiphytes on trees in general (Muňoz et al., 2003; Mehltreter et al., 2005) and oil palm trunks specifically (Nadarajah and Nawawi, 1993; Prescott et al., 2015).

Our surveys of oil palm epiphytes cannot be seen as representative of plantations in general in Peninsular Malaysia as the plantations were selected only if they supported *F. deltoidea* as epiphytes. In all the plantations the percentages of bare trunk were low. Some of the bare trunks come from extensive shedding especially in very old oil palm trees but most comes from the trunk surface where no epiphytes were present.

The epiphyte communities on the oil palms differed between study sites and among tree within sites. Affield (2008) similarly found a high variability in species composition of epiphytes on *Metrosideros robusta* A. Cunn. (Myrtaceae) in different study sites and trees within sites and suggested this may be due to the irregularity of spore and seed colonisation and variable success in establishment on the host plants. He also postulated that the presence of vascular epiphytes depends on there also being non-vascular epiphytes on the trees, because a significant relationship between them was detected. Epiphytic mosses and lichens are said to aid vascular plant establishment as they provide a rough surface and moist environment for seed germination (Zotz, 1998).

In oil palm plantations, variable management practices are also likely to have influenced the composition of the epiphyte communities we recorded. Large companies often routinely clear epiphytes, but one of the five plantations included in this study is managed by a big company and had many epiphytes on the trees. Whether this was deliberate policy or an oversight by the company is unknown.

Pteridophytes were important components of the epiphyte floras at all the sites other than Banting. Apart from at Banting, ferns covered more than 30% of the trunk surfaces. Mosses were also frequent, as were a variety of angiosperms. These included several species of fig trees, a group that are particularly important for biodiversity because of the number of animals that eat their fruits (Shanahan et al., 2001) and leaves (Ødegaard, 2000).

3.5.2 *Ficus* species

Globally, hemiepiphytic *Ficus* (strangler figs) include more described species (about 500) than non-hemiepiphytes (about 300 species) (Harrison, 2005), but most stranglers are monoecious, and there are more dioecious than monoecious species in SE Asia. Among the ten species of *Ficus* identified as epiphytes in this study, eight were hemiepiphytes. These included some of the more common hemiepiphytic *Ficus* present in Malaysia (Sreekar et al., 2010; Hodgkison et al., 2013; Badron et al., 2014). There were also the two varieties of *F. deltoidea* and the creeper *F. sagittata*, which ascended the palm trunks after germinating on the ground.

Ficus species can have roots up to 100 m long, but the epiphytic *F. deltoidea* in the oil palm plantations rarely had roots longer than 1 m. Only certain varieties of this species are sometimes or always epiphytic and it also commonly grows on rocks, (Berg and Corner, 2005). It grows more often as an epiphyte in the lowlands, and can be particularly abundant in riparian situations (Corner, 1969). The abundance of strangler figs may reflect their high tolerance to drought and other adaptations for the conditions that epiphyte seedlings are subjected to (Holbrook and Putz 1996a, 1996b; Hao et al., 2010, 2013, 2016). Hemiepiphytic figs are often bigger than non-hemiepiphytes and produce very large numbers of figs (Lambert and Marshal, 1991). These are often dispersed by birds, which are likely to be the main seed dispersal agents in oil palm plantations, so the seeds of epiphytic *Ficus* may also be deposited on to palms in larger numbers than the seeds of fig trees with other growth forms (Peh and Fong, 2003; Hao et al., 2013).

Larger palm trees were expected to support more epiphytic *Ficus* as their larger size can accommodate more individuals (Wagner et al., 2015) and fig trees are more likely to flourish upon larger host trees (Male and Robert, 2009). Larger palms are also likely to be older and to have had more time to be colonised. According to Boelter et al. (2014), soils and host size control the structure of epiphyte communities. In this study, Banting, Bagan Serai and Batu Pahat are on peat soils, Dengkil is on a silt soil and Tembila is on a sandy soil. Peat soil has a fibre content, low permeability and low shear strength (Teong et al., 2016). The sample size is too limited to say whether soil may have influenced which *Ficus* species were on the palms, and the range of palm heights may have been too small to detect size effects, though the trees were significantly smaller at Tembila, where the tallest palm was only 4.8 m high and the sandy soil there has less ability in retaining water and nutrients (Weber et al., 2007).

Dengkil had the highest number of *Ficus* species growing on the oil palms (8 species) while the fewest species were recorded in Batu Pahat, with only one species. *F. deltoidea* var. *trengganuensis* has a very restricted range, and the plantation in Terengganu was the only one sampled within its range. The other species and varieties are more widely distributed and could potentially have been recorded at most or all of the sites.

The amount of light received during seed germination and the heights on the trunk that provide more water, nutrient and humus are important for the establishment of hemi-epiphytes (Putz and Holbrook, 1986; Daniels and Lawton, 1991). Most individuals of all the *Ficus* species occupied medium to higher parts of the trunks, which is consistent with studies by Wyse and Burns (2011) and Benzing (2004) who found most epiphytes inhabit the upper regions of host trees. Within sites, there were rarely differences between the heights occupied by different *Ficus* species.

3.5.3 *Ficus deltoidea* as an epiphyte

The massive transformation from forest to oil palm plantation in Malaysia has provided *F. deltoidea* with more suitable habitat and it has probably extended its range. Before the transformation to oil palms, the distributions of the varieties of *F. deltoidea* in Peninsular Malaysia were mainly on the limestone hills, mountains, mossy forests, rocks and the upper dunes on the sea-shore. These are areas where the quality of the soil is poor and well-drained. Some of the varieties were predominantly epiphytes (var. *deltoidea*, var. *kunstleri,* var. *trengganuensis*) (Corner, 1969). At least three different forms of *F. deltoidea* were recorded to colonise oil palm plantations in Peninsular Malaysia namely var. *angustifolia* (in Johor, Perak and Selangor), var. *deltoidea* (Johor) and var. *trengganuensis* (Terengganu).

In the plantations surveyed near Kuala Lumpur, *F. deltoidea* var. *angustifolia* either existed in large numbers or there were none at all. Some plantations are likely to have had all their *Ficus* epiphytes removed, but this also suggests that the seeds are dispersed within a short range. Long distance dispersal can increase genetic diversity and reduce population genetic structuring (Mondragon and Calvo-Irabien, 2006), so the extent of seed dispersal can be assessed in the future using genetic techniques.

The higher number of *F. deltoidea* (belonging to two varieties) at Batu Pahat may be because the plantation is very old and the palm trees barely produce fruit. Due to this fact, the owners may neglect cutting the epiphytes since they no longer need to collect the palm fruit. This plantation also acts as source of *F. deltoidea* leaves for commercial products like tea and also for scientific research so cutting the fig trees would reduce their stocks.

The slow growth of *F. deltoidea* and its production of figs sequentially and asynchronously (Chapter 6) may be linked to their occurrence in nutrient poor habitats (Berg and Corner, 2005). Traits developed in response to poor soils, including the unusually large seeds of this species (Chapter 4) may have represented a pre-adaptation that allowed them to survive as epiphytes. Species with larger seeds have the ability to survive poorer quality environments like deep shade, drought, physical damage and competition with other vegetation (Westoby et al., 1996). Larger seeded species also tend to have a lower specific leaf area (SLA) (leaf area per leaf dry mass), which causes their slow growth. At least under glasshouse growing conditions, the seedlings of *F. deltoidea* var. *angustifolia* have stems (hypocotyls) of up to five centimetres before the cotyledons, which should increase their chance of reaching sunlight through competing vegetation (unpublished observations). This is made possible by the greater reserves they have available to support respiration. The unusually large seeds of *F. deltoidea* are surrounded by a mucilaginous exocarp and seeds kept in warm damp conditions are resistant to mould growth, unlike the other parts of the figs (S.G. Compton, personal communication). Mucilage has also been suggested in other *Ficus* species to inhibit germination until gut-passage by birds, and to act as an ant-attractant (Kauffman et al., 1991).

Only Batu Pahat supported two varieties of *F. deltoidea* within the same plantation. Some of the trunks supported both varieties and one trunk had a total of 9 individuals of both varieties. Living on the same trunk triggers competition for light, humidity and space (Sales et al., 2016), and although there was no significant difference between the varieties in terms of abundance and height preferences, most of the *F. deltoidea* sharing a trunk did not produce figs and were often smaller in size than elsewhere. Lack of nutrients may be inhibiting fig production.

3.5.4 Frugivores

Figs are eaten by many vertebrates, especially birds (Shanahan and Compton, 2001; Mackay et al., 2018). The most commonly recorded birds eating figs in SE Asia are bulbuls (Pycnonotidae). The small size of the figs of *F. deltoidea* suggests that their seeds are likely to be dispersed mainly by birds, but Orang Utans were the only published feeders on these figs in Malaysia (Shanahan et al., 2001). At Banting, two individuals of *Pycnonotus goiavier* (Yellow-vented bulbul) were recorded by the camera traps placed opposite a female *F. deltoidea* tree crown. However, as there was no film of the bulbuls eating the figs, it cannot be confirmed that this species eats the figs and is a likely seed dispersers for this species. A study by Rajpar and Zakaria (2010) found that this was the most abundant bird species in Paya Indah Wetland (Peninsular Malaysia). This is also a common species across southern Thailand and Malaysia (T. Kerdkaew personal communication). Many of the fig tree species recorded as epiphytes of the oil palms grow into large trees and only fruit once they have reached a large size (Berg and Corner, 2005). Plantation managers are unlikely to allow them to ever reach so big, so they are not likely to ever be allowed to provide figs that could be used by birds.

3.6 Conclusions

The diversity and abundance of fig trees and other epiphytes on the oil palm trunks was high. The plantations were not necessarily typical but show that given suitable management epiphytes can improve biodiversity in this form of monocultural agriculture. However many of the fig tree species recorded have to be large before reaching maturity and they would not normally be allowed to reach this size because they would be seen as damaging their host trees. *F. deltoidea* is the exception, and its abundant fruiting suggests it is particularly important for biodiversity in oil palm plantations.

Chapter 4

Characterisation of *Ficus deltoidea* varieties

4.1 Introduction

4.1.1 *Ficus*

Ficus (Moraceae) is among the most studied plant genera due to its unique closed inflorescence, variable breeding systems, different growth forms and unique relationship with its pollinators. With more than 800 described species (Weiblen, 2002), fig trees represent more than 75% of all the species in the family Moraceae (Zhang et al., 2018). This genus was also among the earliest domesticated crops and today is widely planted (Lansky et al., 2008), with the edible fig (*Ficus carica* L.) and ornamental species cultivated worldwide.

The genus *Ficus* as a whole is characterised by having an urn-shaped inflorescence commonly known as the fig or syconium (Chen et al., 2009). Entry to the flowers requires passage through a typically bract-lined ostiole (Thorogood et al., 2018). Male and female flowers or only female flowers are present in each fig. Fig trees have two breeding systems, dioecy and monoecy (Dunn et al., 2008). Roughly half of all fig species are monoecious, with individual inflorescences providing both female (seed production and dispersal) and male (pollen production and dispersal) functions (Herre et al., 2008). The remaining *Ficus* species are functionally dioecious.

In these species, there are two types of trees; female trees that produce only seed-bearing fruits; and male trees with figs that produce only pollen and support development of pollinator wasp progeny to transport the pollen (Patel and Hossaert-McKey, 2000). In a dioecious fig tree, the female fig wasps that enter the female figs pollinate flowers but they produce only seeds (Ghana et al., 2015a). The long styles of flowers in the female figs and the structure of the stigmas prevent the wasps from galling the flowers and laying their eggs and so allow only for seed production (Kjellberg et al., 2014). On male trees, the female flowers are modified for receiving a pollinator egg and only fig wasp larvae develop inside. Even pollinated flowers with no eggs deposited do not produce seeds. Approximately one to several months after oviposition, which is usually in flowers that have also been pollinated, the male fig wasp offspring emerge from their galls and mate with the still gall-enclosed females (Liu et al., 2013). As in monoecious figs, all the female flowers inside a fig become receptive simultaneously several weeks before the male flowers mature (protogyny) (Patel, 1996). The galled ovules of the short styled flowers in male figs are each consumed by a single pollinator larva which completes its development there.

The outer wall (receptacle) of a fig varies in colour depending on the species and developmental stage (Lansky et al., 2008). The flowers inside figs are tiny and simplified, with no petals, but sometimes with tepals, which can be prominent in some *Ficus* species. Tepals are elements of the perianth that cannot be distinguished as either sepals or petals (Warner et al., 2009). Among plants in general, sepals typically have a protective function, while petals are involved in pollinator attraction (Bedinger et al., 2017). Tepals can be involved with one or both of these functions. In one study of tepal function in *Chamissoa altissima* (Jacq.) Kunth (Amaranthaceae), the tepals were found to have protective and photosynthetic functions before they obtain a red colour due to betalain pigments and developed a later function in seed dispersal (de Oliveira et al., 2010). Another study on early-flowering common snowdrop (*Galanthus nivalis* L.) recorded tepal function as attracting the pollinators and facilitating the photosynthetic process (Aschan and Pfanz, 2006). The tepals of this species contains chlorophyll that provides photo-assimilates to the flower and the developing seeds. Other functions for tepals have also been described, including pollen capture (Cornille et al., 2012) and possibly as footholds for insects when pollinating the flowers in the genus *Oxygonum* (Polygonaceae) (Hong et al., 1998).

4.1.2 *Ficus* pollination

Fig trees are pollinated exclusively by fig wasps (Agaonidae). The pollination mutualism between fig trees and fig wasps is one of the classic examples of obligate mutualism (Thorogood et al., 2018). The unique enclosed uniovulate flowers inside the figs act as the venue for the mutualism with the figs wasps (Cook and Rasplus, 2003). The fig trees depend on fig wasps to carry their pollen and produce seed, whereas the fig wasps need the *Ficus* inflorescences (figs) to provide food for developing larvae and complete their life cycles (Herre et al., 2008). Pollen produced by a fig is transported by the pollinators that develop in the fig. The morphology of fig wasps is well adapted to their biological cycle starting from entering the figs, oviposition, pollination, mating and finally departure from their natal figs. The wings and the antennae also routinely detach easily when she enters the figs (Kjellberg et al., 2005b). Most of all, pollinating fig wasps are very uniform in that they have similar life cycles (Wiebes, 1982).

The pollen-bearing adult female fig wasp is called a foundress when she reaches a suitable fig and they are responsible for the pollination in the fig trees as well as the production of their progeny. The foundress detects volatiles emitted by receptive figs of their particular host species and locates them using their antennae (Hossaert-McKey et al., 2016). Once inside they pollinate the flowers after entry through a narrow often bract-lined ostiole (Nefdt and Compton, 1996) and at the same time she lays eggs in some of the flowers if she has not entered a female fig. The ostiole is the only route to the inner surface of the figs and the narrow slit-like condition of the ostiole only allows the specific pollinator to get through it (Liu et al., 2013). In many species the foundresses rarely or never emerge from the first figs they enter, but in other species several figs can be entered by a single foundress. After the eggs are laid, and after larval feeding, the fig wasp’s offspring emerge, mate within the figs and the female offspring will carry the pollen out from their natal figs and disperse.

The wingless male wasps hatch first from their galls and search for galls containing females. The males bite holes into the female galls and insert their genitalia in order to mate (Cook and Segar, 2010). Female wasps then emerge into the fig cavity and at this time the male fig flowers are mature and have mature pollen. After the females collect some pollen either actively or passively, the male fig wasps bore a hole to let the pollen-bearing female fig wasps get out from the natal fig. The females then search for other receptive figs to deposit their eggs and pollinate the flowers (Nefdt and Compton, 1996). It used to be thought that the wingless male fig wasps always will spend their entire life in the figs (Compton and McLaren, 1989). However, there are a few cases where males go outside their natal figs and even enter other figs looking for mates (Greeff, 2002). They can even help protect the females from ant predation on the surface of the figs (Zachariades et al., 2010).

In some pollinator species, the female fig wasps actively collect pollen into thoracic pollen pockets and later deposit it deliberately onto flower styles (Jousselin et al., 2003). In others, pollination is passive via transport on the wasp's body, as in most other insects (Waser and Ollerton, 2006). This occurs when the foundress gets coated by dehiscing pollen shed by the anthers on their way out of her natal fig (Kjellberg et al., 2014). In fig wasps that display passive pollination, the pollen grains are trapped in the abdominal pleura and are released as a wasp's gaster swells in the water-saturated fig cavity atmosphere (Galil and Neeman, 1977). Fig trees with passive pollinators have mature anthers that burst open naturally and have figs with high ratios of anthers to female flowers and so produce more pollen per fig to facilitate passive collection by their pollinators (Kjellberg et al., 2001). About two thirds of pollinating fig wasps display active pollination and have offspring that mostly develop in fertilized flowers (Kjellberg et al., 2001). This behaviour is assumed to have evolved because fertilized flowers provide better nutrients for fig wasp larvae (Parrish et al., 2003) and there is experimental evidence that this is the case (Tarachai et al., 2008).

4.1.3 Species concepts in *Ficus*

In 1686, the term species was proposed by John Ray in his book Historia Plantarum saying that a species is a group of individuals that can interbreed together despite having 'accidental' variations between them. A broader concept was proposed by Carolus Linnaeus in his book Species Plantarum (1753) who classified all the known living organisms by looking at their morphology. He also proposed a binomial system and renamed plants and animals in the two word latinized system. In plants, he differentiated between them based on floral morphology and sexual characters. The most widely accepted species concept was later proposed by Ernst Mayr in 1942. He described species as "groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups". This concept is used as the basic definition of species today.

In recent classifications, defining a species also requires taking into account variation within and between the species (Aldhebiani, 2018). In classification systems of plants, the kingdom is divided further by classes, orders, families, genera and species. They can be further described by using variety, cultivar, hybrid and other notations. Varieties indicate variation in inheritable differences within species. The difference between sub-species and varieties is not clear, but varieties tend to be used more frequently when dealing with cultivated plants and do not necessarily reflect natural variation within species.

Ficus is considered as a ‘difficult’ genus by many tropical botanists. This results from similarities in ecology and the sharing of common traits, together with often highly variable leaf morphologies (Harrison, 2005). In determining the boundaries of *Ficus* species, plant anatomy is used as the main reference and current species names are based mainly on anatomical features of leaves, inflorescences and flowers. Luckily, the highly specific interaction with particular species of their obligate pollinators, together with the current development of molecular techniques, has enhanced the modern classification of *Ficus* and provided additional ways of recognising boundaries between species.

The most widely used current classification of *Ficus* is based on Berg and Corner (2005). It includes a contribution by the late ECJ Corner, but the species boundaries were largely determined by the late C.C. Berg, who generally opted to assume that two morphologically distinct entities belonged to the same species, if intermediates were also present between them. Biological features, including the identity of pollinators (the behaviour of which largely determines gene flow in *Ficus*) were not taken into account. Consequently, sympatric biologically distinct taxa (with gene flow not taking place between them) are not always recognised as being distinct species using the current morphological species concept.

Specific volatile compounds are released from the figs to attract the fig wasps associated with each species of fig tree (Hossaert-McKey et al., 2016). The volatiles that attract fig wasps largely determine where fig pollen is deposited. Most fig wasps show a high degree of host-plant specificity and are known from only a single fig tree species. However, in some cases wasps may be shared across closely related fig species (Cook and Segar, 2010). A single fig tree species has up to eight or more pollinator species, but most probably have one or a small number of associated pollinators.

The external morphology of fig wasps reflects variation in fig structure, particularly features of the ostiole and the length of the styles. Female pollinators need special adaptations especially on the head to pass through the ostiole as they enter the fig

(Liu et al., 2013). They have a head that has sunken membranous region that allows it to be deformed when entering the ostiole while protecting parts of the antennae that do not detach (Boucek et al., 1981). The ostiole length reflects head shape as there is a positive correlation between head shape and fig size (van Noort and Compton, 1996).

The style length of the flowers also influences the morphology of the fig wasps. *Ficus* species with longer styles of female flowers in monoecious figs are associated with longer ovipositor lengths of their particular pollinators, though monoecious fig pollinators in general have longer ovipositors than those associated with dioecious figs (Nefdt and Compton, 1996; Compton et al., 2010). Successful entrance into the fig cavity and subsequent oviposition does not guarantee that a female can gall the flowers where her eggs are laid. The galling process is poorly understood (Elias et al., 2018), but galled ovules and their pedicels enlarge rapidly. This is believed to be in response to chemicals released by the ovipositing females and may reflect competition for space inside the figs (Ghana et al., 2015a). The larvae may continue to produce gall-inducing chemicals during their development.

For a viable seed to be generated, pollen compatibility is important. For example in *Flourensia cernua* (Asteraceae), when non-compatible male pollen and female stigmas expressed the cognate S-allele, it will produce none or very low numbers of seed due to inhibition at the S-locus (Ferrer et al., 2009). The S-allele is self sterility gene where the function of this gene is to prevent self fertilisation (Ferrer et al., 2009). Non-compatible Pollen incompatibility seems to be poorly developed in fig trees, perhaps because heterospecific pollen is rarely encountered due to the specificity of the plants' vectors. However, when pollen of other species makes its way into figs, hybridisation and introgression can occur.

In the fig and fig wasp cases, the reproductive success of any hybrids depends on the ability of the foundress offspring to develop in their host plants and whether the hybrids can produce viable seeds (Ghana et al., 2015b). In experimental crosses, hybrids between two closely related but not sympatric species *Ficus montana* Blume and *Ficus asperifolia* Miquel were generated by the routine pollinators of F. *montana* (*Kradibia tentacularis* (Grandi). F1 male plants were sterile as the ovules failed to enlarge and the embryos failed to develop. Conversely, F1 females produced a lot of viable seeds. So it is possible for hybrid male figs to be unsuitable for reproduction of a particular species of fig wasp, but hybrid female figs can have high reproductive success. In this species pair, at last, introgression between the *Ficus* species is therefore more likely to occur through entry into female than male plants.

4.1.4 *Ficus deltoidea*

Ficus deltoidea Jack. sometimes known as the mistletoe fig because of one of its growth forms, is one of the more commonly found fig trees in South East Asia. Its distribution includes Thailand, Indonesia and Malaysia (Cardellina, 2012). They are locally known as “Mas Cotek” by Malaysians because of having gold (in Malay gold means emas) coloured fine spots on the surface of the leaves (Mat et al., 2012). In Thailand some varieties are referred to as ‘golden tongue’, perhaps because of the orange-brown undersides to the leaves. Traditionally, *F. deltoidea* is widely used to cure a variety of diseases such as rheumatism, diabetes, toothache, headaches, colds and sore throats using the powdered roots and leaves (Bunawan et al., 2014). More recently it has been shown that *F. deltoidea* has anti-hyperglycaemic (Adam et al., 2012), anti-diabetic, anti-oxidant (Misbah et al., 2013) and wound-healing properties (Abdulla et al., 2010). Adam et al. (2007) stated that all parts of *F. deltoidea* have potential medicinal properties

F. deltoidea is dioecious, with separate male and female plants that either support fig wasp pollinators or produce seeds, respectively. Female figs of this group are exceptional insofar as they only contain small numbers of flowers. This allows individual seeds to be far larger than is normal for fig trees and is a presumed adaptation for an epiphytic lifestyle, as it means that young seedlings have more resources available initially. The small size of the figs suggests that their seeds are dispersed mainly by birds, but Orang Utans are the only published feeders on the figs (Shanahan et al., 2001). The *F. deltoidea* complex includes varieties that are commonly grown as ornamentals, usually as pot plants. They can thrive in dry atmospheres because of their thick cuticle, and small number of stomata both of which reduce water loss (Hao et al., 2016). *F. deltoidea* can be propagated from seed but many of them are propagated through cuttings and almost all the plants offered for sale by nurseries are female (Starr et al., 2003). Little is known about the *Blastophaga* species that pollinate *F. deltoidea* figs, but there are indications that different varieties may support different species of pollinators (Chapter 5).

4.1.5 *F. deltoidea* classification

Corner (1969) asserted that the *F. deltoidea* complex displayed most clearly the interplay of speciation and geographical distribution within the genus. *F. deltoidea* is currently placed in Subgenus *Ficus*, Section *Ficus*, subsection *Frutescentiae* (Berg and Corner, 2005). The subsection was further divided into two species groups containing about 25 species (Shi et al., 2006). The small *F. deltoidea* species group is equivalent to series *Erythrogyneae* of Corner (1960) and contains three species (*F. deltoidea*, *F. oleifolia* and *F. kofmaniae*), distributed in SE Asia. These species are true, short-rooted epiphytes (as opposed to hemi-epiphytic stranglers) or epilithic and when growing on the ground are usually found on nutrient poor soils (Berg and Corner, 2005). A characteristic feature is the small number of flowers in their female figs with sometimes as few as a single unusually large seed being produced per fig (Corner, 1969). In the first two species the presence of modified 'furcate' leaves on mature plants is another feature. Leaf morphology and anatomy are often used for identification of *F. deltoidea* varieties because they are particularly discriminative (Nur Fatihah et al., 2014), but leaf shape often varies greatly between young and mature plants. Trees in section *Ficus* and series *Erythrogyneae* also have ostiolar male flowers and reduced root systems (Corner, 1969).

F. deltoidea is highly variable in anatomy and this led Corner (1960) to recognise 13 varieties under *F. deltoidea*, namely var. *angustifolia*, var. arenaria, var. *bilobata*, var. *borneensis*, var. *deltoidea*, var. *intermedia* var. *kinabaluensis*, var. *kunstleri*, var. *lutescens*, var. *motleyana*, var. *oligoneura*, var. *peltata*, and var. *trengganuensis*. Given the presence of intermediate individuals between varieties and further variation within varieties, Berg (in (Kjellberg et al., 2005b) simplified the intraspecific classification of *F. deltoidea*. Two subspecies were recognised based mainly on leaf differences, namely *F. deltoidea deltoidea* and *F. deltoidea motleyana*. Most of the varieties described by Corner were placed under *F. deltoidea deltoidea* except for var. *motleyana* and var. *olioneura* that were placed under subspecies *F. deltoidea motleyana*. However, most studies of *F. deltoidea* have continued to use the more fine-grained classification of Corner (1960, 1969) and this classification is retained within this thesis.

4.1.6 *F. deltoidea* varieties

In the early classification of (Corner, 1969) he considered that the most primitive or basal variety was var. *motleyana*. This variety is predominantly in Borneo and resembles *Ficus oleaefolia* that is also common in Borneo (and Sumatra). He also noted that the most widespread variety was var. *angustifolia* and that this variety showed the most reduction in leaf, fig and seed size. Falling next in terms of widespread distributions is var. *deltoidea* and this variety was suggested as a possible ancestor of var. *angustifolia* and var. *lutescens*. In Peninsular Malaysia, seven varieties are endemic namely var. *angustifolia*, var. *bilobata*, var. *deltoidea*, var. *intermedia*, var. *kunstleri*, var. *motleyana*, and var. *trengganuensis* while the other Malaysian varieties can be found in Sabah and Sarawak. The varieties are usually easily distinguished by looking at their leaf structure (Figure 4.1). The leaf forms of male and female plants are not distinguishable. The only way to know the gender of the trees is by opening the figs and looking at their inner structure.

All varieties show a presence of fine spots with a golden colour on the surface of the leaves and the vernacular name of Mas cotek is given in Peninsular Malaysia because of it. Morphological studies of *F. deltoidea* show that all characters portray a high variability among the varieties (Awang et al., 2013). This variation between and within species may be due to cross-pollination, sexual recombination and mutations (Corner, 1997). All the fig species in the *F. deltoidea* species group exhibit red tepals, numerous ostiolar male flowers, a reduced root system and large seeds (Corner, 1969). Among 40 morphological characters used in differentiating the varieties in *F. deltoidea*, the three most significant characters were found to be the shape and size of the leaves, the number and colour of the dots on the leaf's surface and fig structure (Awang et al., 2013). Some of the varieties have long spathulate leaves when saplings and these include var. *deltoidea*, var. *angustifolia*, var. *intermedia* and var. *motleyana* (Corner, 1969). In addition, a recent study by Mat et al., (2012), found different leaf forms between juvenile and mature individuals of var. *bilobata* and var. *trengganuensis*.

A tepal is present which encloses the ovary and their colour and structure varies between varieties and sexes. In female *F. deltoidea* the figs are particularly simple, because they contain unusually few pistillate female flowers. There were also sterile male flowers in the female figs. In the male figs, there are staminate flowers to produce the pollen and pistillate flowers to support development of fig wasp offspring (Kjellberg et al., 2014). Male figs of *F. deltoidea* are more typical of figs in general, and contain many more flowers than the female figs. In male figs, the developing galls in *F. deltoidea* is unique externally, being rugose and angular, unlike other *Ficus* with rounded galls (F. Kjellberg pers. Communication).

Figure 4.1 Differences in leaf form between seven endemic varieties of *Ficus deltoidea* in Peninsular Malaysia. From top left, var. *trengganuensis*, var. *kunstleri*, var. *intermedia*, var. *angustifolia*, var. *deltoidea*, var. *bilobata* and var. *motleyana*.

4.2 Objectives

The group of fig trees comprising *F. deltoidea* and its relatives is one of the most interesting within the whole genus, displaying apparently recent evolution, diversification and novel growth forms in and around the Sunda Shelf. The taxonomic and biological status of these varieties is unclear, as is the extent to which different varieties support different fig wasp pollinators. Until now, most published studies discussed the differences in the leaf forms and the chemical and pharmaceutical properties of the leaves, but no study has been conducted on the differences in fig anatomy between varieties and their relationship with their associated *Blastophaga* fig wasps.

Whether the varieties are actually distinct biological species is unclear. *F. deltoidea* often exhibit different states of leaf characters between the young and mature plants that has often led to misleading identifications of the varieties. Whether the figs of varieties are different is unknown. Leaf forms do not influence gene flow but fig morphology does (S. G. Compton pers. Communication).

This study aims aimed to provide biological evidence relating to whether some of the Malaysian varieties of *F. deltoidea* are likely to be distinct species. Specific questions were (1) are there morphological differences (in the fig and the fig wasp (size of figs, floral numbers, ovipositor length, femur length) between different populations of *F. deltoidea* var. *angustifolia* in Peninsular Malaysia? (2) Are there morphological differences between the figs and the fig wasps of this and other varieties of *F. deltoidea*? (3) are any differences in morphology in the fig wasps related to variation in their host figs?

4.3 Methods

4.3.1 Study varieties

Morphological comparisons were made between the figs produced by seven varieties of *F. deltoidea*. The varieties were var. *angustifolia* (Miq.), var. *deltoidea* Corner, var. *trengganuensis* Corner, var. *kunstleri* King, var. *bilobata* Corner, var. *motleyana* (Miq.) and var. *intermedia* Corner. All these varieties are native to Peninsular Malaysia, but natural populations of only three varieties were sampled.

4.3.2 Study sites and sampling methods

The samples of var. *angustifolia*, var. *deltoidea* and var. *trengganuensis* were obtained from natural wild fig trees growing as epiphytes in oil palm plantations in Peninsular Malaysia. The samples of var. *kunstleri*, var. *bilobata*, var. *motleyana* and var. *intermedia* were obtained from potted trees housed in the Terengganu Germplasm Collection (TGC) in Terengganu and planted trees housed at Batu Pahat, Johor. Details of these locations including related climate data were provided in Chapter 2. All the samples were collected to see the differences in the fig's characterisation of all varieties of *F. deltoidei*.

Fig development stage classification follows Galil and Eisikowitch (1968). B-phase is the receptive (female) phase when the figs are ready to be entered by pollinator fig wasps. D-phase is when the next generation of wasps are mature and ready to emerge from the galls (E phase). At each study site, B-phase, D or E phase and aborting figs were selected at random and collected into plastic bags. Since fruiting of all the trees was asynchronous, the figs at different phases were collected from each trees at the same time. The male fig collections were taken at early D phase where no fig wasps including the male had emerged from their galls. In female figs the equivalent early E phase was sampled. Five trees of each sex were sampled at each site.

For *F. deltoidea* var. *angustifolia*, four different oil palm plantations provided the samples. The plantations were located in Banting and Dengkil (Selangor state), Batu Pahat (Johor state) and Bagan Serai (Perak state). 5 B-phase male and female figs and 5 early D-phase male/early E-phase female figs were collected from each tree. The B-phase and D/E-phase figs were collected from the same five trees. A total of 400 figs from 20 male and 20 female trees were brought to the Lab. to be dissected (Table 1.1). The same sample sizes were obtained at single sites for populations of var. *deltoidea* (at Batu Pahat in Johor,) and var. *trengganuensis* (at Tembila, Terengganu), providing totals of 100 figs for each variety (Table 4.1). The fig trees of the remaining varieties were growing in pots and did not show any signs of pollination. Approximate B phase and the largest figs present were sampled. The maximum diameters of their figs were measured. Sample sizes varied according to availability (Table 4.2). Preliminary studies where figs were opened in the field allowed the likely developmental stage of figs to be assessed visually. Any sampled figs that were found to be at other stages of development when opened were rejected. Figs at the appropriate stages on each tree were sampled at random.

Table 4.1 The collections of male and female figs of var. *angustifolia*, var. *deltoidea*, and var. *trengganuensis*, at each study site.

Variety	Date	Location	Male			Female		
			Trees	B phase figs	D phase figs	Trees	B phase figs	D phase figs
angustifolia	15.04.2017	Bagan Serai	5	25	25	5	25	25
angustifolia	03.11.2016	Batu Pahat	5	25	25	5	25	25
angustifolia	20.07.2016	Banting	5	25	25	5	25	25
angustifolia	20.07.2016	Dengkil	5	25	25	5	25	25
deltoidea	10.11.2016	Batu Pahat	5	25	25	5	25	25
trengganuensis	28.10.2016	Tembila	5	25	25	5	25	25

Table 4.2 The collections of male and female figs of six varieties of *F. deltoidea*. (TGC = Terengganu Germplasm Collection).

Variety	Date	Location	Male			Female		
			Trees	B phase figs	D phase figs	Trees	B phase figs	D phase figs
bilobata	28.10.2016	TGC	5	25	25	1	5	5
kunstleri	10.11.2016	Batu Pahat	2	10	10	5	25	25
kunstleri	28.10.2016	TGC	2	10	10	-	-	-
intermedia	28.10.2016	TGC	1	5	5	-	-	-
motleyana	28.10.2016	TGC	-	-	-	5	25	25

4.3.3 Fig characterisations

The diameters of the figs were measured using Vernier callipers, and they were then dissected to record their contents. The diameters were measured at a right angle to the ostiole-pedicel axis. Later, pi is used to calculate the surface area. With B-phase (receptive) female figs, the parameters counted and measured were the number of female flowers, the style lengths of the female flowers, the number of tepals, the lengths of the tepals and the diameter. The same features were recorded in B-phase male figs, together with the number of male flowers present. Each male flower has two anthers.

Tepal and style lengths were measured using an image analyser. The style length measurements followed those described by Nefdt and Compton (1996) where the style length was taken as the distance from the top of the stigma to where the style reached the edge of the ovary. Ghana et al. (2017) noted that maximum style lengths in both male and female figs are reached at about the time the figs are receptive (B phase). In female figs all the available styles at or near B phase were measured. The maximum lengths of five randomly chosen tepals were measured in each fig. The diameters of pollinated figs were measured at late D- or early E- phases as before.

No pollinated figs of some varieties were available. The diameters of these varieties were measured in the same way using the biggest figs on the trees. D- phase male figs of var. *angustifolia*, var. *deltoidea* and var. *trengganuensis* were examined to record pollinator offspring numbers. The following were recorded: the number of galls that contained adult pollinator offspring, the number of empty (failed) galls, maximum gall length and the number of unused flowers. No E phase male figs (where an exit hole was present) were examined. Each gall was squashed gently between two microscope slides to allow the sex of the wasp inside to be recorded (if any were present). No non-pollinator fig wasps were present in the figs of any of the varieties. E phase female figs for the pollinated varieties were examined and the seed lengths and number of seeds produced were counted.

4.3.4 Fig wasp characterisations

The pollinators of three varieties of *F. deltoidea* were examined to see any differences between them (var. *angustifolia*, var. *deltoidea* and var. *trengganuensis*). Ovipositor lengths were measured using pollinators from var. *angustifolia* figs collected at Bagan

Serai, Banting, Batu Pahat ande Dengkil. Batu Pahat also provided the figs for var. *deltoidea* and Tembila provided the figs for var. *trengganuensis*. Measurements were made using 10 females from each of 5 D phase male figs collected from five different trees of each variety (n = 250 females from each variety). The figs from the same tree were placed together in a container covered by fine mesh to allow the pollinators to emerge naturally. Individuals were selected at random from those that emerged from the figs. Ovipositors were measured by placing the females in a drop of water on a glass slide under a cover slip and squashing with moderate pressure to displace the ovipositor sheaths without breaking the ovipositor. The measurements were conducted using image-analysis software, as before.

Hind femur length was used as an indicator of overall body size because it is the longest leg segment, readily distinguishable and easy to measure. Measurements were made as before. From each of the four var. *angustifolia* study sites, groups of about 5-10 D phase figs from five different trees at each site were collected and placed in containers as before. Femurs of 30 individuals from each site were measured (six from each tree). Similar measurements were made using 30 randomly sub-sampled female fig wasps reared from figs of var. *deltoidea* and var. *trengganuensis*. The wasps were obtained from five trees of each variety.

4.3.5 Statistical methods

For the variation between four populations of var. *angustifolia* and variation across all varieties of *F. deltoidea*, ANOVA followed by Tukey tests were conducted to examine differences in means between populations and between trees within populations if the data were countinuos and had a normal distribution. Count data was analysed using GLM with Poisson error. For non-normal distributions, Kruskal-Wallis tests were conducted followed by Mann-Whitney tests. If there was over-dispersion in Poisson distributions for count data, the data were analysed using quasi-Poisson errors. Correlation tests were conducted to examine relationships between pairs of variables within the same variety only in var. *angustifolia* because four study site provided the samples. Linear regression were used together with ANOVA to examine relationships across and within variables where site is used as a random factor. GLMs, ANOVAs correlation and regression were all performed in R studio (1.0.153) and SPSS Statistic 20.

4.4 Results

4.4.1 Variation among populations of var. *angustifolia*

4.4.1.1 Variation in female figs of var. *angustifolia*

Receptive (B phase) female figs were small, with a diameter of about 4 mm (Table 4.3). There was no significance difference in diameter between sites (Kruskal-Wallis, $\chi^2 = 5.06$, df = 3, $P > 0.05$) and trees (Kruskal-Wallis, $\chi^2 = 29.51$, df = 19, $P > 0.05$). After pollination, at E phase they reached maximum diameters of 7 or 8 mm (Table 4.3). A significant difference between sites was found (ANOVA, F = 10.50, df = 3, 96, $P < 0.01$) and between trees (ANOVA, F = 5.01, df = 19, 80, $P < 0.01$).

From 3 to 6 female flowers were present in the female figs of var. *angustifolia* (Table 4.4). There were significant differences in flower numbers between sites (Kruskal-Wallis, $\chi^2 = 11.35$, df = 3, $P < 0.05$) and trees (Kruskal-Wallis, $\chi^2 = 30.95$, df = 19, $P < 0.05$). Mann-Whitney tests showed that only the Banting site differed from other sites. Usually four tepals were present in each female flower (Table 4.4), but their numbers ranged from three to five. Kruskal-Wallis followed by Mann-Whitney Test tests found a significant difference in tepal numbers between the Banting and other sites (Kruskal-Wallis, $\chi^2 = 22.01$, df = 3, $P < 0.01$), reflecting the smaller number of flowers in the figs at this site.There were also significant differences in tepal numbers between trees (Kruskal-Wallis, $\chi^2 = 48.06$, df = 19, $P < 0.01$).

Table 4.3. The diameters of receptive phase and maximum diameters of female var. *angustifolia* figs from four sites in Peninsular Malaysia. Values with different superscripts in a column differed significantly in Tukey tests ($P< 0.01$).

Sites	n (trees)	n (figs)	Receptive diameter (mm)		Maximum diameter (mm)	
			Mean ± SD	Range	Mean ± SD	Range
Bagan Serai	5	25	4.2 ± 0.02	3.9-4.5	7.1 ± 0.03^{a}	6.7-7.8
Banting	5	25	4.3 ± 0.03	3.9-4.7	7.4 ± 0.04^{b}	6.7-8.3
Batu Pahat	5	25	4.3 ± 0.02	4.0-4.7	7.2 ± 0.04^{a}	6.7-8.1
Dengkil	5	25	4.3 ± 0.03	3.9-4.7	7.1 ± 0.04^{a}	6.7-7.8

Table 4.4. The numbers of female flowers and tepals in female figs of var. *angustifolia* from four sites. Values with different superscripts in a column differed significantly in Mann-Whitney tests ($P< 0.05$).

Sites	Female flower number		Tepal Number		No. of tepals per flower	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Bagan Serai	4.96 ± 0.61^{b}	4-6	20.36 ± 2.74^{b}	16-27	4.11 ± 0.30^{b}	3.60-4.67
Banting	4.28 ± 0.79^{a}	3-6	16.48 ± 3.18^{a}	12-24	3.87 ± 0.43^{a}	3.25-4.80
Batu Pahat	4.48 ± 1.00^{b}	4-6	19.24 ± 1.90^{b}	15-24	4.03 ± 0.33^{b}	3.33-4.75
Dengkil	4.80 ± 0.76^{b}	4-6	18.96 ± 3.12^{b}	12-24	3.95 ± 0.14^{b}	3.75-4.40

Between one and six seeds were present in the pollinated female figs (Table 4.5). The highest mean number of seeds was in figs from Batu Pahat. Kruskal-Wallis tests detected a significant difference in the numbers of seeds in figs between sites (χ^2 = 14.53, df = 3, $P < 0.05$) and between trees (Kruskal-Wallis, χ^2 = 37.99, df = 19, $P < 0.05$). The shortest and longest seeds were from figs in Dengkil measuring 3.54 mm and 4.43 mm respectively (Table 4.5). Seed lengths varied significantly between sites (ANOVA, F = 8.12, df = 3,196, $P < 0.01$) and specifically between Bagan Serai and the three other sites. Seed lengths also varied significantly between trees (ANOVA, F = 3.68, df = 19,180, $P < 0.01$).

Unused flowers represent non-pollinated, undeveloped flowers within figs that contained seeds and so had been entered by pollinators. More unused flowers were present in female figs from Bagan Serai than elsewhere (Kruskal-Wallis, χ^2 = 21.23, df = 3, $P < 0.01$) (Table 4.6). Trees also differed significantly in the numbers of unused flowers present in their figs (Kruskal-Wallis, χ^2 = 42.94, df = 19, $P < 0.05$). These differences were reflected in differences in pollination efficiency (the proportion of female flowers that developed into seeds) with figs from Bagan Serai having a higher proportion of unused flowers than elsewhere (Table 4.6).

Table 4.5. The numbers of seeds and their maximum lengths in female figs of var. *angustifolia* from four sites. Values with different superscripts in a column differed significantly (Seed number Mann-Whitney tests, Seed length Tukey tests P< 0.05).

Sites	n trees (figs)	Seed number		n trees (seeds)	Seed length (mm)	
		Mean ± SD	Range		Mean ± SD	Range
Bagan Serai	5 (25)	3.44 ± 1.29^{a}	1-6	5 (50)	3.90 ± 0.16^{a}	3.72-4.35
Banting	5 (25)	3.56 ± 0.89^{a}	1-5	5 (50)	4.03 ± 0.18^{b}	3.67-4.42
Batu Pahat	5 (25)	4.48 ± 1.00^{b}	1-6	5 (50)	4.09 ± 0.24^{b}	3.59-4.21
Dengkil	5 (25)	4.08 ± 1.22^{ab}	1-6	5 (50)	4.04 ± 0.23^{b}	3.54-4.43

Table 4.6.The unused female flowers in female figs of var. *angustifolia* from four sites. Values with different superscripts in a column differed significantly (Mann-Whitney tests, P< 0.05).

Sites	n (trees)	n (figs)	Unused female flowers		Proportion of unused flowers	
			Mean ± SD	Range	Mean ± SD	Range
Bagan Serai	5	25	1.52 ± 1.19^{a}	0-4	0.31 ± 0.24^{a}	0 - 0.80
Banting	5	25	0.56 ± 0.96^{b}	0-4	0.11 ± 0.19^{b}	0 - 0.80
Batu Pahat	5	25	0.32 ± 0.90^{b}	0-4	0.06 ± 0.18^{b}	0 - 0.80
Dengkil	5	25	0.72 ± 0.98^{b}	0-3	0.15 ± 0.22^{b}	0 - 0.75

4.4.1.2 Between-population variation in male figs of var. *angustifolia*

Receptive (B phase) male figs were small with a diameter of about four mm (Table 4.7). There were no significant differences in diameter between sites (Kruskal-Wallis, $\chi^2 = 6.10$, df = 3, $P > 0.05$) and trees (Kruskal-Wallis, $\chi^2 = 19.14$, df = 19, $P > 0.05$). There were also no significant differences in maximum diameters at early E phase between sites (ANOVA, $F = 2.62$, df = 3, 96, $P > 0.05$) and between trees (ANOVA, $F = 1.24$, df = 19, 80, $P > 0.05$).

Male flowers only exist in male figs. They ranged between 21 to 37 per fig (Table 4.8). There were no significant differences in male flower numbers between sites (ANOVA, $F = 1.72$, df = 3, 96, $P > 0.05$), but differences between trees (ANOVA, $F = 5.69$, df = 19, 80, $P < 0.05$) (Table 4.8). There were more female than male flowers in the male figs. Figs from Dengkil had the most variation in female flower numbers, from 94 to 259 in different figs. There were significant differences between sites (ANOVA, $F = 8.18$, df = 3, 96, $P < 0.01$) and trees (ANOVA $F = 12.56$, df = 19, 80, $P < 0.01$) (Table 4.8).

Reflecting the large variation in female flower numbers in male figs at Dengkil, there was also large variation in the numbers of tepals present in the figs (Table 4.9). ANOVA test showed there were significant differences in tepal numbers between sites ($F = 8.87$, df = 3, 96, $P < 0.01$) and between trees (ANOVA, $F = 6.92$, df = 19, 80, $P < 0.01$).

Table 4.7 The diameter of receptive male var. *angustifolia* figs and their maximum diameters of male figs at four sites.

Sites	n (trees)	n (figs)	Receptive diameter (mm)		Maximum diameter (mm)	
			Mean ± SD	Range	Mean ± SD	Range
Bagan Serai	5	25	0.41 ± 0.03	0.37-0.45	0.71 ± 0.03	0.67-0.78
Banting	5	25	0.42 ± 0.03	0.38-0.47	0.74 ± 0.04	0.67-0.83
Batu Pahat	5	25	0.43 ± 0.02	0.38-0.44	0.72 ± 0.04	0.67-0.81
Dengkil	5	25	0.41 ± 0.03	0.36-0.44	0.71 ± 0.04	0.67-0.78

Table 4.8 The numbers of male and female flowers in male figs of var. *angustifolia* from four different sites. Female flower numbers with different superscripts differed significantly between sites (Tukey tests, P < 0.01).

Sites	n (trees)	n (figs)	Male flower numbers		Female flower numbers	
			Mean ± SD	Range	Mean ± SD	Range
Bagan Serai	5	25	29.88 ± 3.54	21-34	173.96 ±16.44^{c}	150-209
Banting	5	25	29.64 ± 3.49	23-35	142.76 ± 12.21^{a}	121-163
Batu Pahat	5	25	31.20 ± 3.60	25-41	156.08 ± 16.61^{b}	130-185
Dengkil	5	25	28.64 ± 5.17	21-37	176.08 ± 48.44^{c}	94-259

Table 4.9 The number of tepals in male figs of var. *angustifolia* from four sites. Tepal numbers with different superscripts differed significantly between sites (Tukey tests, P< 0.01).

Sites	n (trees)	n (figs)	Tepal numbers	
			Mean (± SD)	Range
Bagan Serai	5	25	645.24 ± 100.93^{b}	557-830
Banting	5	25	499.92 ± 70.29^{a}	336-590
Batu Pahat	5	25	538.28 ± 69.64^{a}	436-708
Dengkil	5	25	648.04 ± 209.64^{b}	288-1052

4.4.1.3 Variation in *Blastophaga* sp. pollinators reared from var. *angustifolia*

Ovule gall length is related to the size of the pollinators that emerge from the galls. Gall lengths ranged from 0.83-1.19 mm (Table 4.10). The longest mean length was recorded from Dengkil (1.03 mm) and the shortest from Batu Pahat (0.92 mm). There were significant differences in gall sizes between sites (ANOVA, $F = 19.02$, $df = 3, 196$, $P < 0.01$) and trees (ANOVA, $F = 8.57$, $df = 19, 180$, $P < 0.05$, Table 4.10). Despite this variation in gall sizes, the lengths of the hind femurs of reared female fig wasp offspring (a measure of body size) showed very little variation. They did not differ significantly in length between sites (ANOVA, $F = 1.25$, $df = 3,116$, $P > 0.05$) and trees (ANOVA, $F = 1.03$, $df = 19, 100$, $P > 0.05$).

The contents of five figs from five different trees at each site were recorded (Table 4.11). Total female flower numbers varied greatly, ranging between 94 and 259. Empty galls may have had eggs laid by the pollinator but if so they had failed to develop successfully. Unused flowers had not developed and showed no sign that they had been galled. Around one third to one half of these flowers were galled by the pollinators (Table 4.11). Galls that failed to support successful pollinator development were much less common, with usually two or fewer present per fig, but large numbers of failed galls were present in the 25 figs at Banting. There were significant differences between sites in the numbers of successful galls (ANOVA, $F = 3.21$, $df = 3, 96$, $P < 0.05$) and also between trees (ANOVA, $F = 2.45$, $df = 19, 80$, $P < 0.05$, Table 4.11). The number of empty galls did differ between sites (Kruskal-Wallis, $\chi^2 = 15.86$, $df = 3$, $P < 0.05$). Mann-Whitney tests between sites showed that only empty gall numbers from Banting differed from other sites (Table 4.11). Trees from Banting were the only ones to differ significantly in their numbers of empty galls (Kruskal-Wallis, $\chi^2 = 56.79$, $df = 19$, $P < 0.01$). The numbers of unused flowers did not differ between sites (Kruskal-Wallis, $\chi^2 = 21.25$, $df = 3$, $P < 0.01$) but differed between trees (Kruskal-Wallis, $\chi^2= 42.94$, $df = 19$, $P < 0.05$).

Flower utilisation in the male figs was very similar at the four sites (Figure 4.2). About 5% of the female flowers were empty galls at Banting, more than at the other sites. This difference comes because of a single tree which had figs with high numbers of empty galls.

Table 4.10 The gall lengths in male figs of var. *angustifolia* and pollinator hind femur lengths from four sites. Values for gall length with different superscripts differed significantly (Tukey tests, P< 0.01).

Sites	n figs (n galls)	Gall length (mm)		n fig wasps	Femur length (mm)	
		Mean (± SD)	Range		Mean (± SD)	Range
Bagan Serai	10 (50)	0.94 ± 0.10^{a}	0.77-1.13	30	0.20 ± 0.01	0.18-0.21
Banting	10 (50)	0.99 ± 0.06^{b}	0.89-1.12	30	0.20 ± 0.01	0.18-0.21
Batu Pahat	10 (50)	0.92 ± 0.07^{a}	0.76-1.08	30	0.20 ± 0.01	0.18-0.21
Dengkil	10 (50)	1.03 ± 0.10^{b}	0.83-1.19	30	0.20 ± 0.01	0.18-0.21

Table 4.11 The female flower numbers and utilisation of female flowers in male figs of var. *angustifolia* at four sites. Values with different superscripts in a column differed significantly (Tukey and Mann-Whitney tests, P < 0.05).

Sites	Total female flowers		Total Successful Galls		Empty Galls		Unused flowers	
	Mean (± SD)	Range	Mean (± SD)	Range	Mean (± SD)	Range	Mean (± SD)	Range
Bagan Serai	173.96 ± 16.44^{c}	150 - 209	62.32 ± 23.11^{ab}	24 -120	0.12 ± 0.44^{a}	0 - 2	111.52 ± 22.06^{a}	53-146
Banting	142.76 ± 12.21^{a}	121-163	46.76 ± 26.99^{a}	29 -129	7.6 ± 14.91^{b}	0 - 46	88.4 ± 24.8^{b}	34-122
Batu Pahat	156.08 ± 16.61^{b}	130 - 185	58.76 ± 25.29^{ab}	26 -123	0.24 ± 0.6^{a}	0 - 2	97.08 ± 22.32^{b}	66-138
Dengkil	176.08 ± 48.44^{c}	94 - 259	71.92 ± 38.24^{b}	19 -151	1.08 ± 3.24^{a}	0 -16	103. 08 ± 48.86^{b}	12-213

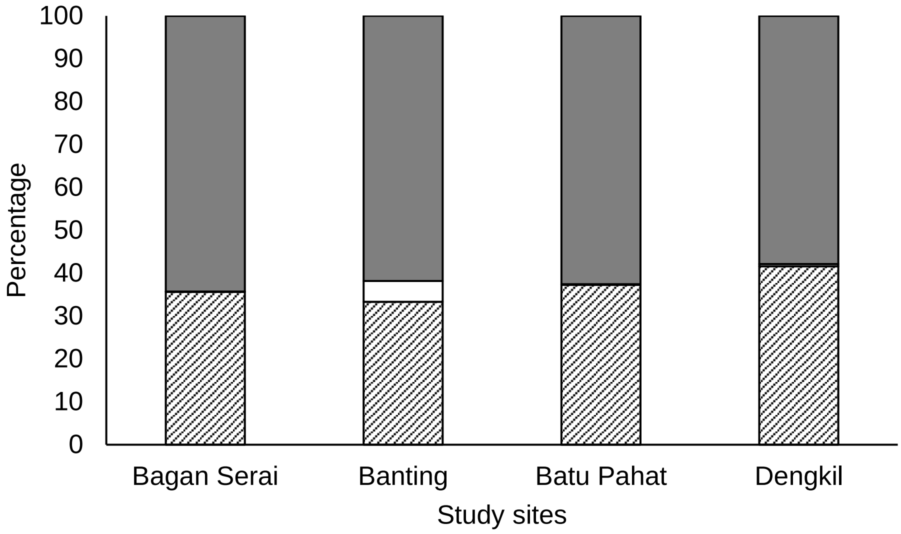


Figure 4.2 Female flower utilisation in male figs of var. *angustifolia* at four sites. Hashed bars = successful galls, open bars = empty galls and solid bars = unused flowers.

4.4.2 Variation between varieties of *F. deltoidea*

4.4.2.1 Characterisation of female figs

Receptive figs of var. *trengganuensis* had the longest diameters (up to 9.40 mm) and receptive figs of var. *bilobata* had the smallest diameters (as small as 2.7mm) (Table 4.12). Two groups of figs are distinguishable based on the size of their receptive figs (Figure 4.3). Varieties with small figs include var. *angustifolia*, var. *deltoidea*, var. *bilobata* and var. *motleyana* while those with large figs are var. *trengganuensis* and var. *kunstleri*.

The receptive and maximum diameters of figs from six varieties were measured (Table 4.12). Only one tree of var. *bilobata* was available. Within the other varieties, there were no significant differences between trees in diameter at receptivity (Kruskal-Wallis $P > 0.05$). Significant differences were present in the diameters of figs at receptivity from different varieties (Kruskal-Wallis, $\chi^2 = 143.62$, df = 5, $P < 0.001$). There were significant differences between the maximum diameters of figs belonging to different varieties using log transformed data (ANOVA, F = 93.92, df = 5,199, $P < 0.001$), but within most varieties there were also significant differences between trees (Tukey tests, Table 4.12).

The number of female flowers in female figs across all varieties was low (Table 4.13). Varieties with smaller figs tended to have fewer flowers (**Error! Reference source not found.**). Uniquely, all the figs from var. *bilobata* contained only one female flower. The largest number of female flowers was in figs of var. *trengganuensis*. The varieties all differed significantly from each other in their flower numbers except for var. *angustifolia* and var. *motleyana* (GLM, $\chi^2 = 100.43$, df = 5, $P < 0.001$) but between trees in the same variety, all varieties showed a significant difference ($P< 0.05$). Longest style lengths were also found in figs of var. *trengganuensis* and the shortest in figs of var. *deltoidea* (Table 4.13). Most varieties differed in mean style lengths (ANOVA on log transformed, F = 694.70, df = 5, 527, $P < 0.001$). Within the same sites, the trees also differed significantly in style lengths.

Table 4.12 The diameters of receptive female figs and the maximum observed diameters of female figs of six varieties of *F. deltoidea*. Values with different superscripts in a column differed significantly (Tukey and Mann-Whitney tests, P < 0.001). * indicates that values differed significantly between trees of the same variety.

Variety	n (trees)	n (figs)	Receptive diameter (mm)		Mature diameter (mm)	
			Mean ± SD	Range	Mean ± SD	Range
angustifolia	20	100	4.3 ± 0.03^{b}	3.9-4.7	$7.2 \pm 0.04^{b*}$	6.7-0.83
deltoidea	5	25	4.6 ± 0.02^{b}	4.4-5.0	$8.1 \pm 0.05^{c*}$	7.2-0.91
trengganuensis	5	25	7.5 ± 0.01^{d}	5.6-9.4	$13.6 \pm 0.08^{d*}$	12.1-1.49
kunstleri	5	25	6.0 ± 0.11^{c}	5.0-8.6	$12.9 \pm 0.15^{d*}$	11.2-1.62
bilobata	1	5	2.9 ± 0.02^{a}	2.7-3.1	3.4 ± 0.01^{a}	3.2-0.36
motleyana	5	25	4.5 ± 0.03^{b}	4.0-5.1	7.5 ± 0.08^{bc}	6.5-0.88

Table 4.13 The number of female flowers and the lengths of styles in female figs of six varieties of *F. deltoidea*. Values with different superscripts in a column differed significantly (Tukey tests, P < 0.001). * indicates that values differed significantly between trees of the same variety.

Variety	n (trees)	n (figs)	Female flower number		Style lengths	
			Mean ± SD	Range (mm)	Mean ± SD	Range (mm)
angustifolia	20	100	$4.72 \pm 0.71^{a*}$	3-6	$1.66 \pm 0.26^{b*}$	1.02-2.12
deltoidea	5	25	$8.48 \pm 1.36^{c*}$	6-11	$1.33 \pm 0.19^{a*}$	0.96- 1.64
trengganuensis	5	25	$23.88 \pm 4.21^{e*}$	18-32	$3.22 \pm 0.44^{e*}$	2.21-4.21
kunstleri	5	25	$19.8 \pm 1.8^{d*}$	17-23	$2.77 \pm 0.25^{d*}$	2.18-3.47
bilobata	1	5	1 ± 0^{b}	1-1	1.49 ± 0.13^{ab}	1.30-1.66
motleyana	5	25	$5.88 \pm 0.6^{a*}$	5-7	$2.31 \pm 0.35^{c*}$	1.48-2.89

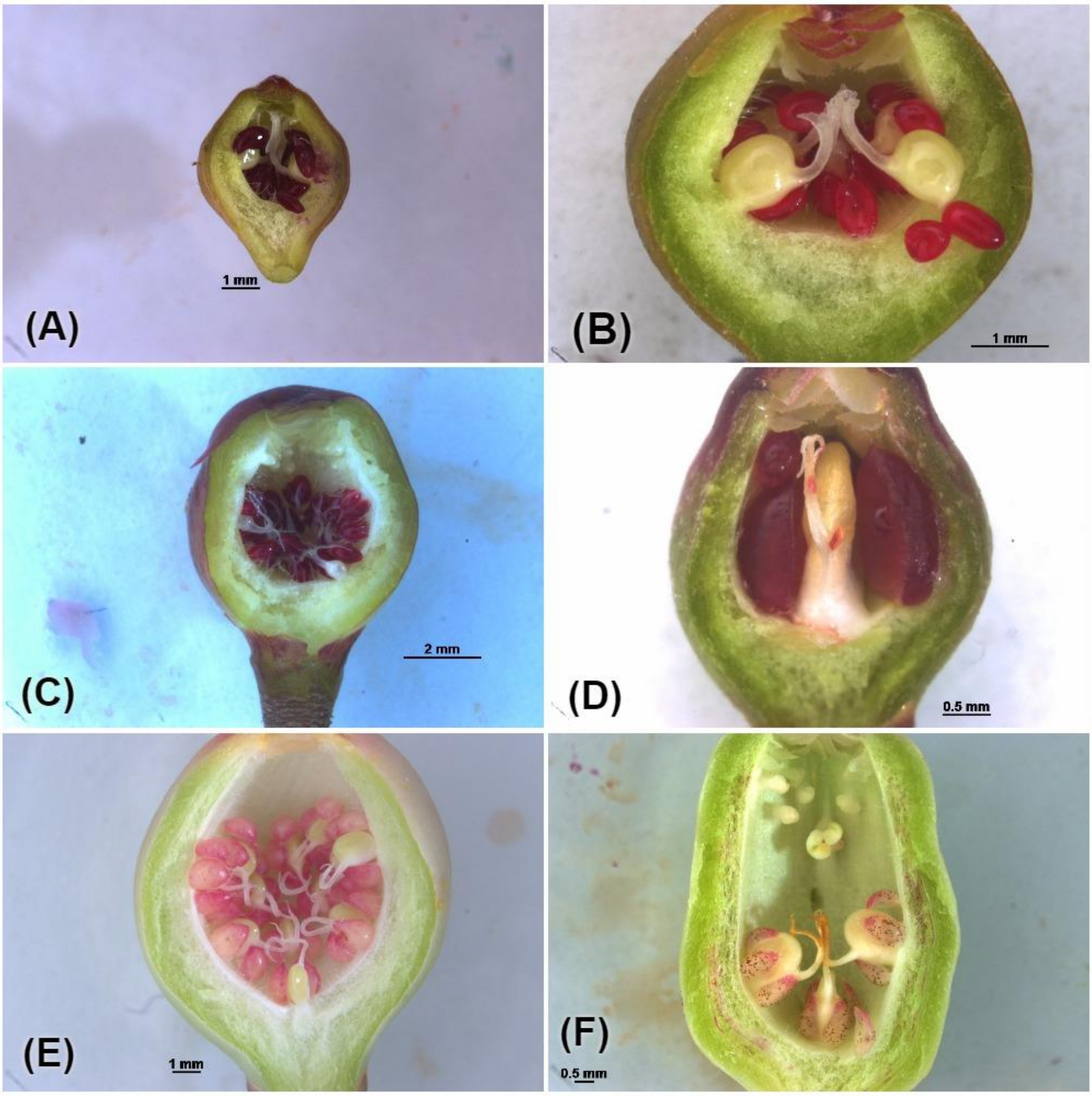
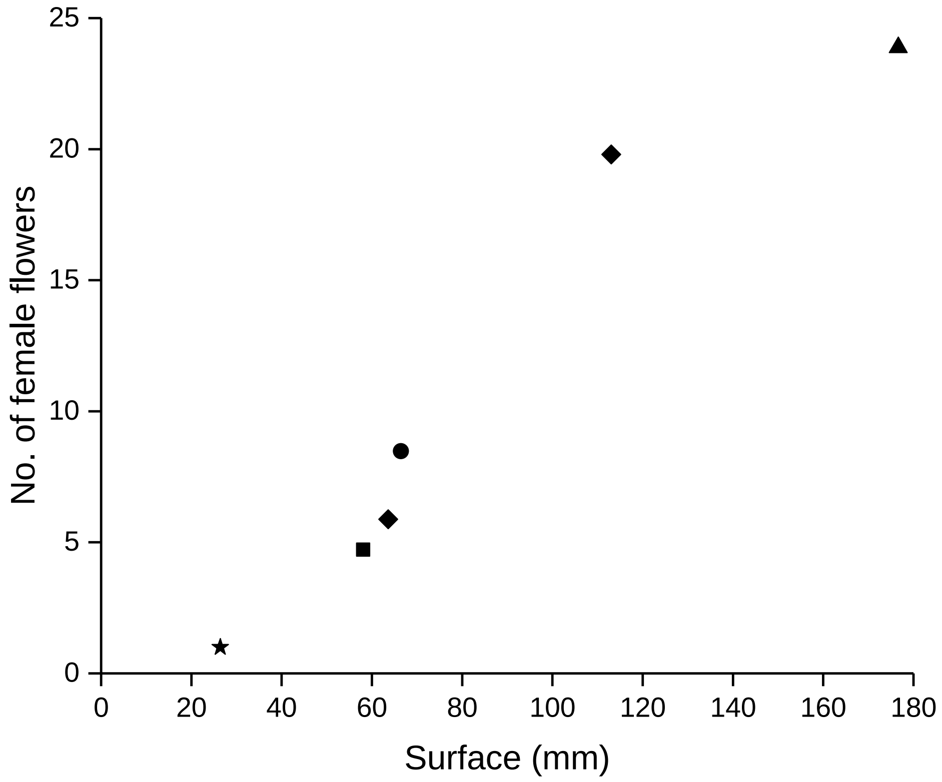


Figure 4.3 The female flowers in receptive female figs of *Ficus deltoidea* varieties. A = var. *angustifolia*, B = var. *deltoidea*, C = var. *trengganuensis*, D = var. *bilobata*, E = var. *kunstleri* and F = var. *motleyana*). The figs had not been pollinated.



Diameter at receptivity and the number of female flowers in female figs of *F. deltoidea* varieties. (■) = var. *angustifolia*, (●) = var. *deltoidea*, (▲) = var. *trengganuensis*, (★) var. *bilobata*, (⬟) var. *kunstleri* and (♦) var. *motleyana*).

Because the tepals enclosed each ovary, their numbers were strongly correlated with flower numbers (Table 4.14). Var. *bilobata* recorded the lowest tepal number with a mean of 4.6 per fig while the most tepals were recorded in var. *trengganuensis* with 87.08. The ranges of numbers of tepal per single female flower were between 3 - 5. All varieties had significantly different numbers of tepals (GLM, $\chi^2 = 822.1$, df = 5, $P < 0.001$). The shape of the tepals in female figs varied little across the varieties but tended to have a rounder shape compared to the long slender tepals in male figs (Figure 4.4). There was some variation in tepal lengths between varieties (ANOVA, F = 123.40, df = 5, 568, $P < 0.001$) (Table 4.14).

Seed lengths could only be measured for the three varieties where pollination took place, namely var. *angustifolia*, var. *deltoidea* and var. *trengganuensis*. The seeds of var. *angustifolia* were the smallest, averaging 4.02 mm and the longest were from var. *trengganuensis* with a mean of 4.54 mm (Table 4.15). Seed lengths varied significantly between varieties (ANOVA, F = 118.90, df = 2, 297, $P < 0.001$). Within trees, only var. *angustifolia* showed differences in seed length ($P < 0.05$). This might be due to the seed lengths in var. *angustifolia* being taken from four different sites. A summary table of plant traits in male figs, which clarify the many ways in which the different varieties varied was depicted in (Figure)

Table 4.14 The number of tepals and tepal lengths in female figs of six varieties of *F. deltoidea*. Values with different superscripts in a column differed significantly (Tukey tests, P < 0.001). * indicates that values differed significantly between trees of the same variety.

Variety	n (trees)	n (figs)	Tepal numbers		Tepal lengths (mm)	
			Mean ± SD	Range	Mean ± SD	Range
angustifolia	20	100	$18.76 \pm 3.9^{b*}$	12-24	$1.34 \pm 0.16^{c*}$	0.89-1.69
deltoidea	5	25	$36.36 \pm 5.61^{c*}$	27-46	$1.12 \pm 0.2^{b*}$	0.96-1.83
trengganuensis	5	25	$87.08 \pm 13.02^{d*}$	70-110	$1.59 \pm 0.19^{a*}$	1.17-1.95
kunstleri	5	25	$75.64 \pm 5.32^{e*}$	62-84	$1.49 \pm 0.16^{a*}$	1.04-1.87
bilobata	1	5	4.6 ± 0.55^{a}	4-5	1.59 ± 0.08^{a}	1.38-1.73
motleyana	5	25	$24.08 \pm 2.71^{b*}$	20-28	$1.16 \pm 0.16^{b*}$	0.85-1.44

Table 4.15. Seed lengths in three varieties of *F. deltoidea*. Values with different superscripts in a column differed significantly (Tukey tests, P < 0.001). * indicates that values differed significantly between trees of the same variety.

Variety	n (trees)	n (figs)	n (seeds)	Seed length (mm)	
				Mean ± SD	Range
angustifolia	20	42	200	$4.02 \pm 0.22^{a*}$	3.54-4.43
deltoidea	5	10	50	4.24 ± 0.28^{b}	3.70-4.64
trengganuensis	5	10	50	4.54 ± 0.18^{c}	4.16-4.93

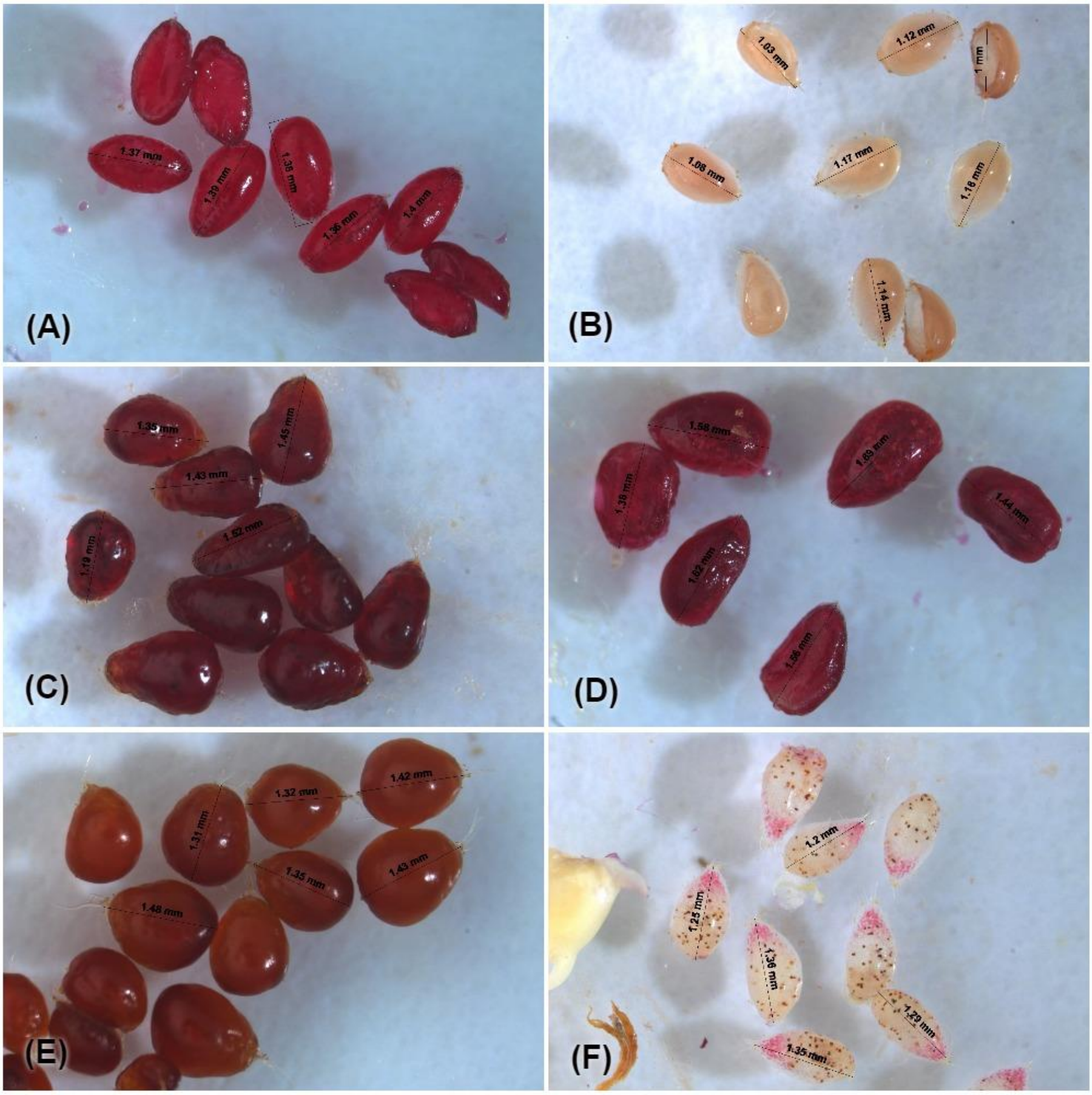


Figure 4.4 The tepals in female figs of *F. deltoidea* varieties. A = var. *angustifolia*, B = var. *deltoidea*, C = v ar. *trengganuensis*, D = var. *bilobata*, E = var. *kunstleri* and F = var. *motleyana*.

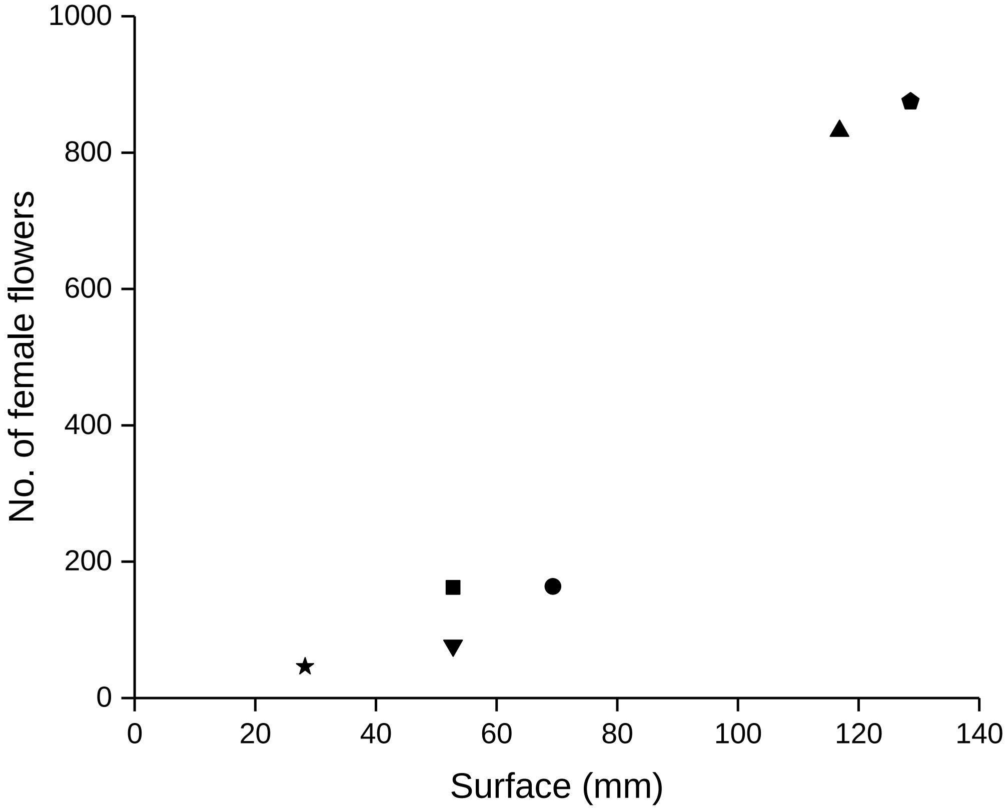
Table 4.16 Summary table of plant characteristics (mean ± SD) in the female fig in six varieties of *F.deltoidea.*

	Varieties					
Characteristic	*angustifolia*	*deltoidea*	*trengganuensis*	*bilobata*	*kunstleri*	*motleyana*
Receptive diameter (mm)	0.43 ± 0.04	0.46 ± 0.02	0.75 ± 0.012	0.29 ± 0.02	0.6 ± 0.11	0.45 ± 0.03
Maximum diameter (mm)	0.71 ± 0.04	0.81 ± 0.05	1.36 ± 0.08	0.34 ± 0.01	1.29 ± 0.15	0.75 ± 0.08
No. of female flowers	4.72 ± 0.71	8.48 ± 1.36	23.88 ± 4.21	1 ± 0	19.8 ± 1.8	5.88 ± 0.6
No. of tepals	18.76 ± 3.9	36.36 ± 5.61	87.08 ± 13.02	4.6 ± 0.55	75.64 ± 5.32	24.08 ± 2.71
Style length (mm)	1.66 ± 0.26	1.33 ± 0.19	3.22 ± 0.44	1.49 ± 0.13	2.77 ± 0.25	2.31 ± 0.35
Tepal length (mm)	1.34 ± 0.16	1.12 ± 0.2	1.59 ± 0.19	1.59 ± 0.08	1.49 ± 0.16	1.16 ± 0.16
No. of seeds	3.93 ± 1.17	6.68 ± 2.08	16.16 ± 7.34	0.00	0.00	0.00

4.4.2.2 Male Figs Characterisation

There was significant variation in the diameters of receptive figs (ANOVA on log transformed, F = 240.00, df = 5, 196, P < 0.001) and the maximum sizes of the figs (ANOVA on sqrt transformed, F = 1416.00, df = 5, 196, P < 0.001) produced by male plants of the six varieties of *F. deltoidea* (Table 4.16). Var. *bilobata* had the smallest receptive figs and var. *trengganuensis* the largest. Most of the pairwise comparisons showed a highly significant difference between varieties but no significant differences within trees of the same variety. Variation between trees of var. *intermedia* could not be assessed as only one tree was available. The aborting figs of var. *bilobata* and var. *trengganuensis* again had the smallest and the biggest maximum diameters respectively. Most varieties varied in the maximum size of their aborting figs (Table 4.16). Only two varieties showed significant differences between trees from the same variety.

The male flowers in male figs were consistently located near the ostiole while the female flowers were present in the basal part of the figs (Figure 4.5). The number of female flowers in male figs across all varieties was high compared to the female figs where varieties with smaller figs tended to have fewer flowers (Figure)The most male flowers were in figs of var. *kunstleri* and the least in var. *bilobata* (Table 4.17). The numbers of flowers varied significantly between (GLM, χ^2 = 119.57, df = 5, P < 0.001). Within a variety, significant differences were found between trees (except for var. *deltoidea*). Female flower numbers were lowest in var. *bilobata* (range = 24-69) and highest in var. *kunstleri* (range = 427–1362) (Table 4.17). There were significant differences overall (GLM, χ^2 = 156.3, df = 5, P < 0.001) with most combinations differ significantly. Anther to ovule ratios were highly variable between figs of the same variety but are consistent with all the varieties being passively pollinated (Table 4.18).



Diameter at receptivity and the number of female flowers in male figs of *F. deltoidea* varieties. (■) = var. *angustifolia*, (●) = var. *deltoidea*, (▲) = var. *trengganuensis*, (★) var. *bilobata*, (⬟) var. *kunstleri* and (▼) var. *intermedia*).

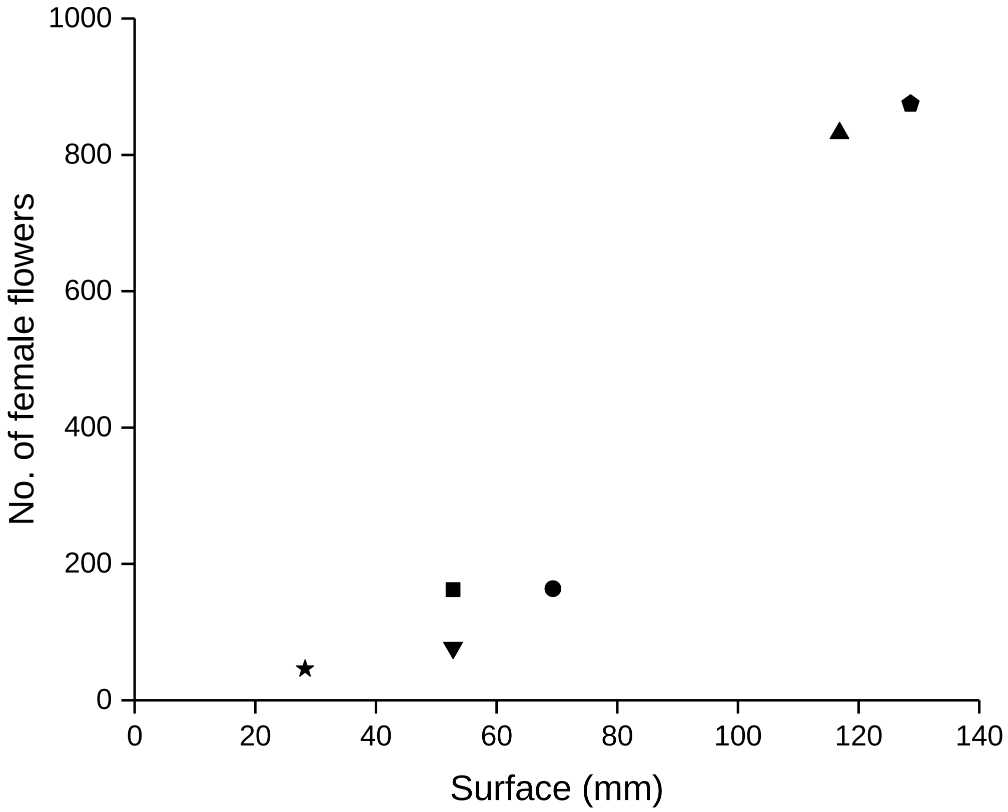
Table 4.16 The minimum and maximum diameters of male figs of six varieties of *F. deltoidea*. Values with different superscripts in a column differed significantly (Tukey tests, $P < 0.001$). * indicates that values differed significantly between trees of the same variety.

Variety	n (trees)	n (figs)	Receptive diameter (mm)		Maximum diameter (mm)	
			Mean ± SD	Range	Mean ± SD	Range
angustifolia	20	100	0.42 ± 0.03^{b}	0.37-0.47	0.63 ± 0.03^{b}	0.55-0.69
deltoidea	5	25	0.47 ± 0.02^{c*}	0.42-0.49	0.84 ± 0.04^{c}	0.76-0.90
trengganuensis	5	25	0.61 ± 0.07^{d}	0.52-0.80	1.31 ± 0.08^{e}	1.20-1.42
kunstleri	4	20	0.64 ± 0.08^{d*}	0.51-0.81	1.18 ± 0.06^{d*}	1.10-1.28
bilobata	5	25	0.30 ± 0.02^{a}	0.27-0.33	0.39 ± 0.04^{a*}	0.34-0.49
intermedia	1	5	0.41 ± 0.01^{b}	0.40-0.42	0.58 ± 0.04^{b}	0.55-0.62

Table 4.17 The numbers of male and female flowers in six varieties of *F. deltoidea*. Values with different superscripts in a column differed significantly (Tukey tests, $P < 0.001$). * indicates that values differed significantly between trees of the same variety.

Variety	n (trees)	n (figs)	Male flower number		Female flower number	
			Mean ± SD	Range	Mean ± SD	Range
angustifolia	20	100	29.84 ± 4.06^{c*}	20-41	162.22 ± 30.43^{a*}	94-259
deltoidea	5	25	50.41 ± 5.65^{a}	42-61	157.41 ± 26.70^{a*}	108-239
trengganuensis	5	25	143.68 ± 26.00^{b*}	91-188	828.48 ± 123.22^{b}	526-1024
kunstleri	4	20	176.75 ± 61.49^{b*}	79-285	874.90 ± 327.72^{b*}	427-1362
bilobata	5	25	13.64 ± 1.89^{d*}	11-17	46.08 ± 12.04^{c*}	24-69
intermedia	1	5	49.80 ± 6.22^{a}	42-57	77.00 ± 6.36^{d}	71-86

Figure 4.5 The location of male and female flowers in the male figs of *Ficus deltoidea* complex. A = var. *angustifolia*, B = var. *deltoidea*, C = var. *trengganuensis*, D = var. *bilobata*, E = var. *intermedia* and F = var. *kunstleri*.



Diameter at receptivity and the number of female flowers in male figs of *F. deltoidea* varieties. (■) = var. *angustifolia*, (●) = var. *deltoidea*, (▲) = var. *trengganuensis*, (★) var. *bilobata*, (⬟) var. *kunstleri* and (▼) var. *intermedia*).

Table 4.18 The ratio of anthers to ovules in male figs of varieties of *F. deltoidea*.

Variety	n (trees)	n (figs)	Ratio of anthers to ovules	
			Mean ± SD	Range
angustifolia	20	100	0.19 ± 0.03	0.14-0.26
deltoidea	5	25	0.31 ± 0.05	0.24-0.46
trengganuensis	5	25	0.18 ± 0.04	0.12-0.25
kunstleri	4	20	0.21 ± 0.04	0.16-0.28
bilobata	5	25	0.31 ± 0.09	0.20-0.58
intermedia	1	5	0.65 ± 0.11	0.59-0.79

Similar to the female figs, tepals enclosed the ovules in the male figs (Figure 4.6). The number of tepals present reflected ovary numbers with var. *kunstleri* and var. *bilobata* again recording the highest and the lowest numbers of tepals respectively (Table 4.19). There was a significant difference across all varieties (GLM, χ^2 = 161.33, df = 5, P < 0.001) but most pairs did not have different tepal numbers. With tepal lengths the pattern was the same, with var, *kunstleri* figs having the longest tepals and var. *bilobata* the shortest (Table 4.19). Tepal lengths varied significantly (ANOVA on log transformed data, F = 485.50, df = 5, 1044, P < 0.001) and there were differences between most pairs of varieties (Tukey tests) as well as between trees of the same variety.

The style lengths of female flowers in male figs ranged from 0.09 mm – 0.29 mm (Table 4.20). The shortest mean style lengths were in var. *bilobata* figs while var. *trengganuensis* figs had the longest mean style length. Most varieties differed in style lengths (ANOVA, F = 1768.00, df = 5, 1044, P < 0.001). Gall length provides an indirect measure of fig wasp body size. Gall lengths were longest in var. *deltoidea* figs with and the shortest in var. *angustifolia* (Table 4.21). There were significant differences in the sizes of galls in the figs of the three varieties (ANOVA, F = 181.20, df = 2, 297, P < 0.001). Tukey tests showed that gall sizes in var. *angustifolia* and var. *trengganuensis* did not differ significantly unlike the other two combinations. Within varieties, only var. *trengganuensis* did not show any significant variation between trees. A summary table of plant traits in male figs, which clarify the many ways in which the different varieties varied was depicted in (Table)

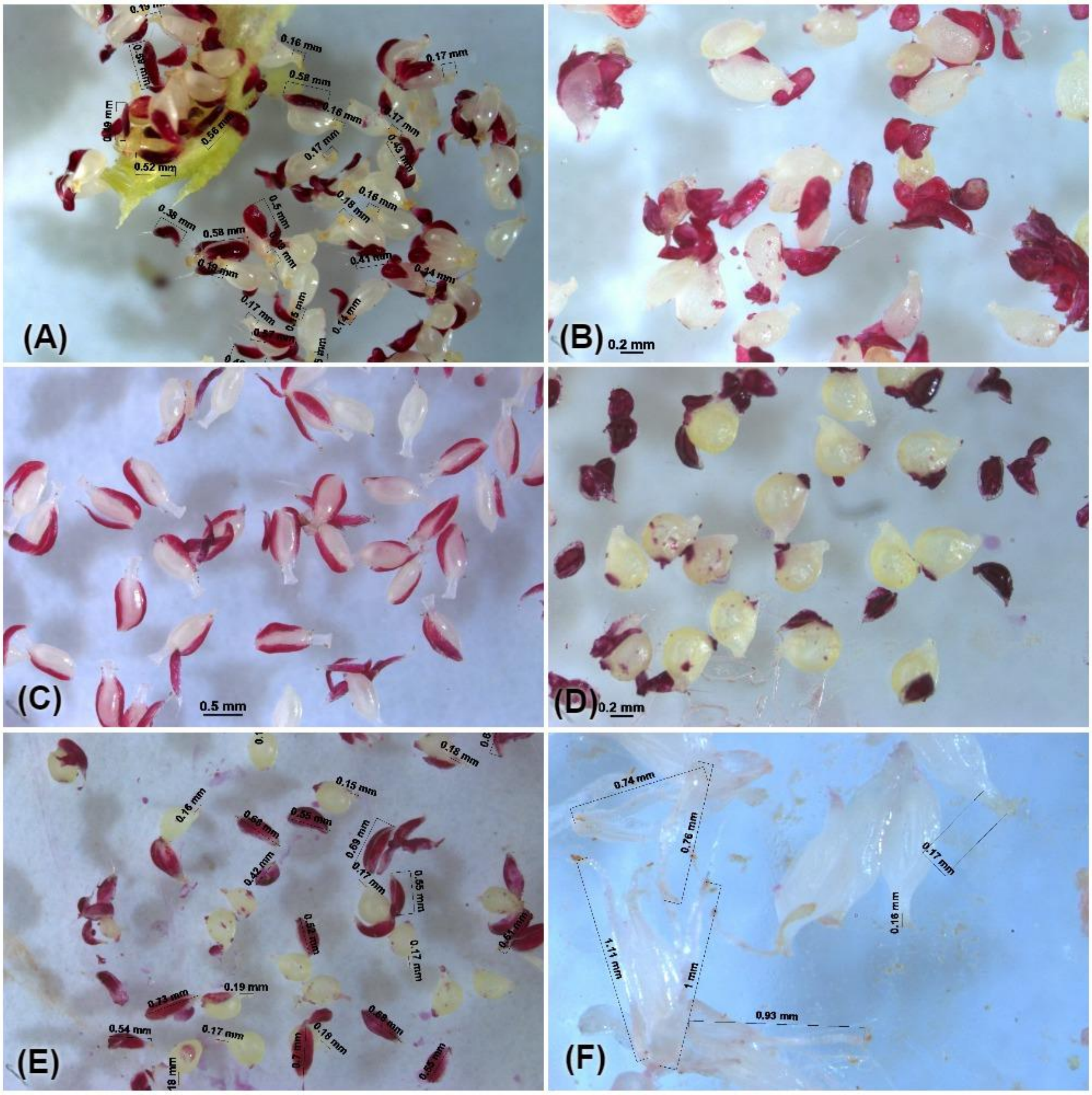


Figure 4.6 The tepals in male figs of varieties in the *Ficus deltoidea* complex. (A = var. *angustifolia*, B = var. *deltoidea*, C = var. *trengganuensis*, D = var. *bilobata*, E = var. *kunstleri* and F = var. *intermedia*).

Table 4.19 The numbers and lengths of tepals in male figs of six varieties of *F. deltoidea*. Values with different superscripts in a column differed significantly (Tukey tests, P < 0.001). * indicates that values differed significantly between trees of the same variety.

Variety	n (trees)	n (figs)	Tepal Numbers		Tepal lengths (mm)	
			Mean ± SD	Range	Mean ± SD	Range
angustifolia	20	100	582.87 ± 140.69^{a*}	336-1052	0.48 ± 0.10^{b*}	0.26-0.74
deltoidea	5	25	620.74 ± 111.39^{a}	399-866	0.81 ± 0.12^{a*}	0.33-1.02
trengganuensis	5	25	3065.44 ± 644.342^{b}	1976-4006	0.68 ± 0.11^{c*}	0.43-0.99
kunstleri	4	20	3310 ± 1268.68^{b*}	1257-4988	0.91 ± 0.14^{d*}	0.54-1.19
bilobata	5	25	155.2 ± 44.14^{c*}	88-257	0.34 ± 0.04^{e*}	0.21-0.44
intermedia	1	5	296.8 ± 16.84^{d}	276-304	0.54 ± 0.09^{a*}	0.33-0.73

Table 4.20 The lengths of the styles of female flowers in male figs of six varieties of *F. deltoidea*. Values with different superscripts in a column differed significantly (Tukey tests, P < 0.001). * indicates that values differed significantly between trees of the same variety.

Variety	Style lengths (mm)				
	n (trees)	n (figs)	n (flowers)	Mean ± SD	Range
angustifolia	5	25	250	0.20 ± 0.05^{b}	0.17-0.24
deltoidea	5	25	250	0.15 ± 0.02^{a*}	0.12-0.19
trengganuensis	5	25	250	0.24 ± 0.01^{c*}	0.21-0.29
kunstleri	4	20	100	0.15 ± 0.01^{a*}	0.13-0.19
bilobata	3	15	150	0.12 ± 0.06^{d}	0.09-0.13
intermedia	1	5	50	0.18 ± 0.01^{e}	0.15-0.21

Table 4.21 The gall lengths of female flowers in three varieties of *F. deltoidea*. Values with different superscripts in a column differed significantly (Tukey tests, $P < 0.001$). * indicates that values differed significantly between trees of the same variety.

Variety	Gall lengths (mm)				
	n (trees)	n (figs)	n (galls)	Mean ± SD	Range
angustifolia	20	20	200	0.96 ± 0.11^{a*}	0.7-1.13
deltoidea	5	5	50	1.27 ± 0.13^{b*}	1.07-1.59
trengganuensis	5	5	50	1.00 ± 0.05^{a}	0.94-1.13

Table 4.23 Summary table of plant characteristics (mean ± SD) in the male figs of six varieties of *F. deltoidea.*

	Varieties					
Characteristic	*angustifolia*	*deltoidea*	*trengganuensis*	*bilobata*	*kunstleri*	*intermedia*
Receptive diameter (mm)	0.41 ± 0.04	0.47 ± 0.02	0.61 ±0.07	0.30 ± 0.02	0.64 ± 0.08	0.41 ± 0.01
Maximum diameter (mm)	0.62 ± 0.04	0.84 ± 0.04	1.31 ±0.08	0.39 ± 0.04	1.18 ± 0.06	0.58 ± 0.04
No. of male flowers	29.84 ± 4.06	50.41 ± 5.65	143.68 ± 26.00	13.64 ± 1.89	176.75 ± 61.49	49.80 ± 6.22
No. of female flowers	162.22 ± 30.43	163.59 ± 26.70	831.88 ± 123.22	46.08 ± 12.04	874.90 ± 327.72	77.00 ± 6.36
No. of galls	59.94 ± 29.96	144.59 ± 28.08	182.56 ± 111.47	-	-	-
No. of empty galls	2.26 ± 8.14	1.30 ± 1.90	24.60 ± 19.49	-	-	-
No. of unused female flowers	100.02 ± 32.23	9.74 ± 10.46	621.32 ± 131.50	46.08 ± 12.04	874.90 ± 327.72	77.00 ± 6.36
No. of tepals	582.87 ± 140.69	620.74 ± 111.39	3065.44 ± 644.342	155.2 ± 44.14	3310 ± 1268.68	296.8 ± 16.84
Style length (mm)	0.20 ± 0.05	0.15 ± 0.02	0.24 ± 0.01	0.12 ± 0.06	0.15 ± 0.01	0.18 ± 0.01
Tepal length (mm)	0.48 ± 0.10	0.81 ± 0.12	0.68 ± 0.11	0.34 ± 0.04	0.91 ± 0.14	0.54 ± 0.09
Gall length (mm)	0.96 ± 0.11	1.27 ± 0.13	1.00 ± 0.05	-	-	-

4.4.2.3 Fig wasp Characterisation

Fig wasps reared from figs of var. *angustifolia*, var. *deltoidea* and var. *trengganuensis* were the only pollinators available. Their ovipositor lengths overlapped (Table 4.22) but the mean length of the ovipositors of pollinators reared from var. *angustifolia* was significantly shorter than those reared from the remaining varieties (ANOVA, F = 1076, df = 2, 747, $P < 0.001$) (Figure 1.6). There were significant differences between the mean ovipositor lengths of wasps that emerged from different trees belonging to var. *trengganuensis* (ANOVA, F = 5.73, df = 4, 245, $P < 0.01$), but not different trees belonging to the other varieties (var. *angustifolia* (ANOVA, F = 1.00, df = 4, 245, $P > 0.05$) and var. *deltoidea* (ANOVA, F = 1.75, df = 4, 245, $P > 0.05$).

Significant differences were present in the hind femur lengths between pollinators of the three varieties (Kruskal-Wallis, $\chi^2 = 118.15$, df = 2, $P < 0.001$). Pollinators from var. *angustifolia* were the smallest and those from var. *trengganuensis* the largest (Table 4.23). Within-variety differences in femur length from different trees were not significant with var. *angustifolia* (ANOVA, F = 0.75, df = 19, 100, $P > 0.05$), var. *deltoidea* (ANOVA, F = 0.69, df = 4, 25, $P > 0.05$) and var. *trengganuensis* (ANOVA, F = 1.49, df = 4, 25, $P > 0.05$). The ratios of ovipositor to femur lengths (Figure 1.8) were very similar in the fig wasps reared from the three varieties.

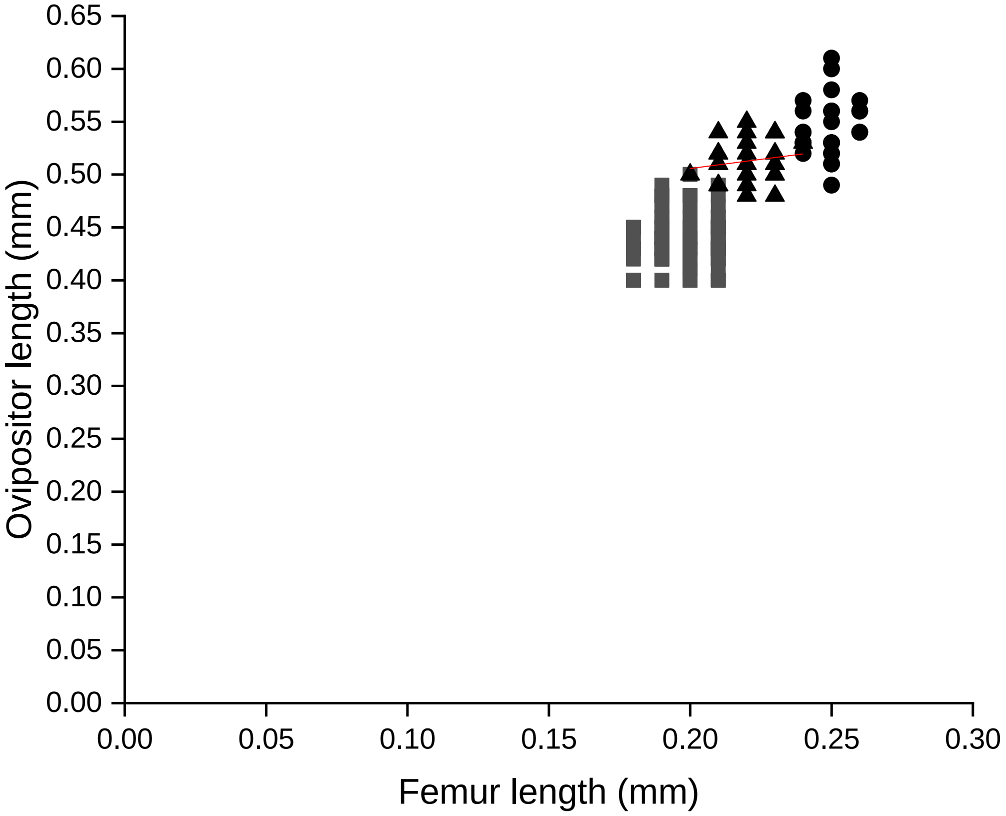
The strong relationship between femur length and ovipositor length present is very clear when varieties are compared, Linear regression, $R^2 = 0.61$, F = 282.96, df = 178, $P < 0.001$). Within varieties, there was no relationship between the femur length and the ovipositor length of the female pollinator.

Table 4.22 The ovipositor lengths of female fig wasps reared from three varieties of *F. deltoidea*. Values with different superscripts in a column differed significantly (Tukey tests, $P < 0.001$). * indicates that values differed significantly between trees of the same variety.

Variety	Ovipositor lengths (mm)				
	n (trees)	n (figs)	n (wasps)	Mean ± SD	Range
angustifolia	5	25	250	0.44 ± 0.02^{a}	0.40-0.49
deltoidea	5	25	250	0.54 ± 0.03^{b}	0.48-0.61
trengganuensis	5	25	250	$0.51 \pm 0.02^{c*}$	0.44-0.55

Table 4.23 The femur lengths of female fig wasps reared from three varieties of *F. deltoidea*. Values with different superscripts in a column differed significantly (Mann-Whitney, tests, $P < 0.001$).

Variety	Femur lengths (mm)				
	n (trees)	n (figs)	n (wasps)	Mean ± SD	Range
angustifolia	20	20	200	0.20 ± 0.01^{a}	0.18-0.21
deltoidea	5	5	50	0.25 ± 0.01^{b}	0.24-0.26
trengganuensis	5	5	50	0.22 ± 0.01^{c}	0.21-0.24



The relationship between femur length and ovipositor length of female pollinators in *F. deltoidea* varieties. (■) = var. *angustifolia*, (●) = var. *deltoidea*, (▲) = var. *trengganuensis*, (★).

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Within-variety relationships between the femur length and ovipositor length of female pollinators using Pearson correlation.

Variety	n (femur)	n (ovi)	Pearson's r	P value
angustifolia	120	120	0.002	0.98
deltoidea	30	30	0.225	0.23
trengganuensis	5	25	0.175	0.36

4.4.3 Fig and fig wasp inter-relationships

4.4.3.1 Style length and ovipositor length relationships

Measurements of the style lengths in male figs of the three varieties and the ovipositor lengths of their respective pollinators showed that all the ovipositors were long enough to reach all the ovaries of the female flowers in all varieties (Figure 4.7). This meant that even the shortest ovipositor length (a 0.4 mm ovipositor from *Blastophaga* sp. var. *angustifolia*) was capable of penetrating the full length of even the longest styles of female flowers in male figs of all six varieties of *F. deltoidea* (Figure 4.8). On the other hand, none of the ovipositors were able to reach to the end of the much longer styles in any of the female figs and the ovipositors of the fig wasps showed that (Figure 4.9).

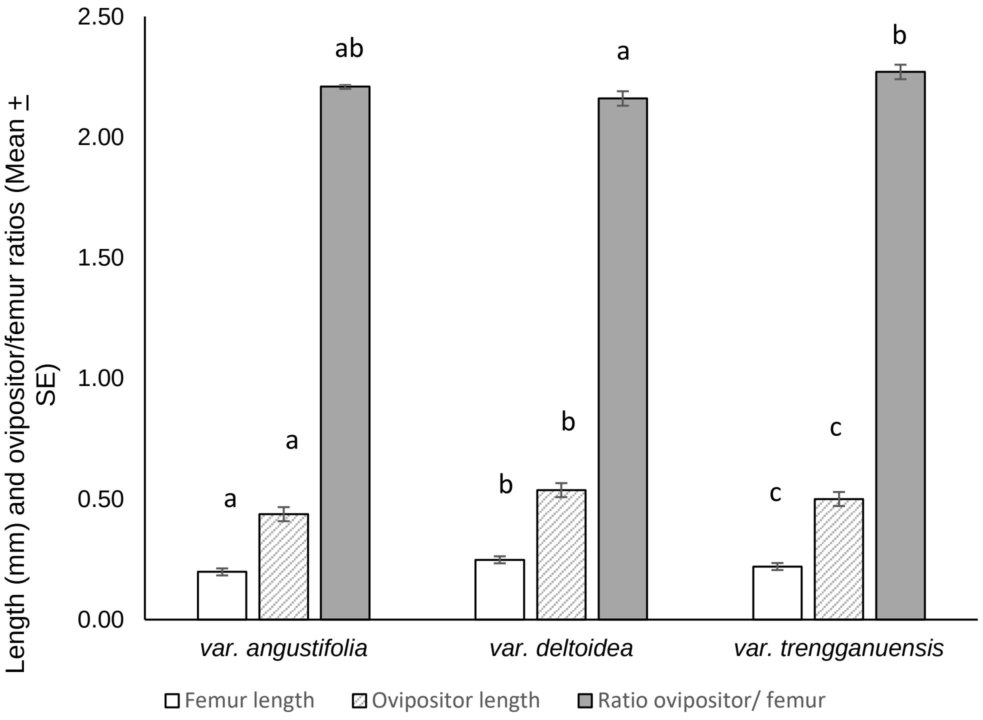


Figure 4.7 Hind femur lengths (open bars), ovipositor lengths (hashed bars) and ratios of ovipositor to femur lengths (solid bars) of fig wasps reared from three varieties of *F. deltoidea*. Values with different superscripts differed significantly (Mann-Whitney tests, P < 0.001).

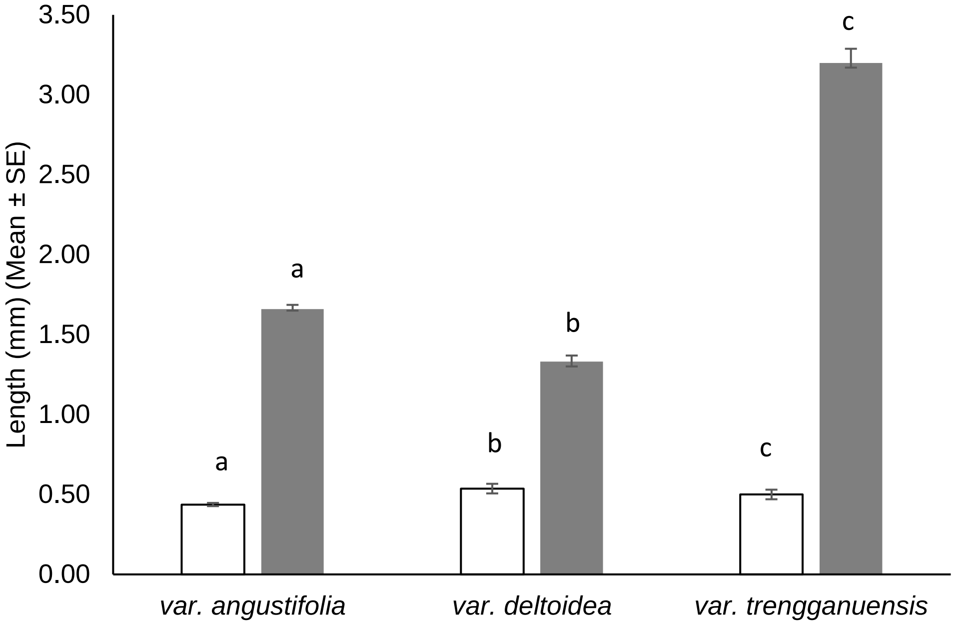


Figure 4.8 A comparison between ovipositor lengths (open bars) and style lengths (solid bars) of female flowers in female figs of three varieties of *F. deltoidea* (Tukey tests, P < 0.001).

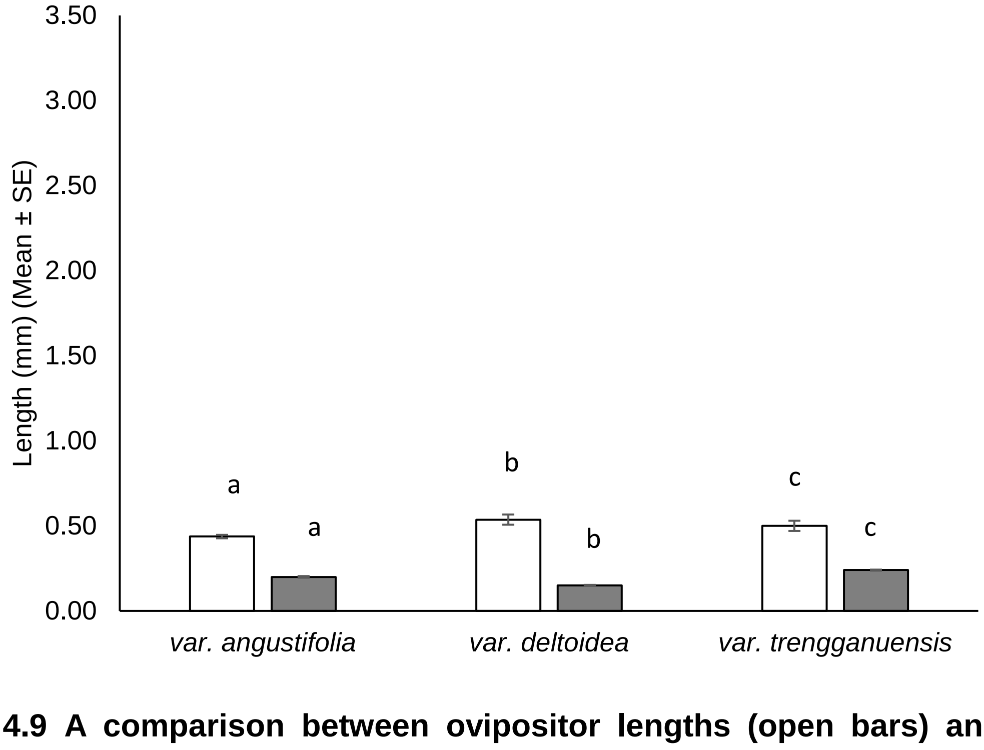
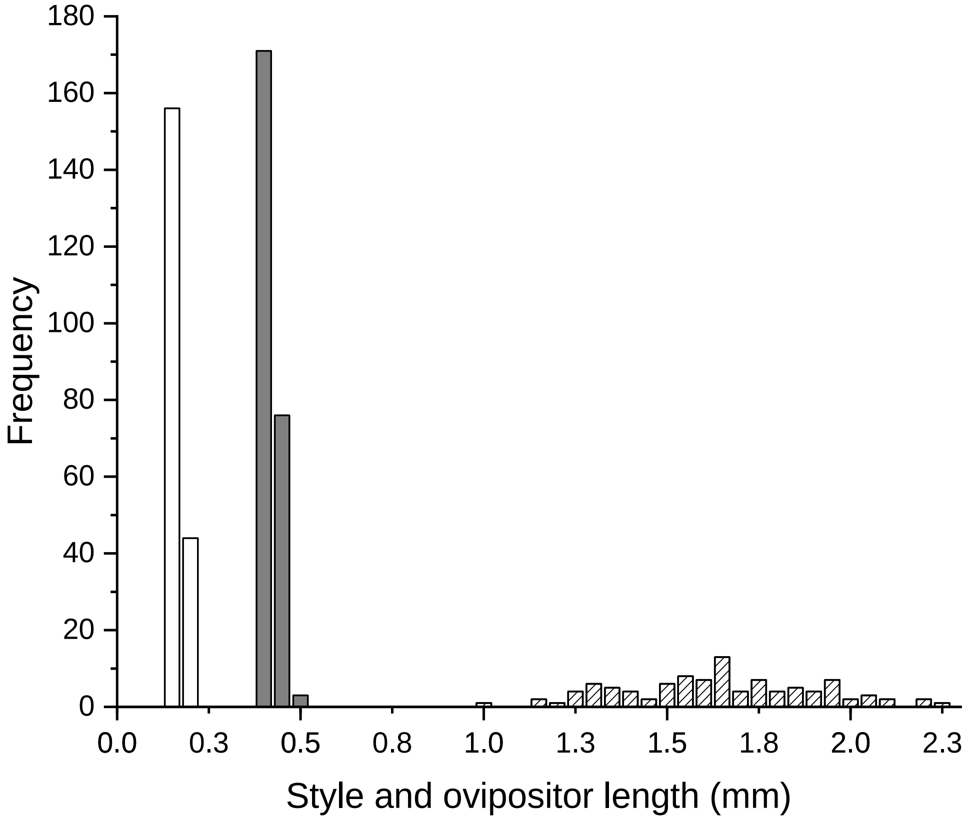
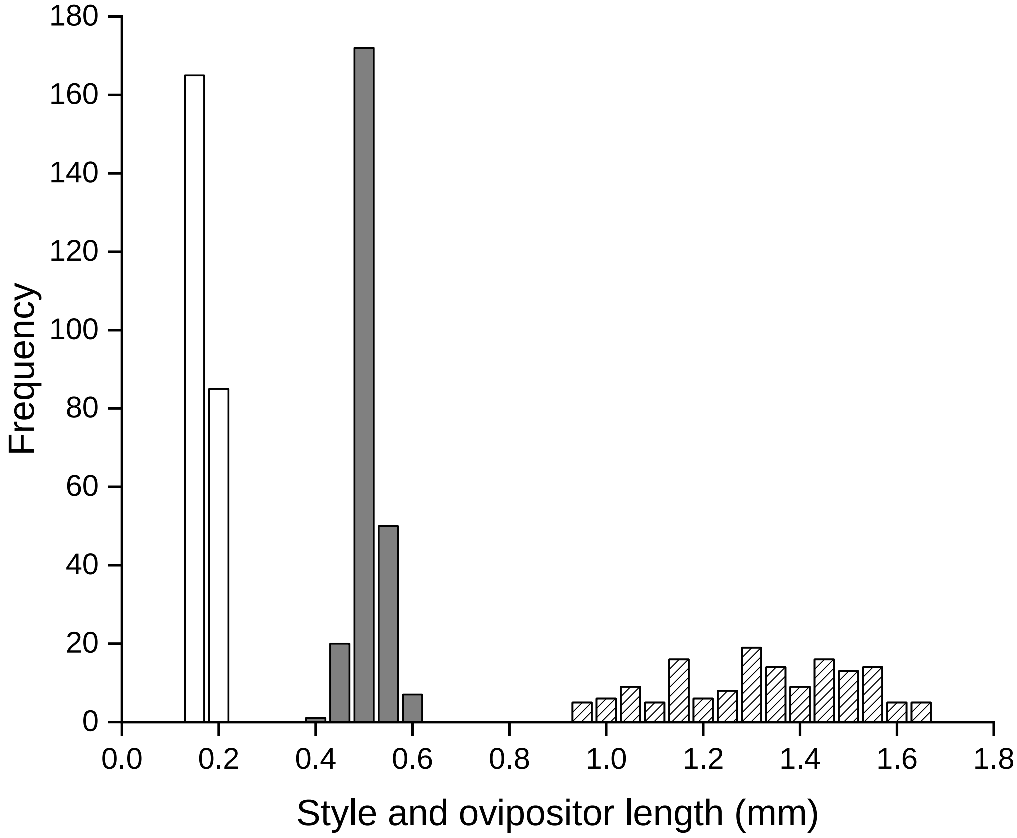


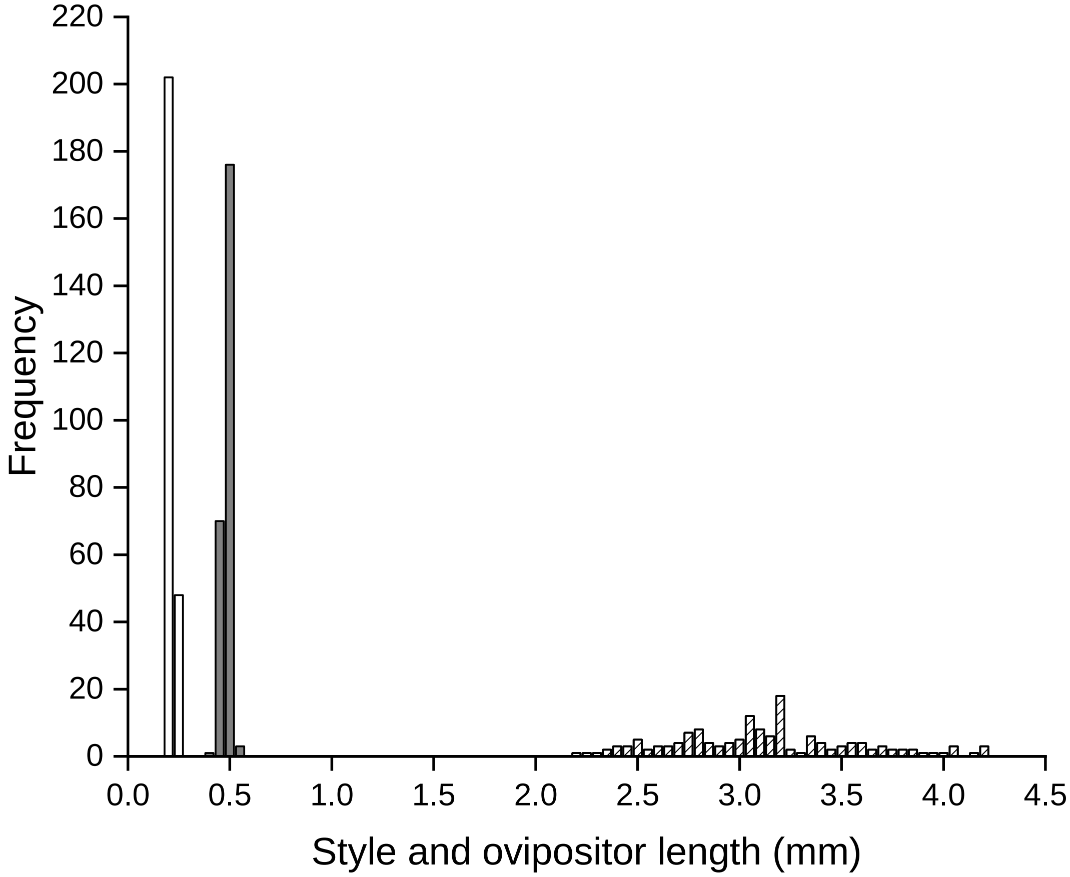
Figure 4.9 A comparison between ovipositor lengths (open bars) and style lengths (solid bars) of female flowers in male figs of three varieties of *F. deltoidea*. Values with different superscripts differed significantly (Tukey tests P< 0.001).



The relationship between the ovipositor length of *Blastophaga* sp. (solid bars) and style lengths in male figs (open bars) and female figs (hashed bars) of *F. deltoidea* var. *angustifolia*.



The relationship between the ovipositor length of *Blastophaga* sp. (solid bars) and style lengths in male figs (open bars) and female figs (hashed bars) of *F. deltoidea* var. *deltoidea.*



The relationship between the ovipositor length of *Blastophaga* sp. (solid bars) and style lengths in male figs (open bars) and female figs (hashed bars) of *F. deltoidei* var. *trengganuensis* .

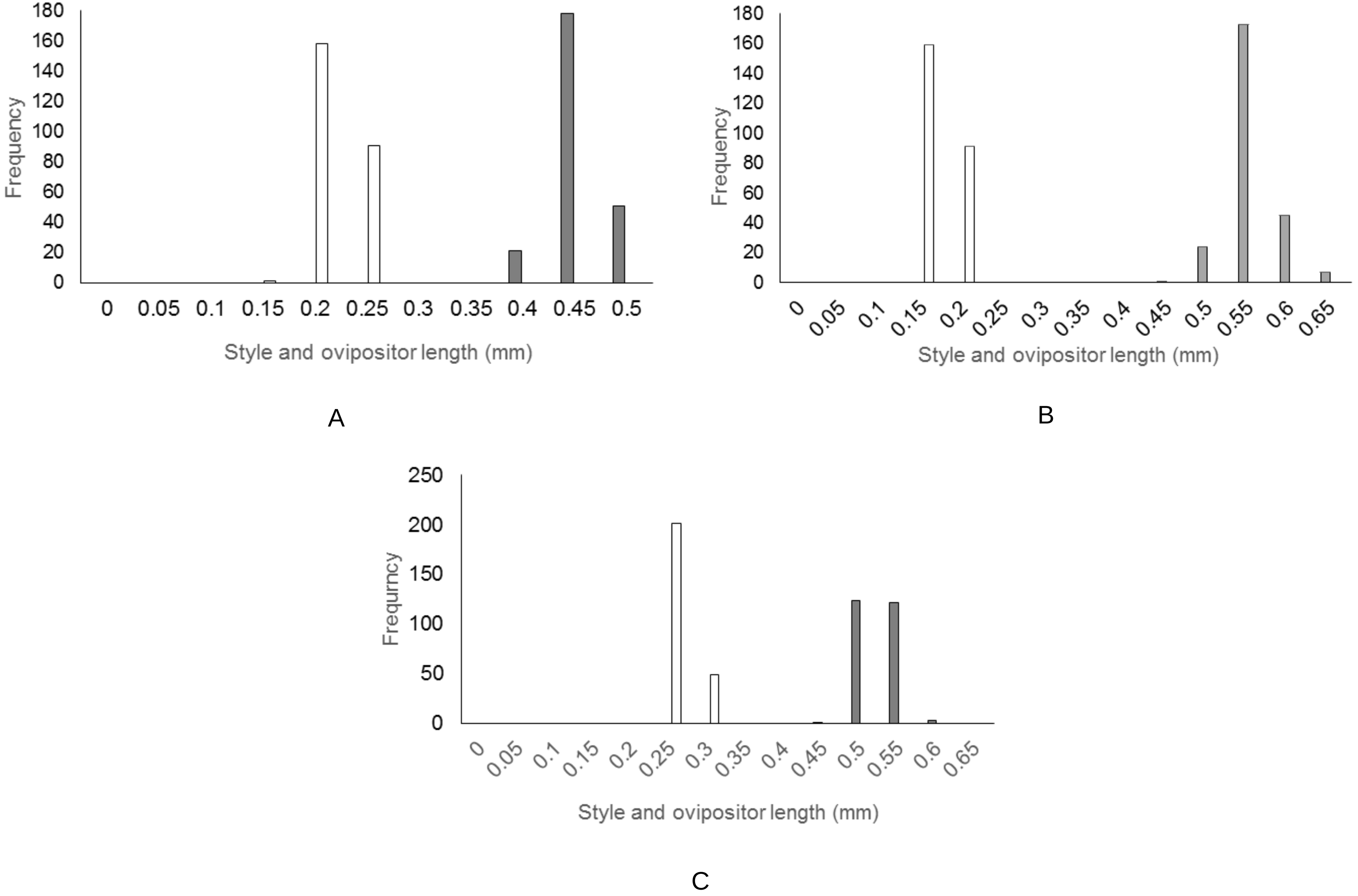


Figure 4.10 The relationship between the ovipositor length of *Blastophaga* sp. (solid bars) and style lengths in male figs of *F. deltoidea* (open bars). A = var. *angustifolia*, B = var. *deltoidea*, C = var. *trengganuensis*.

4.4.4 Flower utilisation and pollination

All three varieties had more than 50% of the flowers in female figs pollinated (Figure 4.11). Var. *angustifolia* had 83.26% of its flowers producing seeds followed by var. *deltoidea* (78.77%) and var. *trengganuensis* (67.67%). The small figs of var. *angustifolia* and var. *deltoidea* contained few female flowers compared to the big figs in var. *trengganuensis*. They produced correspondingly fewer seeds (Table 4.24, GLM, $\chi^2 = 36.72$, df = 2, $P < 0.001$). Within varieties, only var. *deltoidea* did not differ significantly between trees in terms of seed numbers.

Differences between varieties in the contents of male figs are summarised in Figure 4.12. Male figs contained many more female flowers than female figs and differences in the numbers of flowers galled and fig wasp offspring generated by the figs reflected in the differences in flower numbers between varieties (Table 4.25, Figure 4.13). There was a significant difference in the total fig wasp offspring generated in the three varieties (GLM, $\chi^2 = 34.38$, df = 2, $P < 0.001$). This resulted from fewer offspring in the var. *angustifolia* figs than in the figs of the other varieties (Mann-Whitney tests).

Not all the galled flowers supported successful pollinator development. These formed empty galls, which represented only a small proportion of the total galled flowers (Table 4.25). The number of empty galls varied significantly between varieties (GLM, $\chi^2 = 349.7$, df = 2, $P < 0.001$) because var. *trengganuensis* figs contained many more empty galls than the other varieties (Mann-Whitney tests). Within varieties, the numbers of empty galls varied between trees of var. *angustifolia* were significantly different (Kruskal-Wallis, $\chi^2 = 56.77$, df = 19, $P < 0.001$).

The mean number of male offspring per fig varied from about 10 to 50 across the different varieties (Table 4.25). The number of male fig wasp offspring differed between varieties (GLM, $\chi^2 = 261.01$, df = 2, $P < 0.001$). Within varieties, only var. *angustifolia* did not also have a significant difference in male numbers between trees (Table 4.25). The mean numbers of female offspring per fig varied from about 50 to 135 in the three varieties. Significant differences were present in the numbers of female offspring in the figs of var. *angustifolia* compared with the other two varieties (GLM, $\chi^2 = 589.00$, df = 2, $P < 0.001$). Only var. *deltoidea* did not also have significant between-trees differences in the numbers of females present (Table 4.25).

The numbers of unused female flowers differed significantly between all combinations of the three varieties (Poisson GLM, $\chi^2 = 18155.11$, df = 2, $P < 0.001$). Within each variety, only trees of var. *angustifolia* differed significantly (ANOVA, F = 3.64, df = 19, 80, $P < 0.001$). The mean sex ratio (proportion of male offspring) was greatest in var. *trengganuensis* (0.29 ± 0.21) (Mean ± SD) followed by var. *angustifolia* (0.18 ± 0.19) and var. *deltoidea* (0.16 ± 0.07) (Figure 4.14) and they differ significantly (Kruskal-Wallis, $\chi^2 = 9.02$, df = 2, $P < 0.05$).

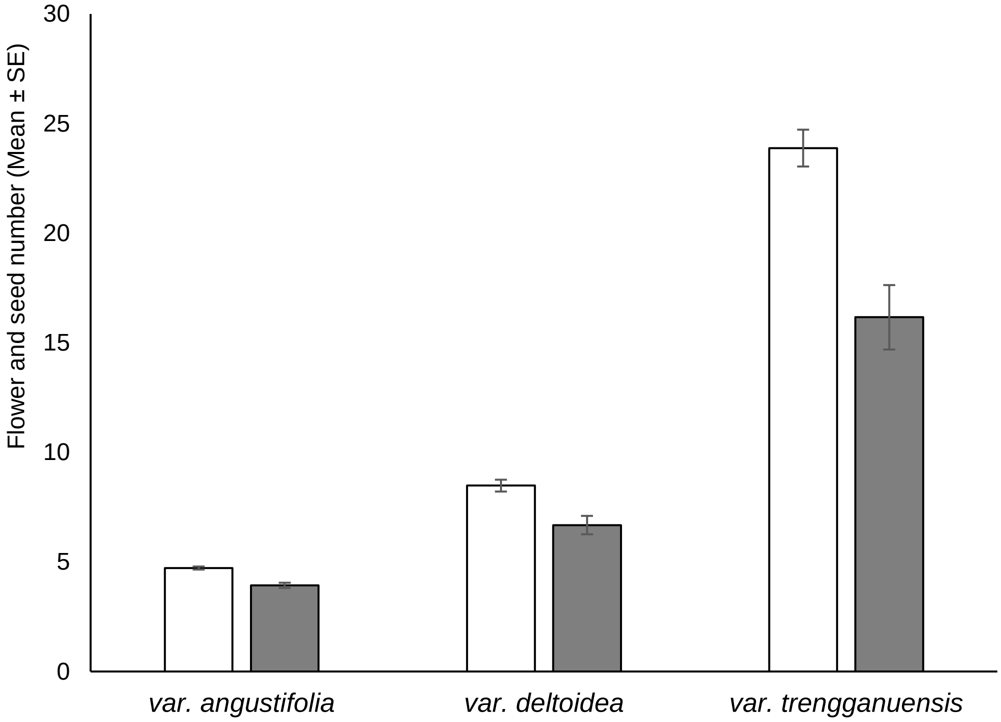


Figure 4.11. The number of female flowers in female figs (open bars) and the number of seeds generated (solid bars).

Table 4.24 The number of seeds in three figs of three varieties of *F. deltoidea*. Values with different superscripts differed significantly (Tukey Tests P< 0.05). * indicates that values differed significantly between trees of the same variety.

Variety	Number of seeds (mm)			
	n (trees)	n (figs)	Mean ± SD	Range
angustifolia	20	100	3.93 ± 1.17^{a*}	1-6
deltoidea	5	25	6.68 ± 2.08^{b}	3-10
trengganuensis	5	25	16.16 ± 7.34^{c*}	2-28

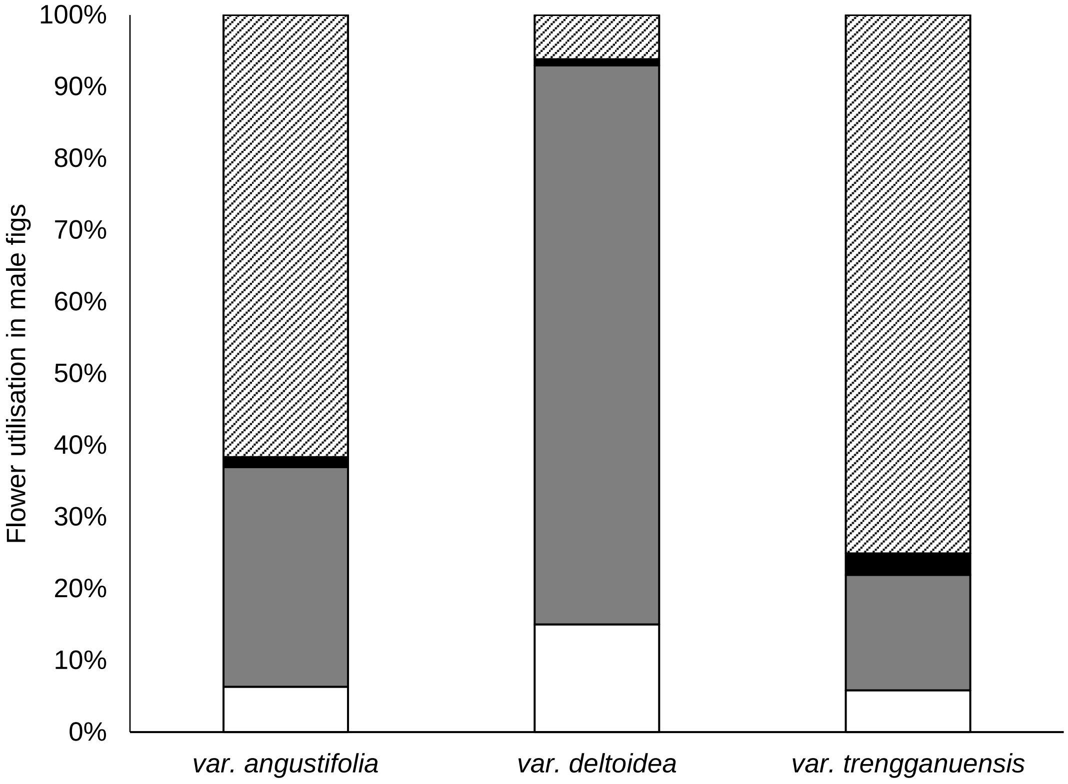


Figure 4.12 A comparison of female flower utilisation by fig wasps in male figs of three varieties of *F. deltoidea*. Open bars = male offspring, grey bars = female offspring, black bars = empty galls, hashed bars = unused flowers.

Table 4.25 The contents of male figs (Mean ± SD) of three varieties of *F. deltoidea*. Values with different superscripts in a row differed significantly (tests vary, see above, P< 0.001). * indicates values differed significantly between trees within the same variety.

Contents/Variety	*angustifolia*	*deltoidea*	*trengganuensis*
Female offspring	49.68 ± 26.34^{a*}	122.70 ± 27.30^{b}	133.04 ± 99.15^{b*}
Range	0-114	70-174	31-299
Male offspring	10.26 ± 10.16^{a}	23.67 ± 11.25^{b*}	49.52 ± 48.40^{c*}
Range	0-51	3-56	5-161
Total offspring	59.94 ± 29.96^{a*}	146.37 ± 29.90^{b}	182.56 ± 111.47^{b*}
Range	18-151	73-203	59-433
Empty galls	2.26 ± 8.14^{a*}	1.30 ± 1.95^{a}	24.60 ± 19.49^{b}
Range	0-46	0-7	9-104
Unused flowers	100.02 ± 32.23^{a*}	9.74 ± 10.46^{b}	621.32 ± 131.50^{c}
Range	12-213	0 - 37	353-797
Total female flowers	162.22 ± 30.43^{a}	157.41 ± 26.70^{ab}	828.48 ± 123.22^{b}
Range	94-259	108-239	526-1024

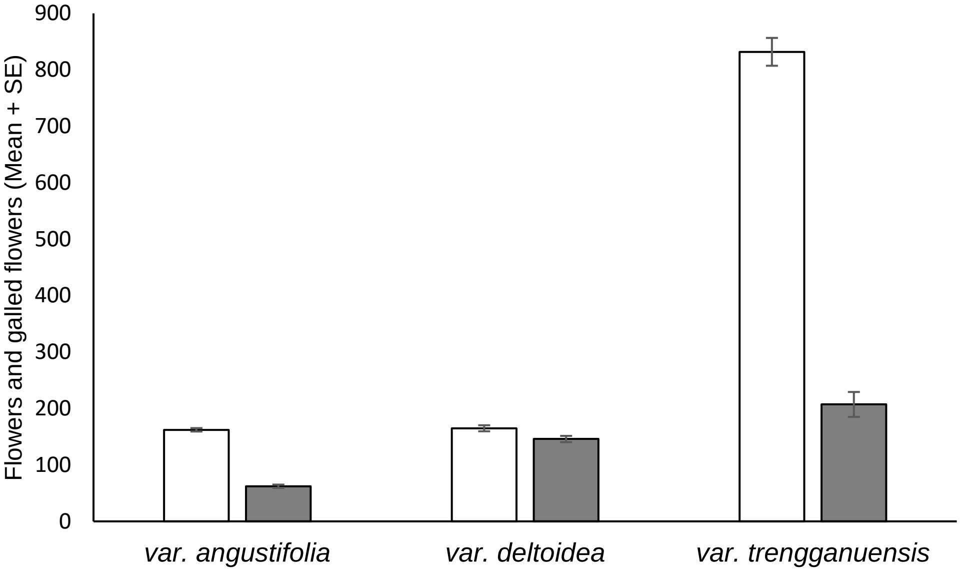


Figure 4.13 Female flower numbers in male figs of three varieties of *F. deltoidea* (open bars) and the numbers of flowers galled (offspring + empty galls) by *Blastophaga* foundresses (solid bars).

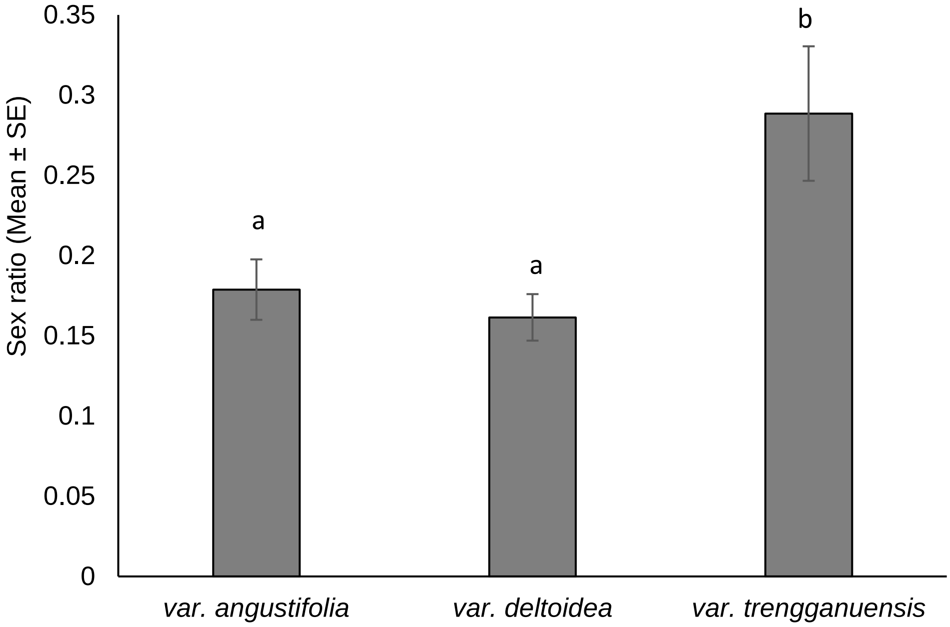


Figure 4.14 The offspring sex ratios in relation to total offspring in three varieties of *Ficus deltoidea*. (Mann-Whitney test, P < 0.05).

4.4.5 Inflorescence design in closely related fig trees: The effects of having different numbers of flowers in the figs

4.4.5.1 Relationship between fig flower numbers and other characters

The strong relationship between maximum diameter of female figs and the number of female flowers present is very clear when varieties are compared (

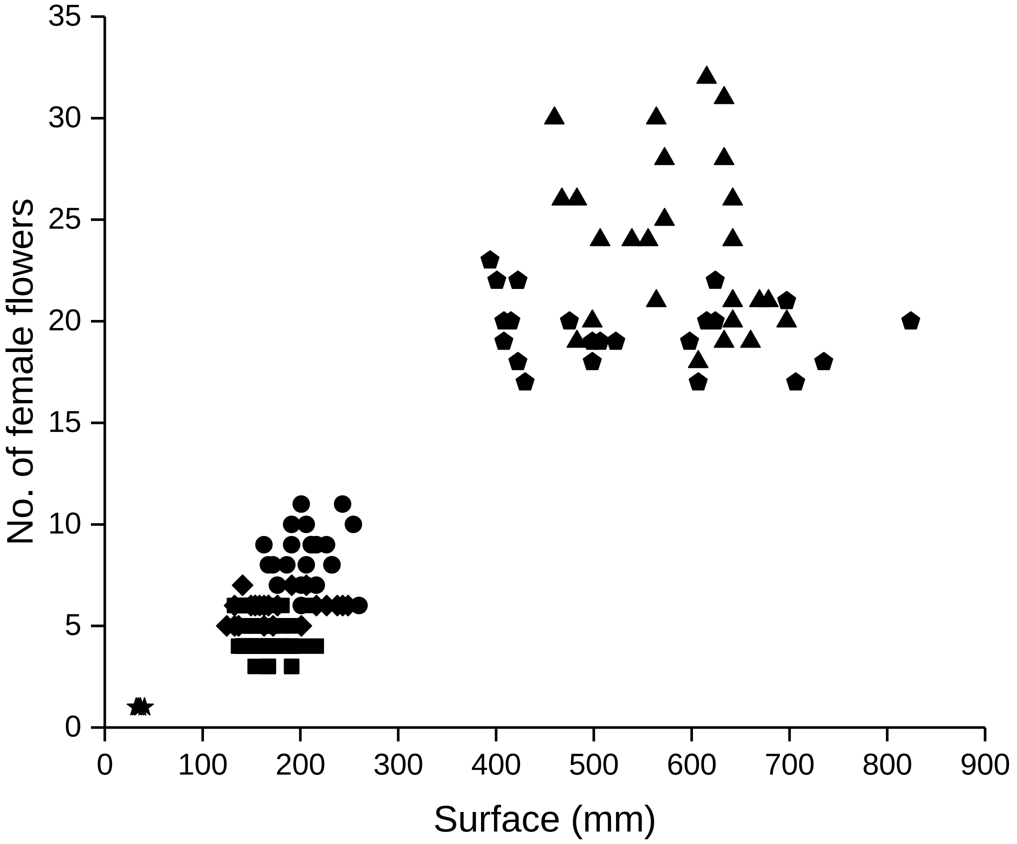


Figure 4.15, Linear regression, $R^2 = 0.84$, F = 1091.87, df = 204, $P < 0.001$). Within varieties, there was no relationship between the numbers of flowers in a female fig and the maximum diameter they reached (Table 4.26).

In the male figs, there was again a strong relationship between the maximum diameter of the figs and the number of female flowers (Figure 4.16, Linear regression, $R^2 = 0.75$, F = 610.52, df = 201, $P < 0.001$). Similar result insofar with female figs for the within varieties, where there was no relationship between the numbers of flowers in a male fig and the maximum diameter they reached (Table 4.27).

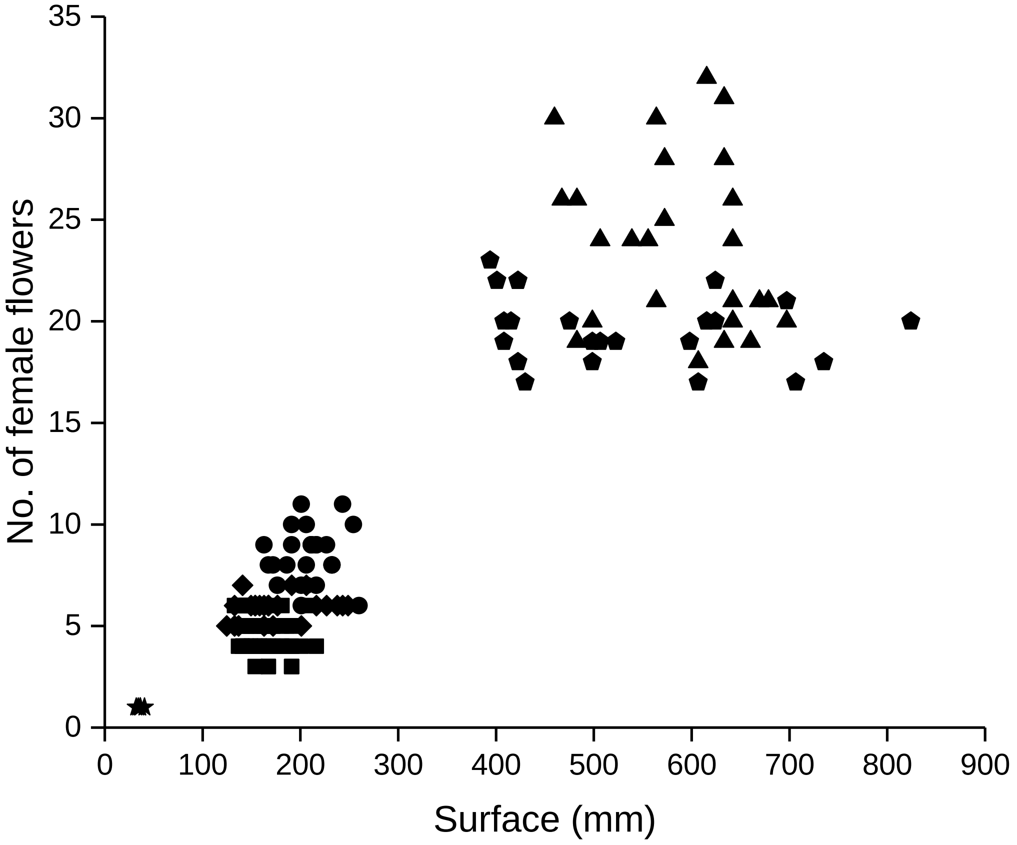


Figure 4.15 The relationship between maximum diameter of female figs and the number of female flowers in *F. deltoidea* varieties. (■) = var. *angustifolia*, (●) = var. *deltoidea*, (▲) = var. *trengganuensis*, (★) var. *bilobata*, (⬟) var. *kunstleri* and (♦) var. *motleyana*).

Table 4.26 Within-variety relationships between the maximum diameter of female figs and the numbers of female flowers they contain using Pearson correlation.

Variety	n (trees)	n (figs)	Pearson's r	P value
angustifolia	20	100	-0.14	0.15
deltoidea	2	27	0.08	0.70
trengganuensis	5	25	-0.23	0.27
kunstleri	5	25	-0.31	0.13
motleyana	5	25	0.27	0.19

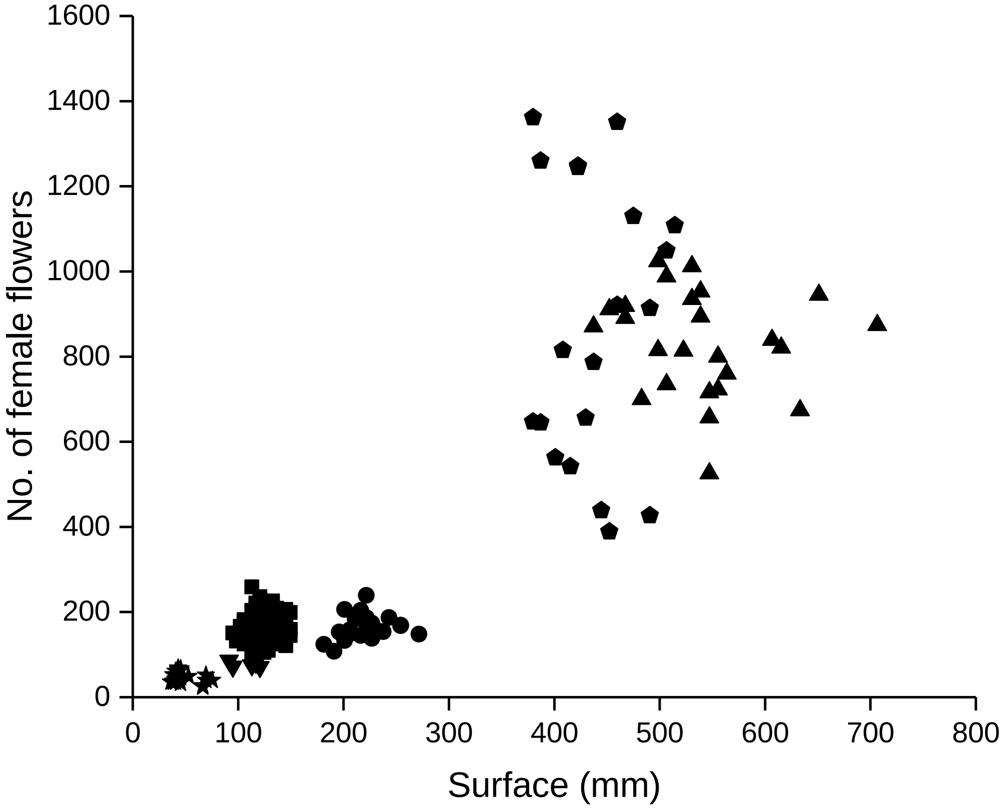


Figure 4.16 The relationship between maximum diameter and the number of female flowers in male figs in *F. deltoidea* varieties. (■) = var. *angustifolia*, (●) = var. *deltoidea*, (▲) = var. *trengganuensis*, (★) var. *bilobata*, (⬟) var. *kunstleri* and (▼) var. *intermedia*).

Table 4.27 Within-variety relationships between the maximum diameter of male figs and the numbers of female flowers they contain using Pearson correlation.

Variety	n (trees)	n (figs)	Pearson's r	P value
angustifolia	20	100	0.05	0.64
deltoidea	2	27	0.24	0.24
trengganuensis	5	25	-0.16	0.43
kunstleri	4	20	0.02	0.92
bilobata	5	5	-0.24	0.18
intermedia	5	25	-0.40	0.51

When figs of all the varieties are considered together, there was a strong relationship between numbers of male and female flowers in male figs (Figure 4.17, Linear regression, $R^2 = 0.90$, F = 1827.06, df = 201, $P < 0.001$). The relationship between numbers of male and female flowers in male figs was not strong, but they were positively correlated in two varieties (Table 4.28). The ratio of male to female flowers numbers was exceptionally high in var. *intermedia*, where more male than female flowers were present (Table 4.29). The rest typically had far more female than male flowers, but one fig of var. *bilobata* was an exception.

Female pollinator offspring provide a measure of the reproductive success of each male fig. When figs of all pollinated varieties are considered together, there was a strong relationship between numbers female flowers and number of female pollinators in male figs (Figure 4.18-Figure 4.20). Linear regression, $R^2 = 0.21$, F = 40.68, df = 151, $P < 0.001$). There was a positive relationship between the number of female flowers in the male figs of var. *angustifolia* and the numbers of female pollinator offspring that had developed there (Table 4.30). No significant relationship was found in the other two varieties.

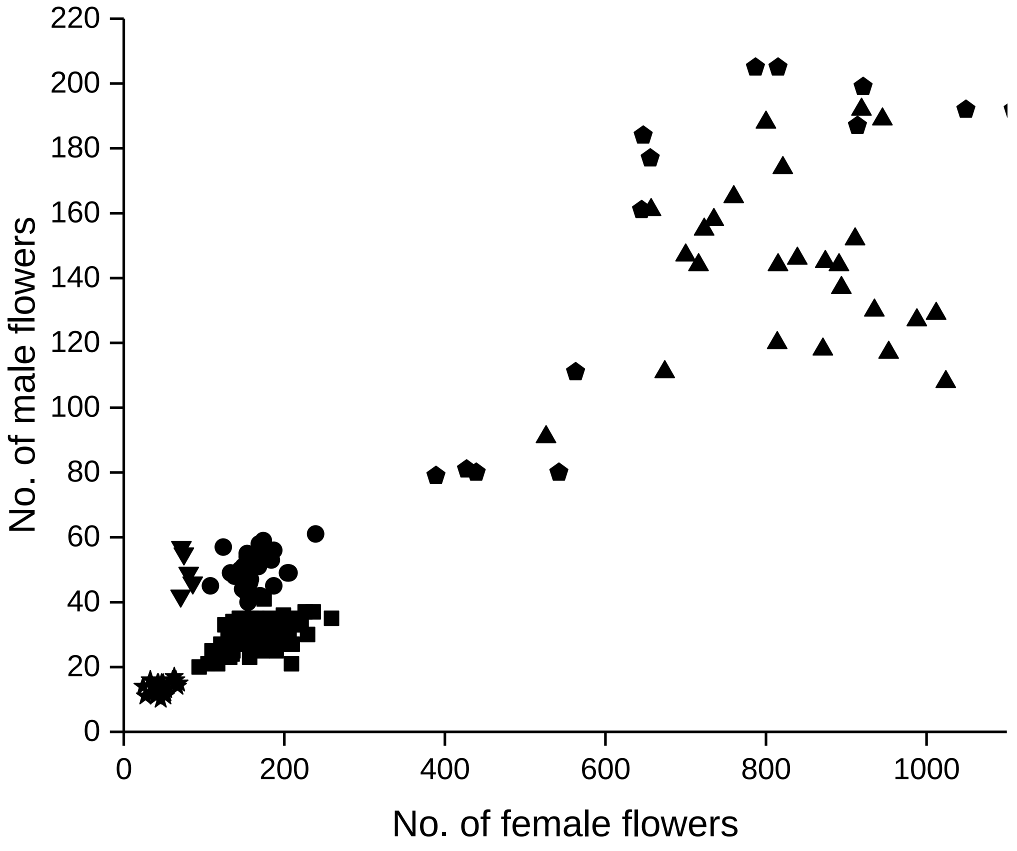


Figure 4.17 Relationships between the number of male flowers and the number of female flowers in male figs (■) = var. *angustifolia*, (●) = var. *deltoidea*, (▲) = var. *trengganuensis*, (★) var. *bilobata*, (⬟) var. *kunstleri* and (▼) var. *intermedia*. Each data point represents the individual fig.

Table 4.28 Within-variety relationships between the number of male flowers and the number of female flowers in male figs of *Ficus deltoidea* using Pearson correlation.

Variety	n (trees)	n (figs)	Pearson's r	P value
angustifolia	20	100	0.95	<0.001
deltoidea	5	27	0.34	0.08
trengganuensis	5	25	0.05	0.79
bilobata	5	25	0.35	0.09
kunstleri	4	20	0.87	<0.001
intermedia	1	5	-0.23	0.71

Table 4.29. The ratio of anthers to ovules in male figs of six varieties of *F. deltoidea*.

Variety	n (trees)	n (figs)	Ratio of male flower numbers to female flower numbers	
			Mean ± SD	Range
angustifolia	20	100	0.19 ± 0.03	0.13-0.26
deltoidea	5	25	0.31 ± 0.05	0.24-0.46
trengganuensis	5	25	0.18 ± 0.04	0.12-0.25
bilobata	5	25	0.31 ± 0.09	0.21-0.58
kunstleri	4	20	0.21 ± 0.04	0.15-0.28
intermedia	1	5	0.65 ± 0.11	0.53-0.79

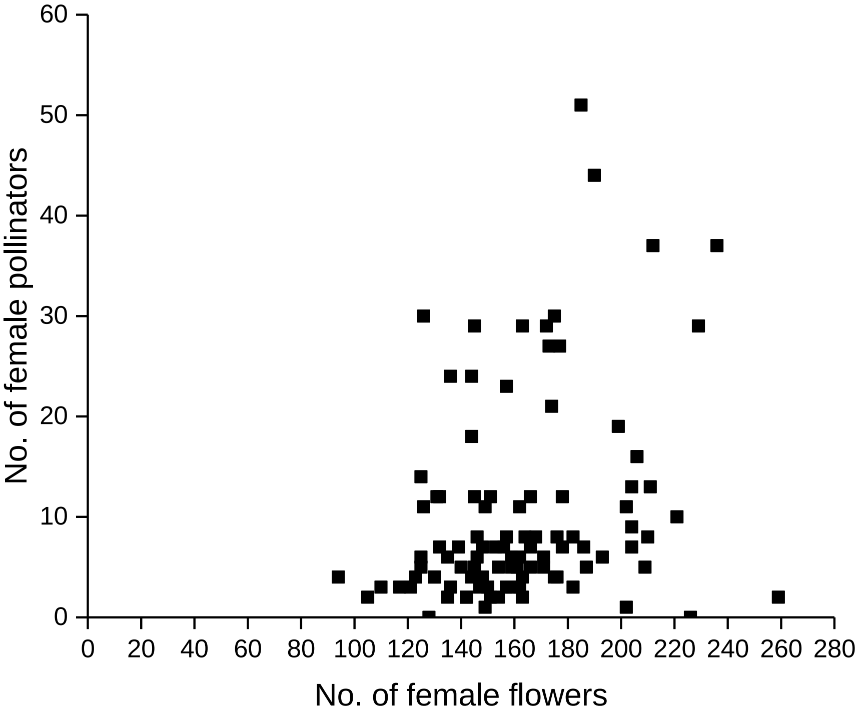


Figure 4.18 Relationships between the number of female flowers and the number of female pollinator offspring in male figs of var. *angustifolia*. Each data point represents one fig.

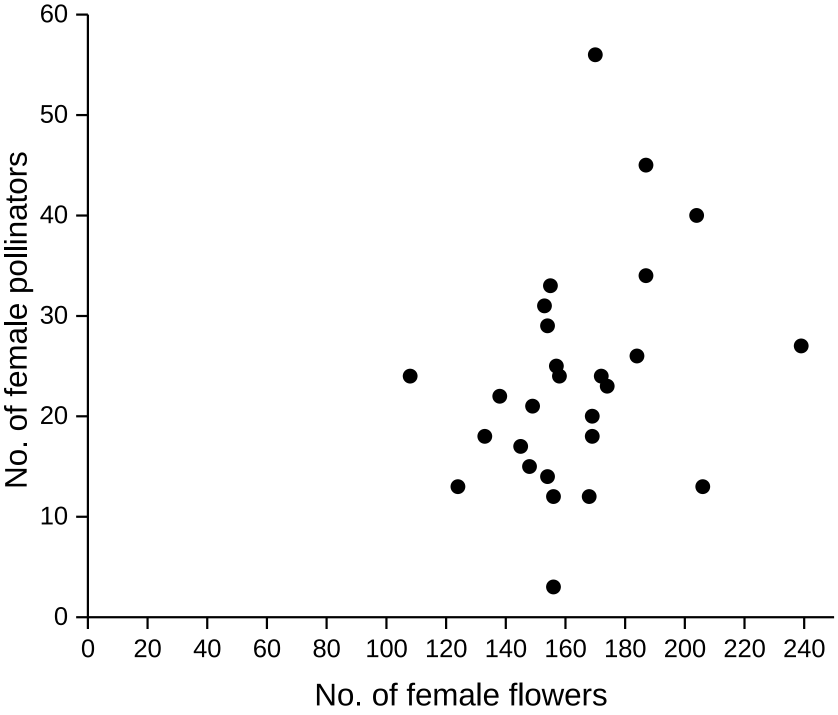


Figure 4.19 Relationships between the number of female flowers and the number of female pollinator offspring in male figs of var. *deltoidea*. Each data point represents one fig.

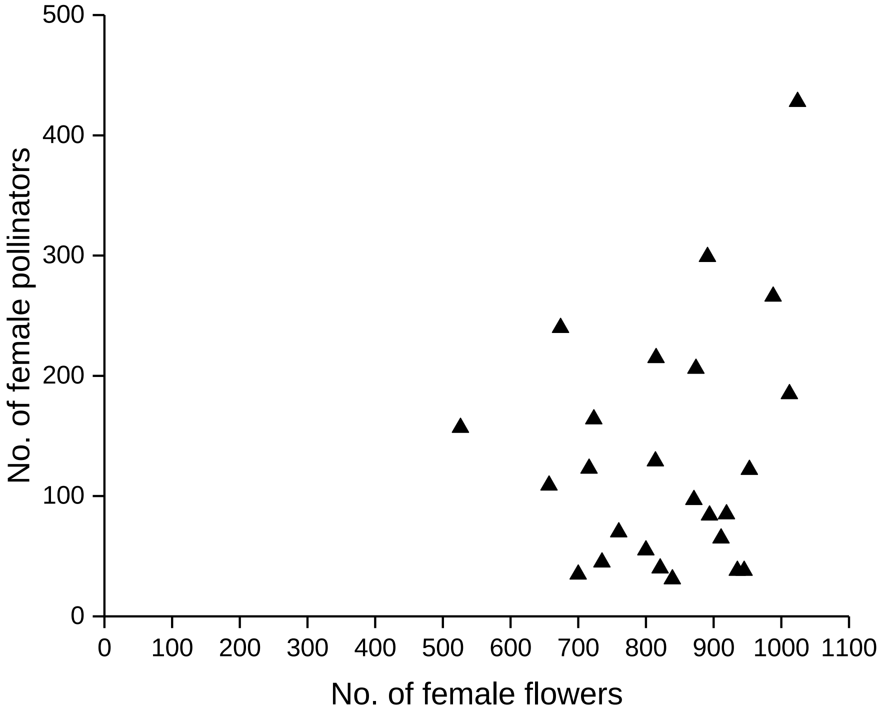


Figure 4.20 Relationships between the number of female flowers and the number of female pollinator offspring in male figs of var. *trengganuensis*. Each data point represents one fig.

Table 4.30 The relationships between numbers of female flowers and the number of female pollinator offspring in varieties of *Ficus deltoidea*.

Variety	n (trees)	n (figs)	Pearson’s r	P value
angustifolia	20	100	0.27	0.01
deltoidea	5	27	0.31	0.12
trengganuensis	5	25	0.13	0.54

The number of female flowers in male figs and the numbers galls and bladders produced were positively correlated across the three pollinated varieties (Figure 4.21, Linear regression, $R^2 = 0.43$, F = 111.79, df = 151, $P < 0.001$). In two of the varieties the total galled flowers (successful and failed galls (bladders) combined) there were more numerous in figs containing a greater number of female flowers (Table 4.31). This relationship was not present in var. *trengganuensis*

Varieties with more female flowers produced more seeds per fig (Figure 4.22, Linear regression, $R^2 = 0.76$, F = 466.91, df = 149, $P < 0.001$). This relationship was also present within the figs of two of the varieties, but not var. *angustifolia* (Table 4.32).

The relationship between the number of female pollinator offspring in a fig and the number of male flowers available to generate pollen for them to carry varied between varieties (Figure 4.23, Linear regression, $R^2 = 0.16$, F = 29.46, df = 151, $P < 0.001$). No significant relationship between number of male flowers in a fig and the number of female pollinators generated to transport the pollen was found in all varieties (Table 4.33. Ratios of the male flowers to the female pollinators was highest in var. *trengganuensis*, where a relatively small proportion of the female flowers in some of the figs had supported pollinator development (Table 4.34).

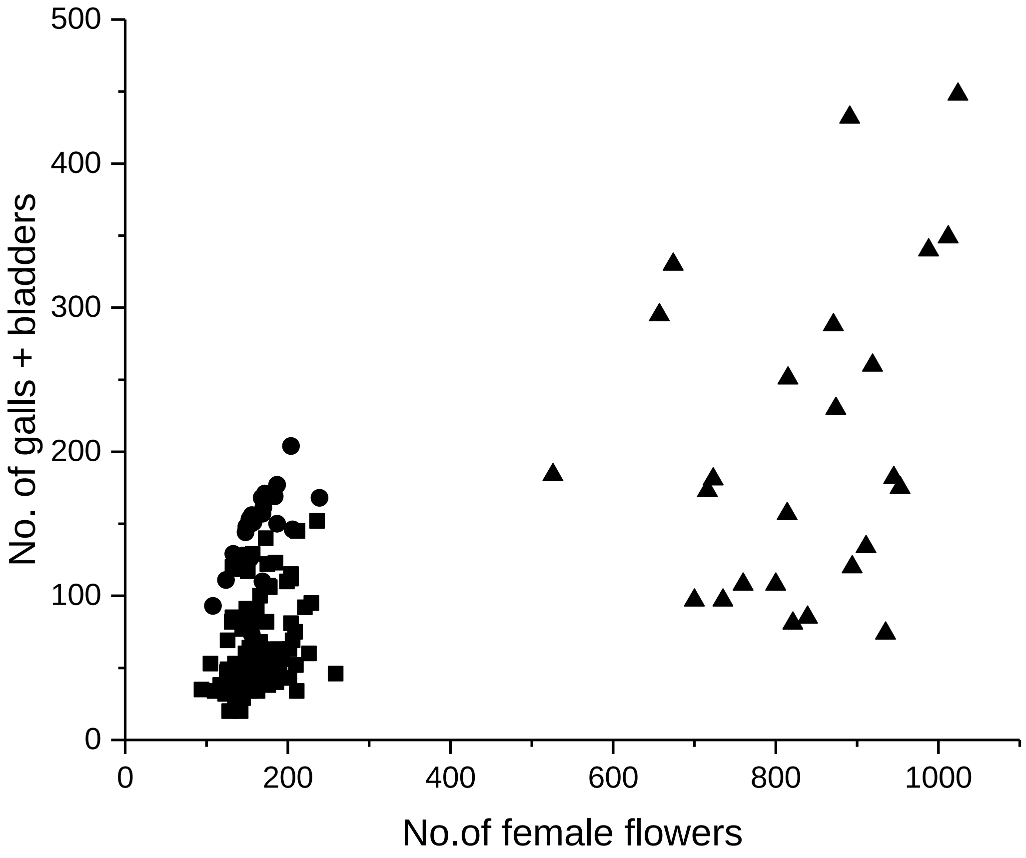


Figure 4.21. Relationships between the combined number of successful and failed galls (bladders) and the number of female flowers in male figs. (■) = var. *angustifolia*, (▲) = var. *trengganuensis*, (●) = var. *deltoidea*. Each data point represents one fig.

Table 4.31 Correlations between the total number of female flowers and the number of galls (successful and empty combined) in male figs of *Ficus deltoidea* using Pearson correlation.

Variety	n (trees)	n (figs)	Pearson's r	P value
angustifolia	20	100	0.42	< 0.001
deltoidea	5	27	0.63	< 0.001
trengganuensis	5	25	0.30	0.14

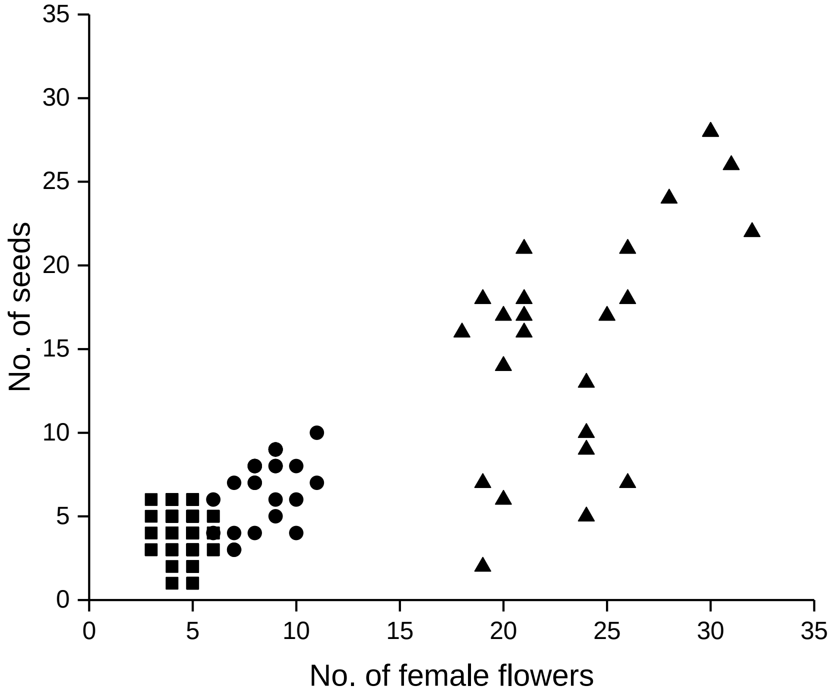


Figure 4.22 Relationships between the mean number of seeds per fig and the number of female flowers in female figs. (■) = var. *angustifolia*, (▲) = var. *trengganuensis*, (●) = var. *deltoidea*). Each data point represents one fig.

Table 4.32 Correlations between the number of female flowers and the number of seeds produced in the female figs of *Ficus deltoidea*.

Variety	n (trees)	n (figs)	Pearson's r	P value
angustifolia	20	100	-0.12	0.24
deltoidea	5	27	0.49	0.014
trengganuensis	5	25	0.61	0.001

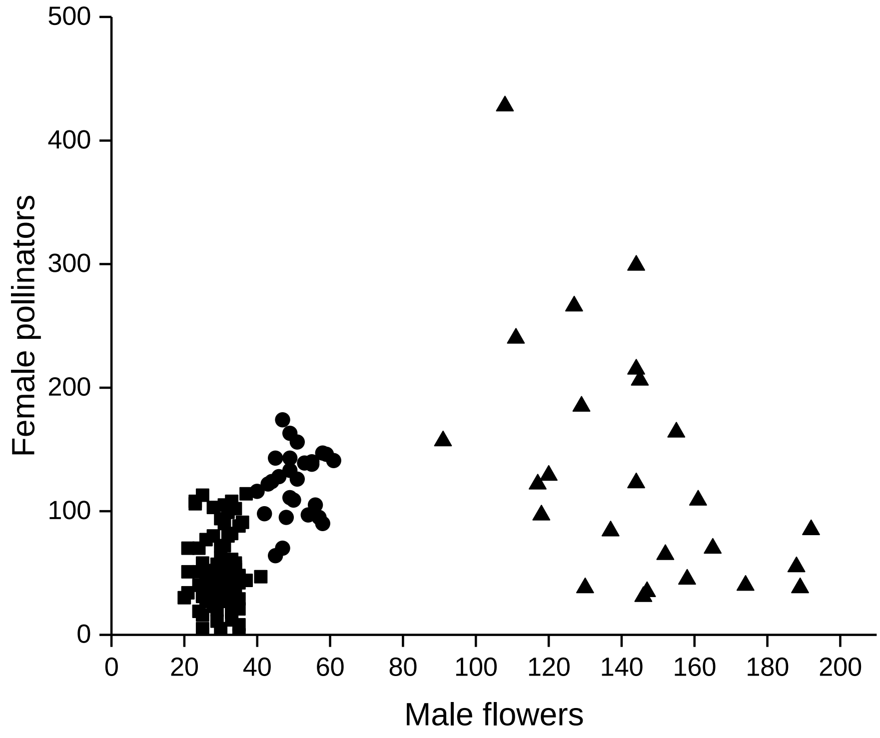


Figure 4.23 Relationships between the numbers of female pollinator offspring with the number of male flowers in the male figs where they developed. (■) = var. *angustifolia*, (●) = var. *deltoidea*, (▲) = var. *trengganuensis*). Each data point represents a single fig.

Table 4.33 Correlations between the number of female pollinator offspring and the number of male flowers in male figs using Pearson correlation.

Variety	n (trees)	n (figs)	Pearson’s r	P value
angustifolia	20	100	0.10	0.30
deltoidea	5	27	-0.18	0.39
trengganuensis	5	25	-0.10	0.67

Table 4.34 The ratios of male flowers to female pollinator offspring in three varieties of *Ficus deltoidea*.

Variety	n (trees)	n (figs)	Male flowers per female pollinators offspring	
			Mean ± SD	Range
angustifolia	20	100	0.86 ± 0.88	0.00-6.00
deltoidea	5	25	0.43 ± 0.12	0.12-0.70
trengganuensis	5	25	1.93 ± 1.52	0.25-5.00

4.5 Discussion

Seven of the 13 varieties in the *F. deltoidea* complex recognised by Corner (1969) are present in Peninsular Malaysia. They formed the basis of this comparative study, which aimed to assess the extent of the variation present in their figs. Natural populations of three varieties were also investigated, allowing their pollination biology and pollinators to be compared.

4.5.1 Variation between populations of *F. deltoidea* var. *angustifolia*

Var. *angustifolia* is the most widespread variety of *F. deltoidea*. It has the most reduction in leaf, fig and seed size and is considered the most derived variety within the *F. deltoidea* complex as described by (Corner, 1969). Comparisons of four populations of var. *angustifolia* in Peninsular Malaysia, which were separated by up to 552 km, found few differences in the structure and contents of their figs. The number of tepals in the female figs varied, and this may have reflected variation in the proportion of flowers that were fully developed. Seed lengths also varied, which may reflect soil nutrient variation (Suleman et al., 2013b) and some variation was also present in the number of unused flowers in female figs (but not the numbers of seeds produced). Differences in foundress numbers may have been responsible.

Populations differed in the total numbers of female flowers (and the tepals they contain) in their male figs. There may be a genetic basis to these differences as long distance gene flow may be rare in this species (Chapter 5). Soil and environment conditions may also have been responsible (Suleman et al., 2013b). The Dengkil plantation is on a silty soil, The Tembila plantation is on a sandy soil and the others on peat soil. Peat soils are very efficient as carbon and energy reservoirs and have very good water retention (Rezanezhad et al., 2016). The type of soil might influence the height of the host trees as Tembila showed the shortest oil palm trunk heights (Chapter 3) which might indicate that ability to retain water and nutrients is poor Weber et al. (2007). The effect of the soil might influence the individual fig characters as well as crop size.

The size of the pollinator females (as measured by gall size, ovipositor lengths and hind femur lengths) did not vary between populations. The number of galled flowers in male figs from different populations differed significantly. The extent of galling depends on the ability of foundresses to oviposit in the female flowers because galling is apparently initiated at the same time as oviposition. There is often a positive correlation between the number of foundresses entering figs and the number of flowers that are galled even if individual clutch sizes per female (Raja et al., 2008a, Suleman et al., 2013b). Foundress numbers are difficult to estimate in this species because they regularly re-emerge from figs (Chapter 7) but it is likely that the number entering male figs varied between populations. This variation was not seen in seed production, perhaps because female figs contain so few flowers that entry by a single foundress results in pollination of almost all the flowers.

4.5.2 Variation between varieties of *F. deltoidea*

The varieties of *F. deltoidea* are distinguishable morphologically based on their size, shape and coloration of the leaves (Nur Fatihah et al. 2014; Mat et al. 2012; Zimisuhara et al., 2015). The figs of some varieties also differ considerably in diameter. Overlap in characters has led to the variation being recognised in the form of many discrete varieties rather than species (Corner 1969) or most varieties not being recognised as distinct (Berg and Corner 2005).

The most obvious differences between the seven varieties (in both plant sexes) were fig sizes and the number of flowers they contain. Varieties with small figs also have small leaves and plants with big figs have big leaves. Varieties with small figs include var. *angustifolia*, var. *deltoidea*, var. *bilobata*, var. *intermedia* and var. *motleyana* while those with big figs include var. *trengganuensis* and var. *kunstleri*. This observation is concordant with the evolutionary trends in *F. deltoidea* described by (Corner, 1969). Morphological features that were less variable (but differed between some pairs of varieties) were tepal lengths in both sexes, style lengths in both sexes, seed lengths in female figs and gall lengths in the male fig. Significant differences between varieties were found in all the traits examined in both male and female figs, but the sampling was limited and the extent of within variety variation is only known in var. *angustifolia*.

The variation between the seven varieties examined and the smaller within variety variation agrees with the leaf and fig characterisation of varieties of *F. deltoidea* by Awang et al. (2013) and Zimisuhara et al. (2015). A molecular approach using chloroplast DNA (cDNA) and nSSR markers from 24 individuals of four varieties of *F. deltoidea* showed there was a complicated diversity present in this complex and divided them into two large groups reflected in leaf size (Tnah et al., 2016). They also concluded that var. *angustifolia* and var. *deltoidea* formed a distinct monophyletic group while var. *kunstleri* and var. *lutescens* were paraphyletic.

There was considerable variation in the numbers of flowers within the male figs but all the varieties had far more female flowers in male than female figs. This is in contrast to more usual pattern. Tarachai et al. (2008) for example recorded a mean of 858 female flowers in female figs of *Ficus hirta* Vahl. compared with a mean of 799 female flowers in male figs of this species. The unusual situation in *F. deltoidea* results from the unusually small numbers of flowers in the female figs (Corner, 1969). The extreme situation was found in female figs of var. *bilobata*, where they only produced a single female flower in each fig. Consistently high ratio of anthers to ovules confirmed that the varieties are likely to be passively pollinated.

4.5.3 Variation between fig wasps from three varieties of *Ficus deltoidea*

The pollinators of only three varieties were available for comparison. In contrast to their host plants, differences between pollinator females were small, although significant differences were present in the lengths of their ovipositors and hind femur. Significant differences were also present in the sizes of the galls they generated in different varieties. Gall size and hind femur length provide indications of the size of the insects. The ratio of ovipositor length did not differ between the pollinators of different varieties. The head shape also different between varieties (pers. Observation) where var. deltoidea tend to have m ore square head shape

In monoecious *Ficus* species mean style lengths are correlated with the ovipositor lengths of a tree's pollinator fig wasp, though not all individuals have ovipositors of sufficient length to oviposit in all the flowers (Nefdt and Compton, 1996). The male trees in dioecious species have figs with flowers that have far shorter styles than those in female figs. The styles grow in length faster in female figs and in both sexes reach maximum length during receptivity before shrinking afterwards (Ghana et al., 2017). As in other dioecious fig trees, the ovipositors of the pollinators of the three varieties were too short to reach the ovules of flowers in female figs but could reach all of the ovules in male figs.

The shortest mean ovipositor length was found in var. *angustifolia* which has small figs and the longest ovipositors were from var. *deltoidea*, which also has small figs. The ovipositor lengths of the pollinator of var. *trengganuensis*, which has big figs, were in between. The shortest ovipositor length (in pollinators from var. *angustifolia*) was still sufficient to penetrate the longest style lengths in male figs (in var. *trengganuensis*). This means the style length differences do not act as a barrier preventing pollinators ovipositing in figs of other varieties. In most *Ficus* species, non-pollinating fig wasps develop alongside pollinators in the figs and their host preferences can give an indication of differences between similar host plants. However, in the three *F. deltoidea* varieties no non-pollinating fig wasps were present.

4.5.4 Variation in reproductive output in different varieties of *F. deltoidea*

Factors that determine the number of fig wasp offspring in figs include the number of female flowers (Suleman et al., 2013a), the size of the foundresses (Herre et al., 1989), the number of foundresses entering the figs (Jousellin et al., 2001; Kjellberg et al., 2001) and non-pollinating fig wasp numbers (Tarachai et al., 2008). Higher temperatures lower the egg-laying capabilities of the pollinators that survive to enter figs (Wang et al., 2011) and temperatures during the development of fig wasp offspring influence offspring survival (Krishnan et al., 2014). Seed numbers are influenced by female flower numbers (Suleman et al., 2013a), the number of foundresses entering the figs (Jousselin et al., 2001) and the amount of pollen carried by the foundresses (Kjellberg et al., 2014). In the passively pollinated *Ficus carica*, only 10% of pollen is successfully transported away from the natal figs and the first foundresses to emerge load more pollen and are capable of pollinating more flowers (Kjellberg et al., 2014).

In this study, the varieties with figs containing more flowers produced more seeds and fig wasp offspring. The efficiency of seed production (the proportion of female flowers producing seeds) was consistently high as all three varieties recorded more than 60% of their flowers being pollinated. *Ficus hirta* Vahl. has female figs with many more flowers and seed production efficiency had a mean at nearly 50% (Yu et al., 2008). The highest efficiency was from var. *angustifolia*. Female figs of *F. deltoidea* contain so few flowers that it might be expected that a single foundress could easily pollinate all the flowers, even if they are passive pollinators like *Blastophaga* sp. This suggests either that a single foundress adequately pollinates var. *angustifolia*, but not var. *deltoidea* and var. *trengganuensis* that have more flowers, or that irrespective of foundress numbers, pollination rates are more efficient in figs with fewer flowers. Figs with a higher number of flowers like var. *deltoidea* and var. *trengganuensis* will need more foundresses to enter the figs, which the plant can control to some extent by extending the time that the ostiole stays open after the first foundress has entered.

Male figs of var. *deltoidea* had the highest efficiency. The number of foundresses that entered each fig is likely to be the major reason for the higher efficiency in var. *deltoidea*. The high density of the *F. deltoidea* in Batu Pahat (Chapter 2 and Chapter 3) should help increase pollinator densities, but in a species like *F. deltoidea* where one foundress can pollinate several figs (Chapter 7), it may be that survival of foundresses walking between figs was also important. Jousellin et al. (2001) inferred that having more foundresses entering a single fig gives more advantage to the figs than the fig wasps as they bring more pollen, but also increase competition for oviposition sites. Having foundresses that can re-emerge from figs may reduce this difference.

4.6 Conclusions

(1) No significant morphological differences were detected between the figs from different populations of *F. deltoidea* var. *angustifolia* in Peninsular Malaysia.

(2) Morphological differences were present between the figs of different varieties, especially in the numbers of flowers that the figs contained, but the fig wasps associated with different varieties were very similar in size.

(3) No differences in the size in the fig wasps could be related to variation in their host figs and style lengths do not act as barrier to pollinator oviposition in different varieties.

Chapter 5

Host specificity of *Ficus deltoidea* pollinators

5.1 Introduction

5.1.1 Specificity of interactions between plants and insects

Interactions between plants and herbivores are often highly species-specific. Among tropical feeding guilds, granivores (seed eaters) showed the highest specificity while root feeders showed the least (Novotny et al., 2010). Among the interactions between plant and herbivores, pollination is a non-antagonistic relationship (mutualism) and co-evolution between these two groups has often led to speciation and an increase in biodiversity (Bruce, 2015). The rewards provided by the plants shape the behaviour of their pollinators (Nepi et al., 2018). Plants evolved their floral traits (colour, shape, odour, size) (Reverté et al., 2016) and floral rewards (pollen, sugar, oils) to attract potential pollinators (Gardener and Gillman, 2002). Some plants have few species of host specific pollinators, while others attract many species of pollinators that are not host specialists. The cues and rewards they provide shape the nature of this interaction (Ashman, 2009).

Nursery pollination differs from conventional pollination because the rewards for the pollinators are the sites for mating and developing their offspring (Sakai, 2002). The specificity in nursery pollination is often high, with one plant species pollinated by only or a few insect pollinators (Rodriguez et al., 2017). Among nursery pollination systems the fig tree (*Ficus*, Moraceae) and fig wasp (Agaonidae) mutualism displays this high specificity. Coevolved mutualisms often exhibit high levels of partner specificity. Obligate pollination mutualisms, such as the fig –fig wasp and yucca–yucca moth systems, represent remarkable examples of such highly species-specific associations. Mutualism favours higher host specificity than does antagonism in plant –herbivore interaction.

5.1.2 *Ficus* pollination

In the fig tree and fig wasp nursery pollination mutualism, the fig wasp progeny feed on the endosperm tissue of the galled ovary (Cook and Segar, 2010) and they are included under group one as classified by Sakai (2002) (see Chapter 1) because the offspring are seed/ovule parasites where the pollinator offspring feed on galled ovules which may or may not have been pollinated. The pollination of figs begins away from the trees when female fig wasps detect volatiles emitted by the receptive figs of their host tree (Wang et al., 2013). The scent acts as signal to the wasp that the fig is receptive and ready to be pollinated (Hossaert-McKey, 2016). This specific scent is usually developmental stage and species specific (Chen et al., 2009).

Once a fig wasp has landed on a receptive fig, it often examines the surface of the fig before deciding to go in and may be responding to physical and surface chemical cues. Their morphology needs to be well adapted to their host figs in order to gain access to the florets via the narrow ostiole (van Noort, 2003), which is the link between the outside and the flowers on the inside of the figs (Liu et al., 2013). The specific pollinator will have a head shape that is correlated to fig size (van Noort and Compton, 1996). Female fig wasps possess several adaptations allowing them to pass through the ostiole such as flattened heads, modified mandibles (Compton, 1993), and spikes on certain body parts (Harrison, 2005). This ostiole acts as a physical barrier preventing females of non-adapted pollinator species from gaining entry into the fig cavity, from where the ovules are accessible (van Noort, 2003; Liu et al., 2003).

During passage through the ostiole, pollinator females lose their wings and most of their antennae (van Noort, 2003). They do not need the wings and all their antennae once they are in the fig cavity, and their loss ensures that the insects cannot fly off again to other trees. Soon after the entrance of the pollinators, the ostiole will close up and seal off the fig cavity (Nefdt and Compton, 1996). Once in the fig cavity, the pollinators need to deposit their eggs into the female flowers and also to gall them. Gall formation involves expansion of the ovary. Gall production by insects is typically highly species specific, and the ability to gall may be an important factor in the host specificity of fig wasps (Ghana et al., 2015a).

An early record of pollinator sharing was in 1989 where the pollinator of *Ficus burtt-davyi* Hutch (*Elisabethiella baijnathi* Wiebes) was introduced to Kirstenbosch Botanical Garden in Cape Town. Not only did it pollinate the *F. burtt-davyi*, it also pollinates and reproduces in two other *Ficus* species namely *Ficus craterostoma* Warb. ex Mildbr. & Burret and *Ficus stuhlmannii* Warb. that were planted in the garden (van Noort, 2003). The seeds were not viable beyond the cotyledon stage (van Noort, 2003). However, the wasps reproduced in all three figs but they can only exit the figs of *F. burtt-davyi*. The wasps were able to reproduce and offspring emerged into the cavity of *F. craterostoma* and *F. stuhlmannii* figs but failed to exit. This suggests another way in which host specificity is maintained

In monoecious fig trees, the styles of the female flowers vary in length. The shorter ones will mainly support the development of fig wasp progeny while the longer styles will support the production of seeds (Herre et al., 2008). In dioecious fig trees, the female fig wasps that enter female figs pollinate the female flowers and these produce seed. The pollinators gain nothing by entering the female figs as no offspring will be produced (Hossaert-McKey, 2016). Pollinators that enter the figs of male trees can deposit their eggs and transform the ovule into a gall where the larvae will feed (Ghana et al., 2015a). For this purpose, they must have an adequate ovipositor length that suits the style length of the receptive female flowers (Nefdt and Compton, 1996; Weiblen 2002; van Noort, 2003). In dioecious fig trees, all female flowers in the male figs are short-styled making them suit the pollinator's ovipositor length (Nefdt and Compton, 1996).

5.1.3 The breakdown of host specificity

It used to be thought that a single *Ficus* species was routinely pollinated by only one specific fig wasp. This was known as the 'one-to-one' rule (Rasplus, 1996; Weiblen, 2002). Some exceptions were known, but the development of a modern molecular approach has shown that one fig tree species often has more than one pollinator (Cook and Segar, 2010) and in rare instances a single fig-wasp can pollinate several fig trees (pollinator sharing) (Kerdelhue et al., 1997; Su et al., 2008) making the relationship less specific than early research suggested.

Co-pollination is a term used when one plant species is associated with more than one pollinator. Based on molecular studies, the occurrence in *Ficus* is more than 30% and this figure will inevitably increase (Yang et al., 2015). One classic finding of co-pollination was from (Michaloud et al., 1985), which discovered two pollinating wasps (*Courtella camerunensis* Wiebes and *Courtella gabonensis* Wiebes) were able to pollinate *Ficus ottoniifolia* Miquel in Gabon (Africa). However, the fig trees occupy different geographical locations. In the rain forest, *C. camerunensis* tend to be more abundant than *C. gabonensis* while in open habitat, the situation was reversed with the almost total exclusion of *C. camerunensis*. On the other hand, in the savannah of Ivory Coast, *C. gabonensis* appear to be the sole pollinator for *F. ottoniifolia* with exclusion of *C. camerunensis*. This result contributed to the suggestion by Kerdelhue et al., (1997) that different geographic distributions of pollinators can lead to two wasp species pollinating the same *Ficus* species.

Ficus sur Forsskal, another African species, also has more than one fig wasp that pollinates their flowers (*Ceratosolen flabellatus* Grandi and *Ceratosolen silvertrianus* Grandi). They can be either sole pollinators or can co-occur in the same fig (Kerdelhué et al., 1997). Other research by Kerdelhue et. al. (1997) and Proffit and Johnson (2009) discovered that another pollinator for *F. sur* was *Ceratosolen capensis* Grandi. The pollinators can exist in the same crops but rarely in the same figs. Inter-specific competition was not recorded in this study as the production of seeds and wasp offspring were similar between a fig that was pollinated by a single wasp species and the presence of both fig wasp species in the same fig (Kerdelhue et al., 1997). The figs are big and can accommodate lots of pollinator foundresses. Whether or not competition occurs will depend on the total number of foundresses that enter. At low densities there will be no competition. Cornille (2012) has proposed that one barrier that can mediate inter-specific competition between two pollinators is if the ostiole will close rapidly once entered, and the arrival times for the species varies. Liu et al., (2013) noted that attractant volatile compounds decrease significantly after foundress entry making it hard for later pollinators to detect.

In the African tree *Ficus sycomorus* L. two different fig wasps colonise the fig trees namely *Ceratosolen arabicus* Mayr and *Ceratosolen galili* Wiebes (Compton et al., 1991). However, only *C. arabicus* pollinates the flowers while *C. galili* does not pollinate. *C. galili* was found to also enter figs of *Ficus mucoso* Ficalho (van Noort, 2003). *C. galili* was found to be smaller than C. *arabicus*. This might be the reason why *C. galili* is able to develop in un-pollinated flowers of *F. sycomorus*. A study by Liu et al., (2013) has found that being smaller has advantages for fig wasps entering figs and they can penetrate the ostiole more easily.

A breakdown of host specificity occurs when a fig wasp responds to the volatile chemicals emitted by non-usual host receptive figs, successfully gains access to the fig cavity via the ostiole and lastly, is able to reproduce inside (van Noort, 2003). Some fig trees produce less specific stimuli that can attract more than one pollinator. Geographical area and season might also cause scent variation even in the same species (Rodriguez et al., 2017). This variation in the floral volatiles may allow a few species of pollinators to co-exist in a single fig tree (Wang et al., 2016). A study by Ware and Compton (1992) found that receptive *Ficus lutea* Vahl were entered by females of *Elisabethiella stuckenbergi* Grandi, *Elisabethiella baijnathi* Wiebes and *Ceratosolen capensis* Grandi. All those pollinators were not the normal pollinator of *F. lutea*. This case was considered as showing mistakes that happens due to the absence of the normal pollinator and the figs remaining receptive for longer (Liu et al., 2013). However, the ostiole of *F. lutea* may have acted as barrier and prevented *E. baijnathi* from entering the figs, leaving only two pollinators able to enter through the ostiole. However, only *E. stuckenbergi* was able to reproduce (Ware and Compton, 1992). In a study by Wang et al., (2016), the pollinator of *Ficus hainanensis* Merr. and Chun was attracted to the volatiles emitted by the receptive figs of *Ficus auriculata* Lour. However, the normal pollinators of F. *auriculata* were not attracted to the receptive figs of F. *hainanensis*, which makes the relationship asymmetric.

Ficus microcarpa Blume is associated with several pollinator fig wasps within its native area (Wang et al., 2015) and a single *Ficus* tree can have up to four different agaonid species (Compton et al., 2009). In Uganda, *Alfonsiella brongersmai* Wiebes, *Alfonsilella natalensis* Wiebes, *Elisabethiella allotriozoonoides* (Grandi) and *Alfonsiella longiscarpa* Joseph were found in figs of *Ficus natalensis* Hochst. and three of the pollinators generated viable seed. Studies by other researchers also discovered different pollinators of *F. natalensis* apart from those four agaonid wasps. A study by Cornille et al., (2012) discovered a single pollinator wasp *Elisabethiella stuckenbergi* Grandi was able to pollinate two different host figs. The first host fig was *F. natalensis* that also has another normal pollinator which is *Elisabethiella socotrensis* (van Noort, 2003) and they can co-occur in the same fig. Another fig species pollinated by *E. stuckenbergi* was *Ficus burkei* (Miq.) Miq. that also has a different routine pollinator (*Alfonsiella brongersmai* Wiebes) in East Africa (Cornille et al., 2012). Similarities in odours released by *F. burkei* and *F. natalensis* might be the reason why they can share pollinators (Cornille et al., 2012).

Some fig wasp species acquire new host species and build new relationships. Wachi et al. (2016) showed that two fig tree species (*Ficus formosana* Maxim. and *Ficus tannoensis* Hayata) have lost their native pollinators and have acquired new pollinators from *Ficus erecta* Thunb. A single *Blastophaga* now pollinates three different fig tree species. Liu et al. (2015) also found that absence of the routine pollinator and host shifting from *Ficus heterosyla* Merr. resulted in new pollinators of *Ficus squamosa* Roxburgh. The male trees of *F. heterostyla* have female pollinators that emerge at the same time as receptive figs of *F. squamosa*. Similarities in flowering phenology may have led to this pollinator sharing in *F. heterostyla* and *F. squamosa* in China (Liu et al., 2015).

Most of the examples of pollinator sharing come from monoecious fig trees (Wachi et al., 2016) but recently a number of breakdowns have been found in dioecious species such as *F. squamosa* (Wang et al., 2016). Pollinator host changes can lead to speciation as the fig wasps adapt to their novel host figs (Wachi et al., 2016). Hossaert-McKey et al. (2010) proposed that co-pollinators were more likely where (1) fig trees live in the same geographical area (2) there is similarity in flowering phenology between species (3) there are similarities in fig morphology (size) and (4) there are similarities in the volatiles emitted by their receptive figs. Liu et al., (2015) added an absence of native pollinators to this list.

5.1.4 Specificity of pollination in *Ficus deltoidea* varieties

Ficus deltoidea Jack. (Moraceae) is distributed across Southeast Asia. Berg and Corner (2005) place *F. deltoidea* in Subgenus *Ficus*, Section *Ficus*, and Subsection *Frutescentiae*. It is mainly distributed in Peninsular Malaysia, Sumatra, Java and Borneo, where it is most diverse and may have originated (Corner, 1969). *F. deltoidea* is dioecious, where the male and female functions are separated. Some varieties of *F. deltoidea* are unusual in that they are true epiphytes whereas most *Ficus* species are stranglers (hemiepiphytes) or free-standing trees (Starr et al., 2003). As well as being epiphytic, this complex contains varieties that can be epilithic (growing on the surface of rocks) or terrestrial shrubs and treelets (Berg and Corner, 2005). Individuals of the same variety can have different growth forms. They are well adapted as epiphytes by having slow growth and large seeds to overcome the nutrient scarcity where they grow (Berg and Corner, 2005).

The numerous varieties under *F. deltoidea* are discriminated through their leaf morphology and anatomy (Nur Fatihah et al., 2014). The most prominent leaf characteristics that differentiate varieties in *F. deltoidea* are their pinnate venation, obtuse deltoid leaf and forked vein (Corner, 1969). Within the same variety, they can have different leaf characters in young and mature leaves (Awang et al., 2013). The varieties also exhibit variation in their figs and flowers (Berg and Corner, 2005). A morphological study by Nur Fatihah et al. (2014) recognised two sub-species and seven varieties of *F. deltoidea* from Peninsular Malaysia. These are var. *deltoidea* Corner, var. *angustifolia* (Miq.) Corner, var. *trengganuensis* Corner, var. *bilobata* Corner, var. *intermedia* Corner, var. *kunstleri* (King) Corner, and var. *motleyana* (Miq.) Corner. Among these varieties, variety *angustifolia* is the most widespread and has been considered as the most derived while variety *motleyana* may be close to the ancestors of all the varieties (Corner. 1969).

Little information is recorded on the pollinator or pollinators of *F. deltoidea*. A general pattern regarding the Subgenus and section *Ficus* has been established where dioecious Subgenus *Ficus* section *Ficus* is usually pollinated by a fig wasp from the genus *Blastophaga* (Wiebes, 1979). *Blastophaga quadrupes* Mayr is the only recorded pollinator of *Ficus deltoidea*. It probably pollinates var. *lutescens* Desf. as it was collected in Java and Sumatra (Wiebes, 1993). In a study by Laman and Weiblen (1998), two different varieties of *Ficus deltoidea* (var. *borneensis* and var. *motleyana*) present in Gunung Palung, Indonesia were also recorded as pollinated by *Blastophaga quadrupes*. However, in apparent contradiction to this and Corner's (1969) conclusions, there is indirect evidence that some at least of the varieties of *F. deltoidea* may be biologically distinct species, because different species of pollinator fig wasps have been reared from them in Brunei (F. Kjellberg, personal communication to S.G. Compton 2016). However, this is not conclusive, because a single *Ficus* species can support more than one pollinator species (Cook and Segar, 2010), often in different areas of the plant's range or when plants are growing in different habitats (Souto-Vilaros et al., 2018).

Floral and inflorescence differences (Chapter 4) also suggest that different pollinators may be associated with different varieties of *F. deltoidea* and that this taxon may be a complex of closely related but biologically distinct species. The taxonomic and biological status of these varieties is unclear, as is the extent to which different varieties support different fig wasp pollinators. There are numerous studies on the pharmaceutical properties of *F. deltoidea* varieties, but no study has been conducted on the composition of the attractant volatiles they release or whether pollinators from one variety are only attracted by the volatiles of that variety.

5.2 Objectives

In this study, the names of *F. deltoidea* varieties are based on Corner (1969). The following questions concern the specificity of its fig wasps and whether they are associated with particular varieties. Specifically, (1) what is the host specificity of the pollinator of *Ficus deltoidea* var. *angustifolia* and (2) will it also pollinate other varieties? We also examine questions related to the pollination of this variety of *F. deltoidea* that provide context for the specificity of its pollination. (3) Do the fig wasps prefer male figs over female figs? (3) How willing are pollinators to move from their natal male trees?

5.3 Methods

5.3.1 Study species

Plants in containers were used in the experiments together with plants belonging to variety *angustifolia* that were growing in situ as epiphytes on oil palms. Pot-maintained plants belonging to seven varieties of *Ficus deltoidea* were obtained on loan from the germplasm collection of Universiti Sultan Zainal Abidin (UniSZA), Tembila campus in Terengganu, an eastern coast state of Peninsular Malaysia. The varieties were var. *angustifolia*, var. *deltoidea*, var. *trengganuensis*, var. *kunstleri*, var. *bilobata*, var. *motleyana* and var. *intermedia*. At the time of collection, none of the figs on the plants in the germplasm collection had been pollinated. They varied from one to 10 years old. The trees originated from cuttings from several places in Malaysia namely Johor, Kelantan, Melaka, Terengganu, Sabah and Sarawak. They were maintained in either 39 cm or 49 cm diameter clay pots. The media for all the fig trees was a combination of top soil, pome, rice husk and peat soil. All of the collection were given NPK Green organic fertilizer and were watered regularly.

In addition, four pot-maintained female trees of *F. deltoidea* var. *angustifolia* were obtained on loan from the glasshouse of Universiti Kebangsaan Malaysia (UKM) and three male plants of this variety were bought from Batu Pahat, Johor. The male trees from UKM were bought from MARDI Serdang in 2013 while the Batu Pahat trees were propagated through cuttings from KESEDAR, Kelantan in 2006.

The collections from UKM and Batu Pahat were maintained in 1 litre poly planter bags with peat soil before being transferred to clay pots with the same diameters as in Terengganu germplasm collection (TGC) collection. Once they were transferred to the TGC, they were given the same fertilizer and the same media. All the trees were healthy and producing new figs before the experiments were conducted.

5.3.2 Study sites

Experiments were carried out at three Peninsular Malaysian study sites. The first was an oil palm plantation in Kampung Olak Lempit which is in Banting district in the state of Selangor. It is located approximately 50 kilometres from Kuala Lumpur city centre. The climate of the area is described in Chapter 2. The plantation is about 2.1 hectares in area and was planted in 2001. This plantation is managed by a small-scale farmer who lives on the site. There are about 113 epiphytic individuals of *F. deltoidea* var. *angustifolia* on the 285 oil palm trees in the plantation (Chapter 3). These include some big male trees of var. *angustifolia* that were convenient for experimental purposes. The phenology of the fig trees is described in Chapter 6.

The second study site was at the Terengganu germplasm collection (TGC) of Universiti Sultan Zainal Abidin (UniSZA). This collection was established in 2006 and contains 165 medium-large individuals of *Ficus deltoidea* varieties and many smaller plants. It contains eight varieties: var. *angustifolia*, var. *deltoidea*, var. *trengganuensis*, var. *kunstleri*, var. *bilobata*, var. *motleyana*, var. *intermedia* and var. *borneensis* Corner. Of all these varieties, only var. *borneensis* is not native in Peninsular Malaysia. They were propagated from cuttings taken from all over Peninsular Malaysia while the var. *borneensis* were received from the Sarawak Biodiversity Centre. Because no permission to use the var. *borneensis* was granted and this variety is not native in Peninsular Malaysia, the experiment only used the other seven varieties. All the pots were maintained in clay pots.

The third study site was at Parit Maimon, situated in Batu Pahat district in the state of Johor. This site is about 239 km (150 miles) south of Kuala Lumpur city centre. Within this site, two epiphytic varieties of *F. deltoidea* (var. *angustifolia* and var. *deltoidea*) have colonised oil palm trunks. They are also planted in the ground within the site’s residential area together with two other varieties, namely var. *kunstleri* and var. *trengganuensis*.

5.3.3 Host choice by *Blastophaga* sp. (var. *angustifolia*)

In this study, the pollinators of *F. deltoidea* var. *angustifolia* were used in several manipulation experiments. *Ficus* species in subgenus *Ficus* and section *Ficus* are pollinated by fig wasps belonging to the genus *Blastophaga*. The identity of the pollinator of var. *angustifolia* is unknown so the pollinator of this variety is called *Blastophaga* sp. (var. *angustifolia*) here. The pollinator of var. *deltoidea* is referred to in a similar way.

5.3.3.1 Transplant experiment one

Twenty-five *Ficus deltoidea* individuals from seven varieties were obtained on loan from TGC and moved by lorry to the oil palm plantation in Kampung Olak Lempit, Banting. The distance between TGC and Banting was about 566 km. The plants were of a convenient size, in pots of 39 cm and 49 cm diameter, and all were producing figs. The varieties were var. *angustifolia*, var. *deltoidea*, var. *trengganuensis*, var. *kunstleri*, var. *bilobata*, var. *motleyana* and var. *intermedia* (Table 5.1). Two males and two females of var. *deltoidea*, var. *trengganuensis* and var. *kunstleri*, and two males and one female of var. *bilobata* were available. Only two and one trees respectively of var. *motleyana* and var. *intermedia* were available.

Two big male epiphytic *Ficus deltoidea* var. *angustifolia* individuals (D7 and D22) with abundant figs were chosen as potential donors of pollinators to other varieties (Table 5.1). The two focal trees were typical of plants at the Banting site (Chapter 6), with non-synchronous production of figs and all the developmental phases present at any one time (Chapter 6). Each tree had more than 5000 figs present at the time of the experiment. The basal height from the ground for D7 was 2.2 m, while for D22 it was 2.6m. The distance between all the transplants in pots and the donor trees ranged between 1 m to 4 m while the figs on different varieties were as little as 20 cm apart. One female and one male plant from each variety were placed in a circle around the bases of the oil palms supporting the two epiphytic male var. *angustifolia* (Figure 5.1) to see whether the pollinators will enter all the varieties or only specific varieties. Individuals of var. *angustifolia* acted as controls. Plants belonging to the same variety were placed apart, to avoid any directional bias.

Table 5.1 *Ficus deltoidea* individuals obtained from TGC and positioned in pots around potential pollinator donor male individuals of var. *angustifolia* growing as epiphytes in an oil palm plantation in Banting.

No.	Variety	Tree code	Sex	Donor tree
1	*angustifolia*	FDA 1	Male	D7
2	*angustifolia*	FDA 2	Male	D22
3	*angustifolia*	FDA 6	Female	D7
4	*angustifolia*	FDA 7	Female	D22
5	*deltoidea*	FDD 1	Male	D7
6	*deltoidea*	FDD 2	Male	D22
7	*deltoidea*	FDD 3	Female	D7
8	*deltoidea*	FDD 4	Female	D22
9	*trengganuensis*	FDT 1	Male	D7
10	*trengganuensis*	FDT 2	Male	D22
11	*trengganuensis*	FDT 3	Female	D7
12	*trengganuensis*	FDT 4	Female	D22
13	*kunstleri*	FDK 1	Male	D7
14	*kunstleri*	FDK 2	Male	D22
15	*kunstleri*	FDK 3	Female	D7
16	*kunstleri*	FDK 4	Female	D22
17	*bilobata*	FDB 1	Male	D7
18	*bilobata*	FDB 2	Male	D22
19	*bilobata*	FDB 3	Female	D7
20	*motleyana*	FDM 1	Female	D7
21	*motleyana*	FDM 2	Female	D22
22	*intermedia*	FDI 1	Male	D7

Figure 5.1 Varieties of *Ficus deltoidea* encircling large epiphytic male *Ficus deltoidea* var. *angustifolia*. Left donor tree D7, right donor tree D22.

The potted plants were moved into position around donor trees using a wheelbarrow. A total of 12 pots were placed around tree D7 and 10 pots around tree D22. For donor tree D7, the pots were placed in an arc of about 180 degree around the oil palm facing the area of the donor where most figs were. The pot plants encircled D22, forming an arc of about 220 degree. Once in position, all the figs on the potted plants were removed to stimulate production of new figs. Removal took two people about three days. The starting date of the experiment was taken to be once all the figs were pulled off (15th January 2017). They remained in place until 15th May 2017, when the experiment was terminated.

The trees in pots were watered regularly, and all survived throughout the experiment. They also all produced new figs except for plant FDK 1 (var. *kunstleri*). The plants were checked twice a month for the presence of any pollinated figs. At the end of the experiment, the numbers of figs at each developmental phase were also counted. Any DE phase figs were scored fortnightly throughout the experiment to make sure that none were missing at the end because the figs had been eaten by birds. To determine whether any figs were being entered by pollinators, but failing to develop, 50 aborting figs of each individual plant were brought to the Lab. and examined using a binocular microscope to check for the existence of wings in fig ostioles and wasp bodies inside the figs.

5.3.3.2 Transplant experiment two

Three additional male *F. deltoidea* var. *angustifolia* (with no pollinated figs) were brought to the Banting oil palm plantation from TGC to act as pollinator donor trees in experiment two (plants FDA 3, FDA 4, and FDA 5). They were placed next to three big male epiphytic *F. deltoidea* var. *angustifolia* individuals growing as epiphytes on oil palms (not D7 and D22) to allow their figs to be entered by pollinators. Together with potted plants FDA1 and FDA2 this produced five male var. *angustifolia* with figs containing developing pollinator offspring.

After four months, the potted plants (all varieties) were taken by lorry back to TGC, where experiment two was conducted from 16th May 2017 until 16th August 2017. A month earlier, all the figs on the plants in the TGC (of all varieties) had been removed to stimulate production of new figs. This removal of figs was carried out by four assistants from UniSZA. For this experiment, the five male var. *angustifolia* were used as a source of pollinators. The five male plants of var. *angustifolia* with fig wasps were put together with the other varieties adjacent to the rest of the germplasm collection (Figure 5.2). The pots were placed back to their original places in the collection because most of the other pots were too large to be moved conveniently.

Data similar to that collected in Experiment one were recorded at the end of the experiment, namely how many figs were available to be entered and how many figs developed in response to entry. Fifty figs from each fig tree of all the varieties were again collected and examined under a binocular microscope for the presence of pollinator wings and fig wasp bodies.

5.3.3.3 Transplant experiment three

Experiment three was a continuation of experiment two. This experiment was carried out after the entire TGC collection was moved to an area in UniSZA about 500 m away. This allowed the experiment to be conducted more systematically because the experimental design was given to the UniSZA staff ahead of the move and the staff unloaded and arranged all the pots accordingly. Seven pollinated male trees of *Ficus deltoidea* var. *angustifolia* were used as donor trees. Five of the trees were the donor trees used in experiment two while two male trees had acquired the pollinators in experiment two. Male and female individuals of var. *angustifolia* with no pollinated figs acted as controls. Six other varieties used in this experiment were var. *deltoidea*, var. *trengganuensis*, var. *kunstleri*, var. *bilobata*, var. *motleyana* and var. *intermedia*. The arrangement of the clusters of plants is shown in Figure 5.2.

Arrangements of different combinations depended on the availability of recipient plants (A-G in Figure 5.3). The experiment was conducted simultaneously around the seven donor plants. The donor trees were placed in the middle of different combinations of varieties and sexes and controls were present except in combinations F and G. The distances apart of donor plants were not less than 10 m to reduce the chance of pollinators moving between tree cluster combinations. All the pots were placed as close as possible to the donor trees (all were less than 1 m away and the fig plants were touching if possible). This was to increase the chances of fig wasps moving from donor plants.

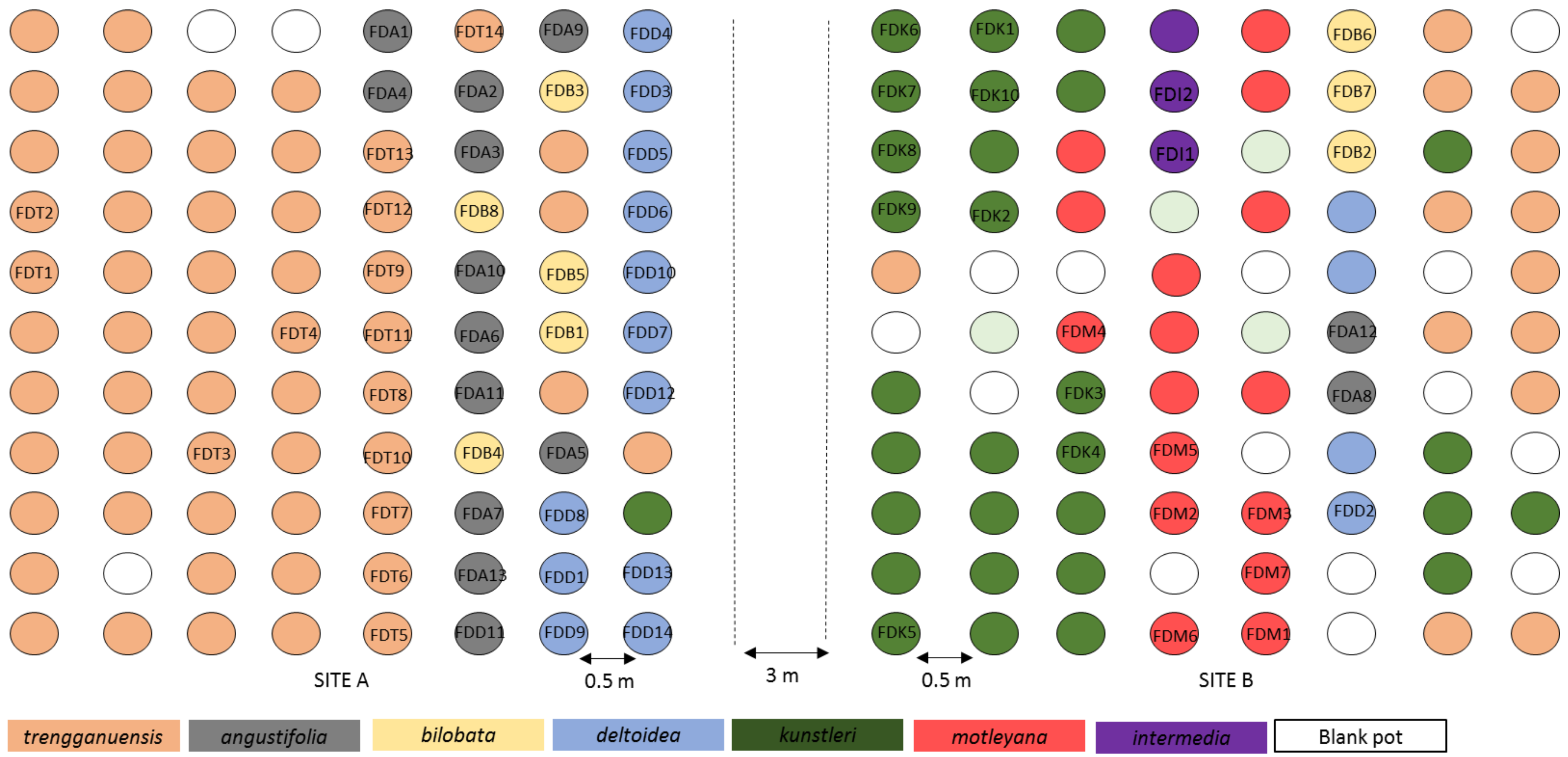


Figure 5.2. The arrangement of pots containing varieties of *Ficus deltoidea* in the Terengganu Germplasm Collection (TGC). The var. *angustifolia* that were releasing pollinators were FDA 1, FDA 2, FDA 3, FDA 4 and FDA 5.

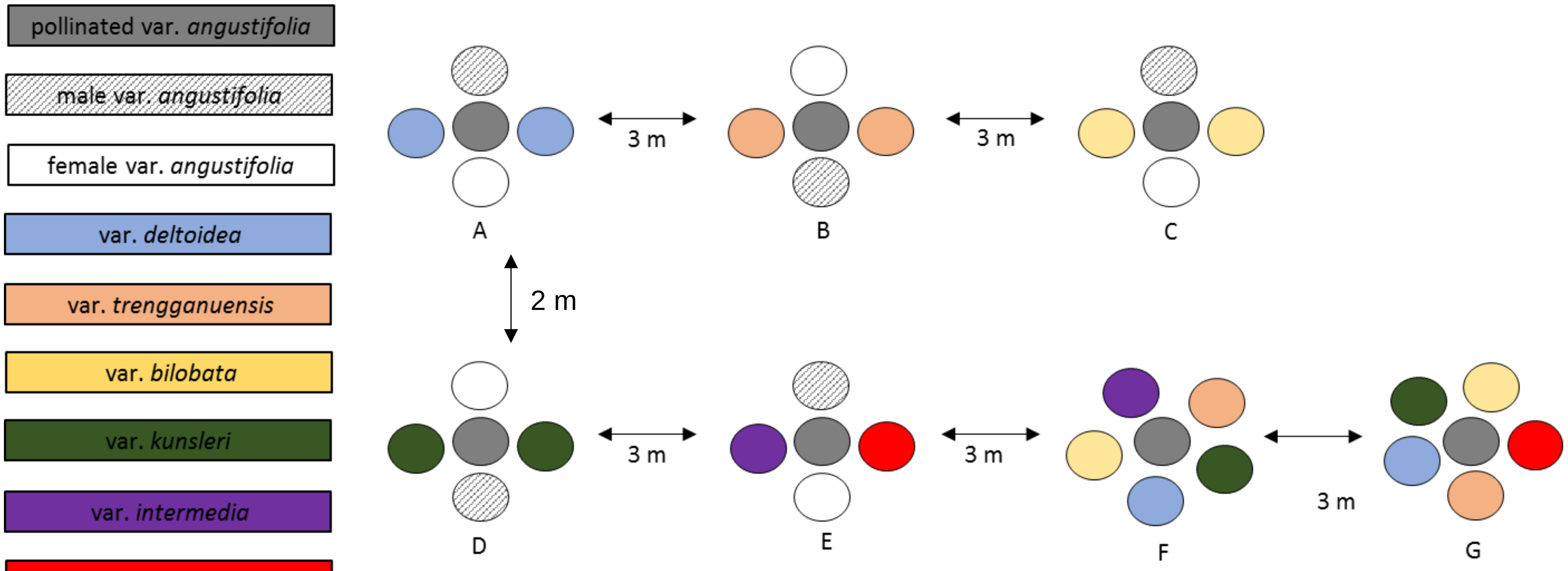


Figure 5.3. The arrangement of pots containing *Ficus deltoidea* varieties during experiment three at UnisZA.

5.3.3.4 Transplant experiment four

This manipulation experiment was similar to experiment three, with all the pots positioned as before. However, in this experiment, all the receptive figs on the donor tree were removed to prevent the wasps that emerged from entering figs on their natal plants. As before, clusters F and G did not offer any receptive figs of var. *angustifolia* to emerging pollinators, while the other versions again offered emerging pollinators a choice between male and female figs of var. *angustifolia*.

5.3.4 Host choice by *Blastophaga* sp. (var. *deltoidea*)

This experiment was conducted in the Banting oil palm plantation using epiphytic var. *angustifolia* plants growing on oil palms as pollinator recipients and pollinators emerging from figs of var. *deltoidea* as donors. The experiment started with bagging pre-receptive figs (A phase) within fine mesh bags to prevent the entry of any pollinators. A total of 27 bags were placed around stems on 5 different trees bearing multiple (9-18) figs. Two of the five trees were female, three were male. The A phase figs from var. *angustifolia* usually take less than a week to become receptive (B phase). During this period mature male figs of var. *deltoidea* were collected from Batu Pahat in Johor.

At Batu Pahat (Johor), mature D/E phase figs of var. *deltoidea* were removed from the trees and brought to a Lab. In Kuala Lumpur where they were kept in containers covered by fine mesh. Once the fig wasps had emerged from the figs they were quickly brought to the plantation where a single foundress was released into each bag where receptive figs were available. The bags were then closed to prevent other pollinators from entering. 30 days later, the bags were opened to check for the presence of wings and the bodies of foundresses and record any developmental signs that pollinators had entered

5.3.5 Statistical methods

Chi-square tests were conducted to determine whether there were significant differences in frequencies of entry of male and female figs of var. *angustifolia*. Chi-square tests were also conducted to examine differences between entry rates of the same gender from different varieties. All analyses used SPSS Statistics 20.

5.4 Results

5.4.1 Host choice by *Blastophaga* sp. (var. *angustifolia*)

5.4.1.1 Transplant experiment 1

After four months, the total numbers of figs on the transplanted potted plants were counted. The figs present on different plants at the end of the experiment ranged from 0 to 4672 (Table 5.2). The only fig tree that did not produce figs was plant FDK 1 (var. *kunstleri*). As mentioned in Chapter 2, different varieties of *Ficus deltoidea* produce either big or small figs. The smallest figs are on var. *bilobata*, followed by var. *angustifolia*, var. *deltoidea*, var. *intermedia* and var. *motleyana*. Conversely, var. *kunstleri* and var. *trengganuensis* produce large figs. The varieties with smaller figs tended to produce more figs.

In this experiment, the largest number of figs found was in var. *bilobata*, with more than 4000 figs. Some plants of var. *angustifolia* and var. *deltoidea* also had more than 2000 figs each. Numbers of replicate individuals were small, but male trees tended to have more figs than females (var. *bilobata*, var. *deltoidea*, var. *angustifolia* and var. *kunstleri*) but var. *trengganuensis* females had more figs. In var. *motleyena* and var. *intermedia*, since only one gender was there, the comparison cannot be made.

The developmental phases of the figs on the male transplants at the end of the experiment showed that only var. *angustifolia* had C phase figs (entered and developing) (Figure 5.4). The experiment was terminated before any of these figs had released pollinator offspring (DE phase). The only phases on plants of the other varieties were either AB (immature or ready to be pollinated) or aborting. Male trees of var. *angustifolia* recorded the highest percentage of AB phase figs (73.6%) and C phase (25.6%). C phase was entirely absent in the other varieties. Aborting phase figs were in a majority on all the other varieties, but were just 0.9% of the total on the var. *angustifolia* plants.

Table 5.2. The entry of pollinators into figs of *Ficus deltoidea* varieties positioned around epiphytic male var. *angustifolia* in Banting plantation.

Recipient variety	Donor Tree	Tree No.	Sex	Total number of figs present on the plants during the experiment		Figs	
				Beginning	End	Entered	Un-entered
angustifolia	D7	FDA 1	Male	0	2175	445	1730
	D22	FDA 2	Male	0	201	162	39
	D7	FDA 6	Female	0	1652	63	1589
	D22	FDA 7	Female	0	1767	88	1679
deltoidea	D7	FDD 1	Male	0	2430	0	2430
	D22	FDD 2	Male	0	493	0	493
	D7	FDD 3	Female	0	54	0	54
	D22	FDD 4	Female	0	248	0	248
trengganuensis	D7	FDT 1	Male	0	215	0	215
	D22	FDT 2	Male	0	76	0	76
	D7	FDT 3	Female	0	836	0	836
	D22	FDT 4	Female	0	202	0	202
kunstleri	D7	FDK 1	Male	0	0	0	0
	D22	FDK 2	Male	0	145	0	145
	D7	FDK 3	Female	0	10	0	10
	D22	FDK 4	Female	0	125	0	125
bilobata	D7	FDB 1	Male	0	4591	0	4591
	D22	FDB 2	Male	0	4672	0	4672
	D7	FDB 3	Female	0	35	0	35
motleyana	D7	FDM 1	Female	0	82	0	82
	D22	FDM 2	Female	0	124	0	124
intermedia	D7	FDI 1	Male	0	135	0	135

Results for female trees of *F. deltoidea* were similar to those of the males, insofar as only figs of var. *angustifolia* provided evidence of pollination, but the females of this variety had a much higher proportion of AB figs than the males (91.1%) and far fewer pollinated figs (1.8% C and 2.6% DE phases). Only 4.5% of the figs of female var. *angustifolia* were aborting, which suggests that many of the female figs were still available to be pollinated. Frequencies of female AB phase figs in the other varieties ranged from 18.5% to 29.8%, and all the remaining figs on these plants were aborting (Figure 5.5).

Among the transplanted individuals, only var. *angustifolia* had pollinated figs (Figure 5.6). All individuals of this variety had some figs that were pollinated. In FDA 1 (male), 445 figs from 2175 figs were entered (20.5 %) and in FDA 2 (also male), 162 figs from 201 figs were entered (80.6 %). Among female trees 63 figs from 1652 figs were entered on plant FDA 6 (3.8 %) and 88 figs from 1767 figs on FDA 7 (5.0 %). The pollinated figs on female trees included both C and DE phases. The entry rates into male transplants (FDA 1 and FDA 2) around different donor trees (D7 and D22) were significantly different ($\chi^2 = 5.07$, df = 1, $P < 0.01$). A similar difference was found among the female transplants FDA6 and FDA7 around different donor trees (Chi square $\chi^2 = 9.904$, df = 1, $P < 0.05$). So for both sexes, higher entry rates were achieved around donor tree D22 than donor tree D7.

Entry rates for the two male var. *angustifolia* were higher than for the two conspecific females. For FDA 1 (male, 20 un-entered, 445 entered) and FDA 6 (female, 92 un-entered, 63 entered) around donor tree D7 (Chi square, $\chi^2 = 238.05$, df = 1, $P < 0.01$. Around donor tree D22 rates for male FDA 2 were 1 un-entered and 162 entered compared with 62 un-entered and 88 entered for female plant FDA7 (Chi square $\chi^2 = 80.57$, df = 1, $P < 0.001$). The sexual difference in entry rates around donor tree D22 appeared to be independent of crop sizes, which were almost the same on the male and female plants.

Between zero and 58 aborting phase figs from all varieties except var. *angustifolia* were dissected, with sample sizes varying according to availability (Table 5.3). This was to check whether pollinators had entered the figs, but they had subsequently failed to develop. None of the figs had wings present in their ostioles or contained the bodies of pollinator wasps.

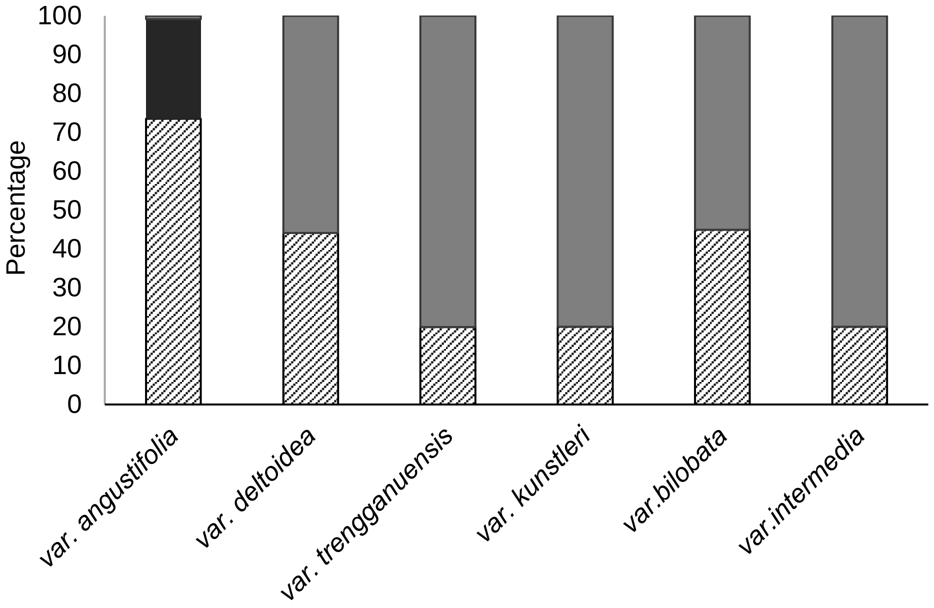


Figure 5.4. Phases of fig development in male *Ficus deltoidea* figs positioned around male var. *angustifolia* in an oil palm plantation. Hatched bars = AB phase, Black bar = C phase, grey bars = Aborting. Combined data – sample sizes vary between varieties.

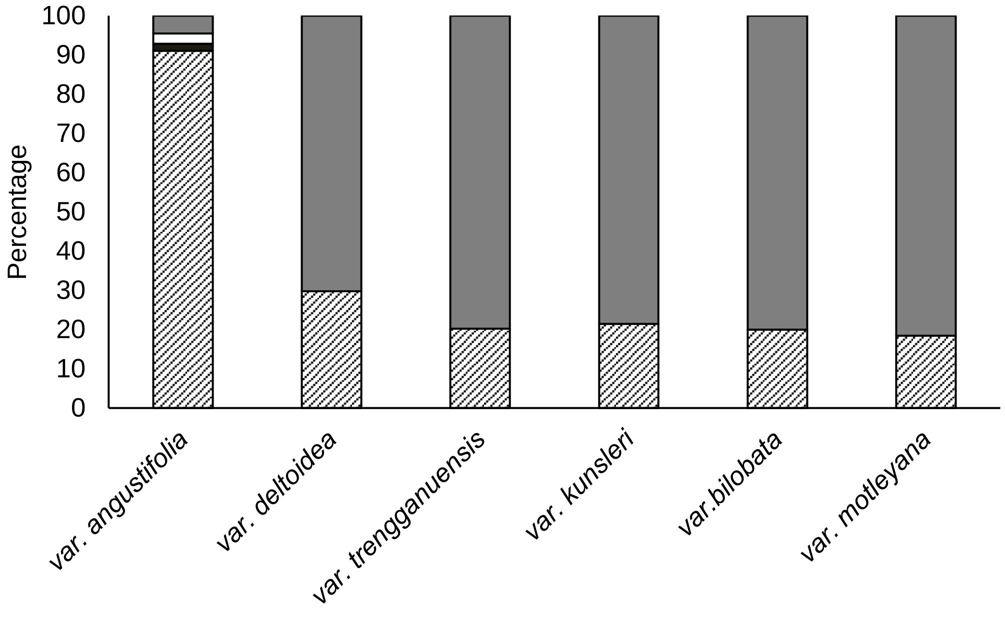


Figure 5.5. Phases of fig development in female *Ficus deltoidea* figs positioned around male var. *angustifolia* in an oil palm plantation. Hatched bars = AB phase, Black bar = C phase, open bar = DE phase, grey bars = aborting. Combined data – sample sizes vary between varieties.

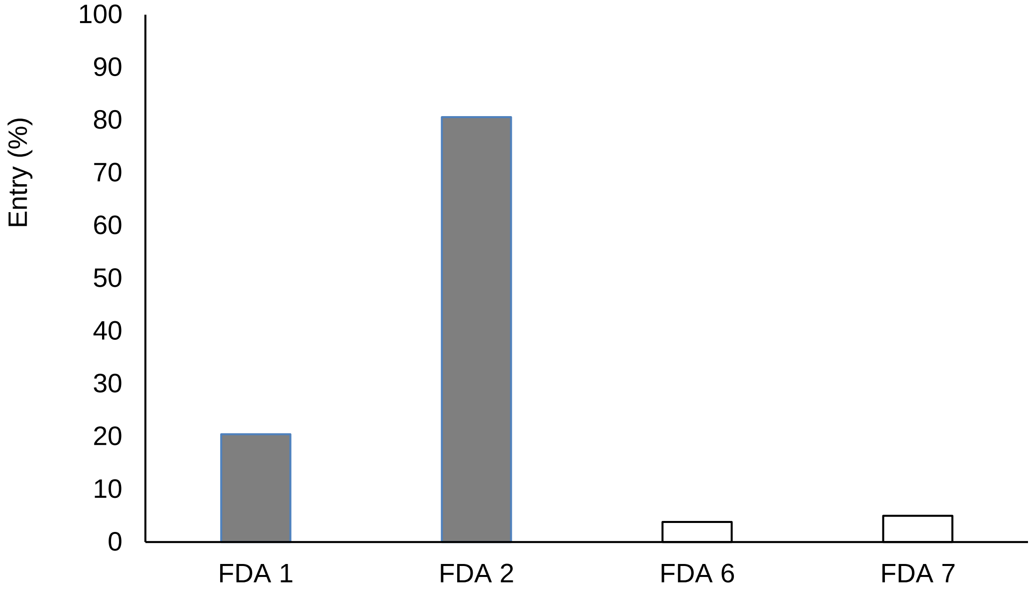


Figure 5.6 Pollination rates in four transplanted *Ficus deltoidea* var. *angustifolia*. Solid bars = male trees, open bars = female trees.

Table 5.3. The contents of aborting figs collected from transplanted trees placed around male var. *angustifolia*.

Variety	Tree No.	Donor tree	Sex	No of figs collected	Figs with wings/wasps
deltoidea	FDD1	D7	Male	54	0
	FDD2	D7	Male	54	0
	FDD3	D22	Female	51	0
	FDD4	D22	Female	57	0
trengganuensis	FDT1	D7	Male	55	0
	FDT2	D22	Male	56	0
	FDT3	D7	Female	55	0
	FDT4	D22	Female	51	0
kunstleri	FDK1	D7	Male	0	0
	FDK2	D22	Male	53	0
	FDK3	D7	Female	10	0
	FDK4	D22	Female	50	0
bilobata	FDB1	D7	Male	56	0
	FDB2	D22	Male	58	0
	FDB3	D7	Female	28	0
motleyana	FDM1	D7	Female	52	0
	FDM2	D22	Female	50	0
intermedia	FDI1	D7	Male	54	0

5.4.1.2 Transplant experiment 2

The transplanted *F. deltoidea* were present at Terengganu Germplasm Centre (TGC) for three months. The number of plants available varied between varieties (Table 5.4). At the end of this period all the figs on the 66 fig trees were counted and examined for evidence of fig wasp entry and pollination. The number of figs on individual plants ranged from 0 (one of the var. *kunstleri*) to over 5000 (a var. *bilobata* individual).

The developmental phases of the figs on the recipient male plants (Figure 5.7) showed that only var. *angustifolia* had been entered by pollinators because some C was present. The only developmental phases on plants of varieties other than var. *angustifolia* were either AB (immature or receptive to pollination) or aborting. AB phase male figs were present on all varieties. This included 60.07% of the figs on male trees of var. *angustifolia*. C phase figs were only found on male trees of var. *angustifolia* with 1.67% of the total figs present. No DE phase was present on all varieties. Aborting figs were present in all varieties. This included 38.26% of the figs on the var. *angustifolia* plants. Among the donor var. *angustifolia* C and DE figs ranged from 4.79% to 65.54% of the total figs on different trees (Table 5.4. The entry into figs of *Ficus deltoidea* varieties in the Terengganu Germplasm Collection (TGC) after sources of var. *angustifolia* fig wasps were added.).

Results for female trees were different to those of the males. None of the female trees showed any evidence of pollination, including recipient female var. *angustifolia* (Figure 5.8). The only phases recorded on all female trees were AB (young and receptive) together with aborting figs. The highest proportion of AB phase figs was in var. *bilobata* (69.6%) while the highest percentage of aborting figs was recorded in var. *kunstleri* (98.2%). This indicate that var. *bilobata* may have only had receptive figs available towards the end of the experiment.

Table 5.4. The entry into figs of *Ficus deltoidea* varieties in the Terengganu Germplasm Collection (TGC) after sources of var. *angustifolia* fig wasps were added.

Variety	Tree No.	Sex	Total number of figs present on the plants during the experiment		Figs	
			Beginning	End	Entered	Un-entered
angustifolia	FDA 1	Male	2175	2943	1031	1912
	FDA 2	Male	201	2876	509	2367
	FDA 3	Male	0	2533	288	2245
	FDA 4	Male	0	2333	1529	804
	FDA 5	Male	0	2899	139	2760
	FDA 6	Female	1652	1673	0	1673
	FDA 7	Female	1767	1266	0	1266
	FDA 8	Male	0	1939	0	1939
	FDA 9	Male	0	2071	213	1858
	FDA 10	Male	0	2989	0	2989
	FD 11	Male	0	3787	0	3787
	FDA 12	Male	0	54	0	54
	FDA 13	Female	0	1470	0	1470
deltoidea	FDD 1	Male	2430	1922	0	1922
	FDD 2	Male	493	597	0	597
	FDD 3	Female	54	84	0	84
	FDD 4	Female	248	294	0	294
	FDD 5	Male	0	1574	0	1574
	FDD 6	Male	0	230	0	230
	FDD 7	Male	0	572	0	572
	FDD 8	Male	0	369	0	369
	FDD 9	Male	0	22	0	22
	FDD 10	Female	0	530	0	530
	FDD 11	Female	0	2337	0	2337
	FDD 12	Female	0	420	0	420
	FDD 13	Female	0	747	0	747
	FDD 14	Female	0	313	0	313
trengganuensis	FDT 1	Male	215	222	0	222
	FDT 2	Male	76	96	0	96
	FDT 3	Female	836	867	0	867

	FDT 4	Female	202	183	0	183
	FDT 5	Male	0	1183	0	1183
	FDT 6	Male	0	885	0	885
	FDT 7	Male	0	217	0	217
	FDT 8	Male	0	1019	0	1019
	FDT 9	Male	0	319	0	319
	FDT 10	Female	0	304	0	304
	FDT 11	Female	0	592	0	592
	FDT 12	Female	0	440	0	440
	FDT 13	Female	0	189	0	189
	FDT 14	Female	0	1229	0	1229
kunstleri	FDK 1	Male	0	0	0	0
	FDK 2	Male	145	350	0	350
	FDK 3	Female	10	25	0	25
	FDK 4	Female	125	94	0	94
	FDK 5	Male	0	210	0	210
	FDK 6	Female	0	806	0	806
	FDK 7	Female	0	891	0	891
	FDK 8	Female	0	700	0	700
	FDK 9	Female	0	1441	0	1441
	FDK 10	Female	0	686	0	686
bilobata	FDB 1	Male	4591	3281	0	3281
	FDB 2	Male	4672	4809	0	4809
	FDB 3	Female	35	217	0	217
	FDB 4	Male	0	3361	0	3361
	FDB 5	Male	0	4703	0	4703
	FDB 6	Male	0	5110	0	5110
	FDB 7	Male	0	3490	0	3490
	FDB 8	Male	0	2861	0	2861
motleyana	FDM 1	Female	82	102	0	102
	FDM 2	Female	124	155	0	155
	FDM 3	Female	0	366	0	366
	FDM 4	Female	0	317	0	317
	FDM 5	Female	0	97	0	97
	FDM 6	Female	0	159	0	159
intermedia	FDI 1	Male	135	92	0	92

	FDI 2	Male	0	37	0	37

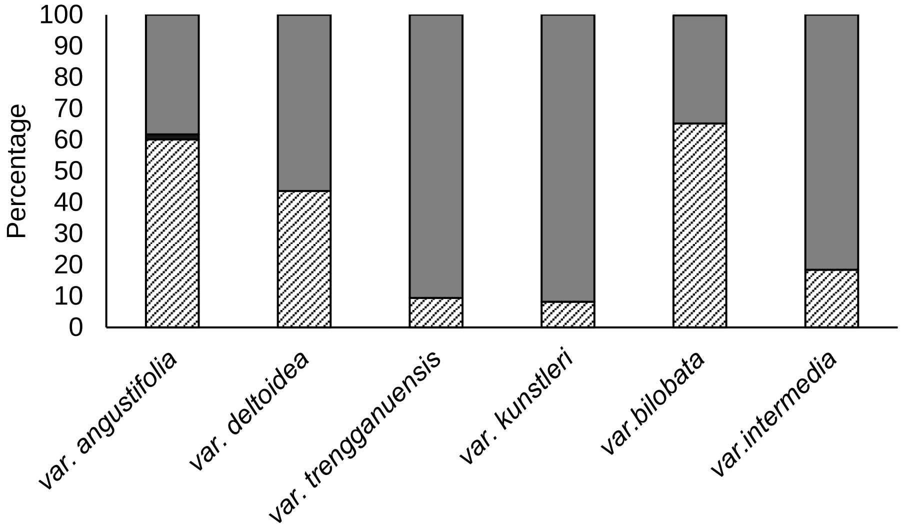


Figure 5.7 The developmental phases of male *Ficus deltoidea* figs recorded at the end of experiment two. Hashed bars = AB phase, Black bar = C phase, open bar = DE, grey bars = aborting.

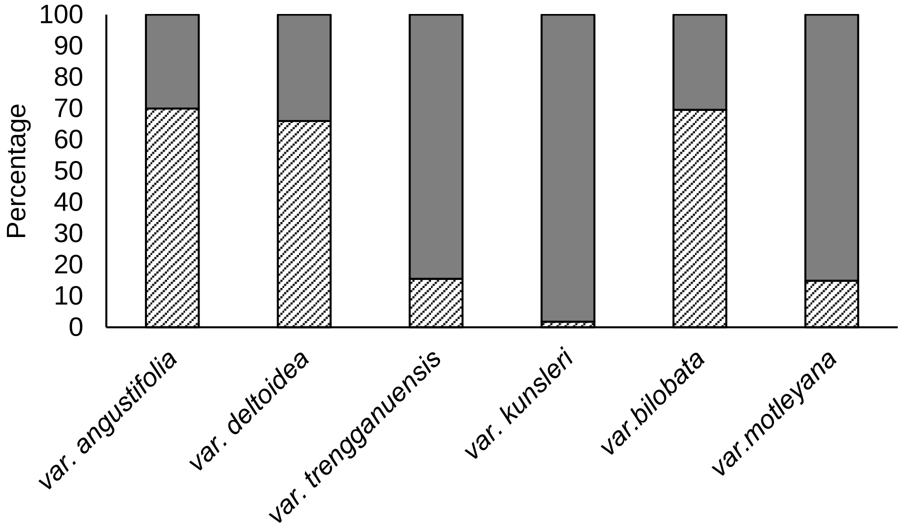


Figure 5.8 The developmental phases of female *Ficus deltoidea* figs recorded at the end of experiment two. Hashed bars = AB phase, grey bars = Aborting. No C or DE phase figs were recorded.

Among the ten male var. *angustifolia* plants, five had figs containing fig wasp offspring at the start of experiment two (Table 5.2). Between 2.1% and 99.0% of their figs had been entered at the start of the experiment (Figure 5.9). At the end of the experiment all the five male trees that had initially had fig wasps continued to have entered figs present (the originally occupied figs had completed their development and fallen from the trees by this time). Male plants with high entry rates at the start of the experiment tended to be the ones that also had many entered figs at the end of the experiment (Figure 5.9).

Among the remaining five male plants, FDA 9 was the only individual to acquire pollinators during the course of the experiment, with 213 of its 2071 figs entered by pollinators. FDA 9 had the highest density of pollinator-releasing trees near it of the five trees, and was located less than 2 meters from four of the male plants that were releasing pollinators (FDA 1, FDA 2, FDA 3, and FDA 4). The three female trees (FDA 6, FDA 7 and FDA 13) were entirely un-pollinated at the end of experiment two Table 5.4). As in experiment one, aborting figs from each plant of each variety were dissected, with sample sizes varying according to availability (Table 5.5).This check for whether or not any fig wasp pollinators had entered the figs found that neither wings nor wasp bodies were present in the ostioles and central cavities of any of the aborting figs.

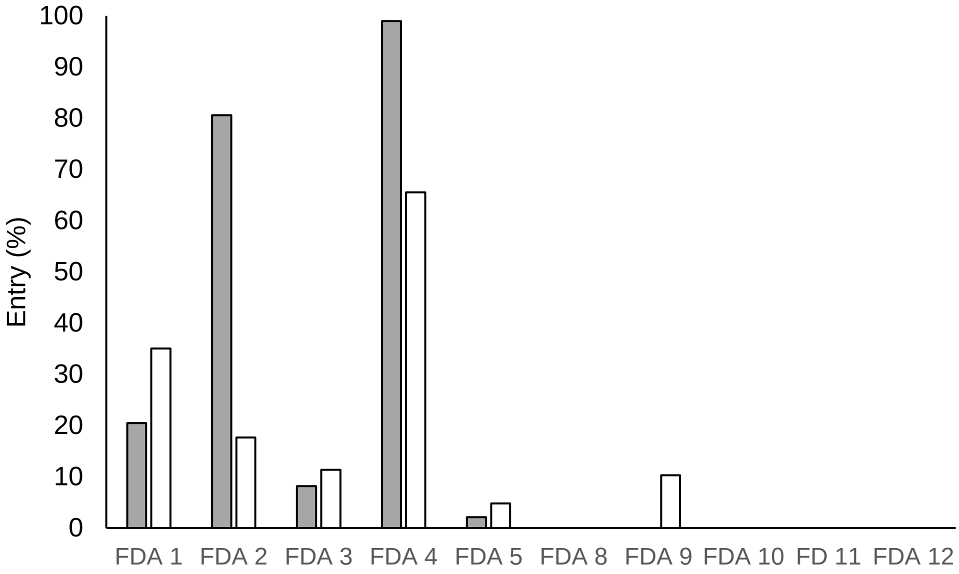


Figure 5.9. Initial and final entry rates on the male trees of var. *angustifolia* in experiment two. Solid bars = entry rates at the start of the experiment, open bars = entry rates at the end of the experiment. FDA 1, FDA 2, FDA 3, FDA 4 and FDA 5 had previously-entered figs at the start of the experiment. FDA 9 was the only control tree with figs that were entered.

Table 5.5 The contents of aborting figs collected from recipient plants in experiment two.

Variety	Tree No	Sex	No. of figs collected	Presence of wings/ wasps
deltoidea	FDD 1	Male	55	0
	FDD 2	Male	52	0
	FDD 3	Female	56	0
	FDD 4	Female	57	0
	FDD 5	Male	55	0
	FDD 6	Male	56	0
	FDD 7	Male	58	0
	FDD 8	Male	53	0
	FDD 9	Male	22	0
	FDD 10	Female	52	0
	FDD 11	Female	53	0
	FDD 12	Female	59	0
	FDD 13	Female	57	0
	FDD 14	Female	57	0
trengganuensis	FDT 1	Male	54	0
	FDT 2	Male	55	0
	FDT 3	Female	52	0
	FDT 4	Female	56	0
	FDT 5	Male	52	0
	FDT 6	Male	55	0
	FDT 7	Male	56	0
	FDT 8	Male	55	0
	FDT 9	Male	54	0
	FDT 10	Female	58	0
	FDT 11	Female	59	0
	FDT 12	Female	52	0
	FDT 13	Female	55	0
	FDT 14	Female	56	0
kunstleri	FDK 1	Male	0	0
	FDK 2	Male	50	0
	FDK 3	Female	22	0

	FDK 4	Female	51	0
	FDK 5	Male	52	0
	FDK 6	Female	55	0
	FDK 7	Female	56	0
	FDK 8	Female	59	0
	FDK 9	Female	55	0
	FDK 10	Female	51	0
bilobata	FDB 1	Male	55	0
	FDB 2	Male	54	0
	FDB 3	Female	56	0
	FDB 4	Male	55	0
	FDB 5	Male	53	0
	FDB 6	Male	57	0
	FDB 7	Male	55	0
	FDB 8	Male	54	0
motleyana	FDM 1	Female	52	0
	FDM 2	Female	55	0
	FDM 3	Female	50	0
	FDM 4	Female	57	0
	FDM 5	Female	58	0
	FDM 6	Female	52	0
intermedia	FDI 1	Male	55	0
	FDI 2	Male	37	0

5.4.1.3 Transplant experiment 3

This experiment had a more systematic placement of the experiment plants, but produced similar results to experiment two. Only var. *angustifolia* had C phase figs, which indicated the entry of pollinators. About 70% of the male figs in the recipient trees of this variety had been entered, but no figs produced by the other varieties had been entered (Figure 5.10). A similar result was found with female figs, with only figs of var. *angustifolia* pollinated, but the percentage of entered figs was only about 18% (Figure 5.11).

Between zero and 56 aborting figs were examined from each variety, depending on availability (Figure 5.6). One individual of var. *motleyana* and one of var. *intermedia* did not produce any figs. There was no sign of wings or pollinator bodies being present in any of the dissected figs.

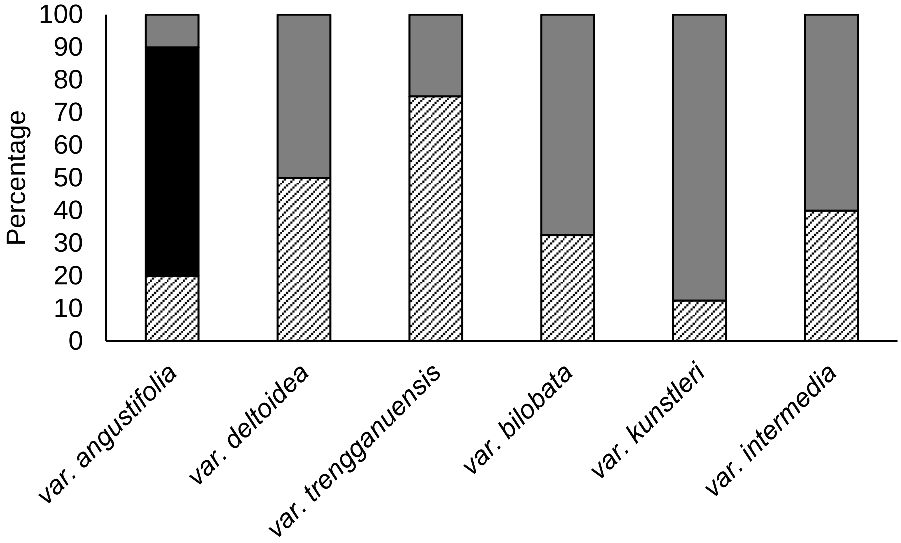


Figure 5.10 The phases of development of male figs placed around var. *angustifolia* that were releasing pollinators, scored at the end of experiment three. Hashed bars = AB phase, black bars = C phase, grey bars = aborting figs. No DE phase figs were recorded.

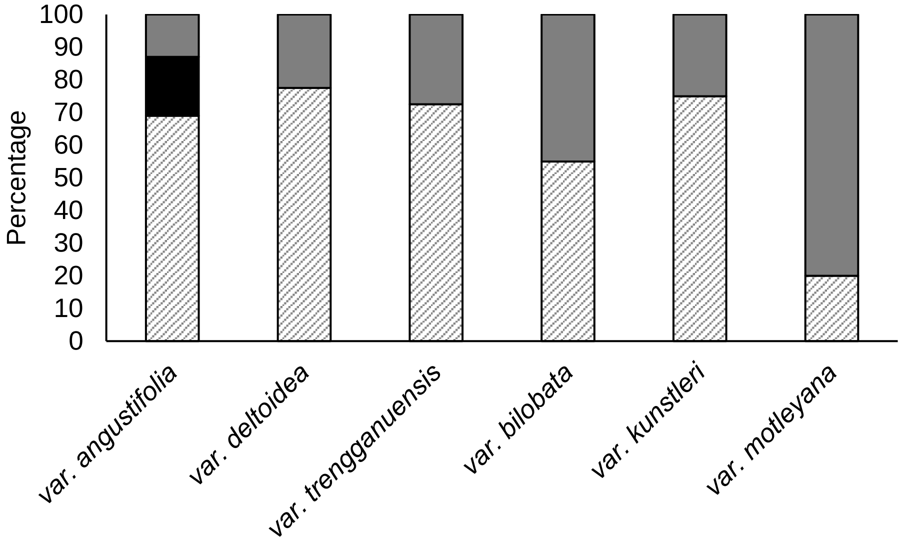


Figure 5.11 The phases of fig development in female figs placed around var. *angustifolia* that were releasing pollinators, scored at the end of experiment three. Hashed bars = AB phase, black bars = C phase, grey bars = aborting figs. No DE phase figs were recorded.

Table 5.6. The contents of aborting figs collected from potted trees placed around male var. *angustifolia* that were releasing pollinators.

Variety	Tree No.	Donor tree	Sex	No of figs collected	Figs with wings/wasps
deltoidea	FDD1	FDA 1	Male	52	0
	FDD2	FDA 9	Male	55	0
	FDD3	FDA 1	Female	50	0
	FDD4	FDA 10	Female	53	0
trengganuensis	FDT1	FDA 2	Male	55	0
	FDT2	FDA 9	Male	56	0
	FDT3	FDA 2	Female	55	0
	FDT4	FDA 10	Female	51	0
bilobata	FDB1	FDA 3	Male	55	0
	FDB2	FDA 9	Male	53	0
	FDB3	FDA 3	Female	54	0
kunstleri	FDK1	FDA 4	Male	4	0
	FDK2	FDA 9	Male	1	0
	FDK3	FDA 4	Female	7	0
	FDK4	FDA 10	Female	21	0
motleyana	FDM1	FDA 5	Female	0	0
	FDM2	FDA 10	Female	11	0
intermedia	FDI1	FDA 5	Male	0	0
	FDI 2	FDA 9	Male	37	0

5.4.1.4 Transplant experiment 4

This experiment was similar to experiment three in terms of the experimental design. However, the receptive figs on all the donor trees were removed before this experiment started. This forced the fig wasps to fly away from their natal trees and make choices about entering figs elsewhere. Under these conditions, small numbers of the emerging female fig wasps were found to enter male figs of another variety as well as male figs of var. *angustifolia* (Figure 5.12). Some of the figs on the donor var. *angustifolia* had reached receptivity and being entered at the end of the experiment.

The fig wasps had entered 3 figs from a total of 39 figs of var. *deltoidea*. The three entered figs of var. *deltoidea* were located on a branch that was touching the donor tree. All three entries were from cluster F of the experiment, where the combination of adjacent plants did not include any control var. *angustifolia*. The three figs of var. *deltoidea* that had been entered were dissected. Wings were found in each fig. The ovules had been galled, but the galls were all empty with no sign of larval development. The entered figs were in mid C phase and usually, in that phase, the larvae of fig wasps can be seen clearly when galls are dissected.

For the female figs, the results were similar to those of the three previous experiments (Figure 5.13). No variety apart from var. *angustifolia* showed signs of fig wasp entry or pollination. From zero to 56 aborting figs were dissected. No aborting figs showed the presence of the wings and fragments of fig wasp body (Table 5.7).

Some of the male figs from var. *angustifolia* had already reached DE phase. Based on personal observations, the development of figs on the potted trees was fast compared to the wild epiphytic fig trees. The nutrients provided by the media might have caused this, or higher temperatures.

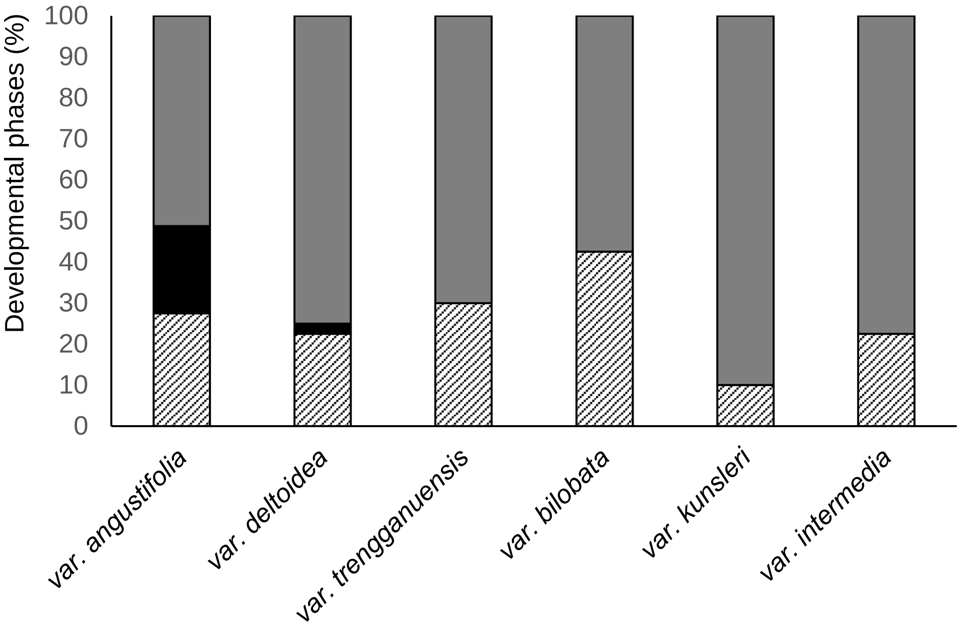


Figure 5.12 The phases of fig development in male figs recorded at the end of experiment four. Hashed bars = AB phase, black bars = C phase, grey bars = aborting figs. No DE phase figs were recorded.

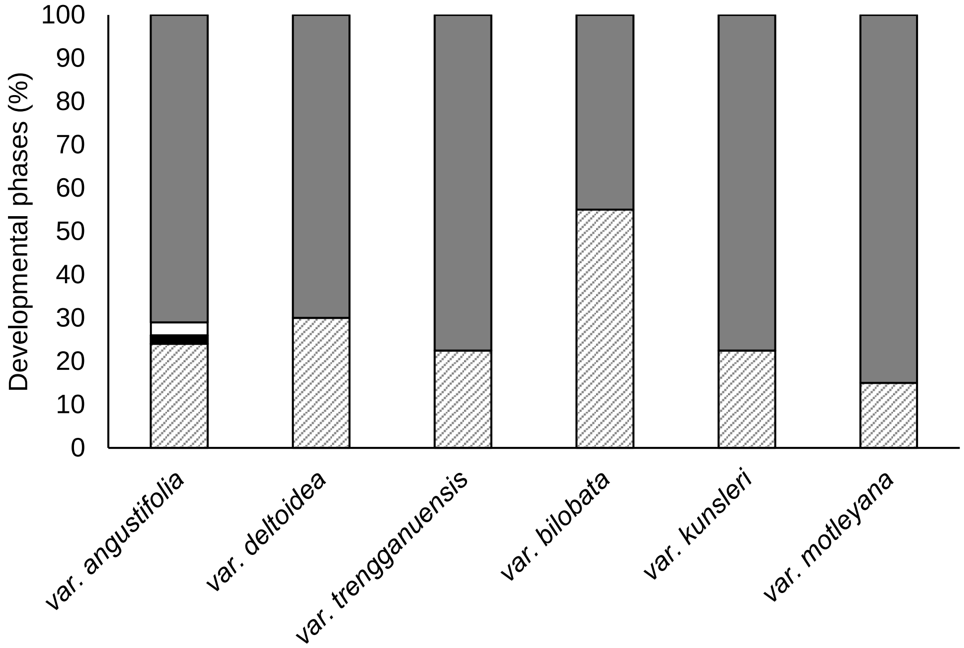


Figure 5.13 The phases of fig development in female figs recorded at the end of experiment four. Hashed bars = AB phase, black bars = C phase, white bars = DE phase, grey bars = aborting figs.

Table 5.7. The contents of aborting figs collected from potted trees placed around pollinated male var. *angustifolia* in Experiment 4.

Variety	Tree No.	Donor tree	Sex	No of figs collected	Figs with wings/wasps
deltoidea	FDD1	FDA 1	Male	55	0
	FDD2	FDA 9	Male	39	3
	FDD3	FDA 1	Female	52	0
	FDD4	FDA 10	Female	54	0
trengganuensis	FDT1	FDA 2	Male	54	0
	FDT2	FDA 9	Male	53	0
	FDT3	FDA 2	Female	52	0
	FDT4	FDA 10	Female	54	0
bilobata	FDB1	FDA 3	Male	54	0
	FDB2	FDA 9	Male	53	0
	FDB3	FDA 3	Female	55	0
kunstleri	FDK1	FDA 4	Male	54	0
	FDK2	FDA 9	Male	51	0
	FDK3	FDA 4	Female	12	0
	FDK4	FDA 10	Female	55	0
motleyana	FDM1	FDA 5	Female	54	0
	FDM2	FDA 10	Female	55	0
intermedia	FDI1	FDA 5	Male	55	0
	FDI 2	FDA 9	Male	38	0

From a total of four donor trees, the fig wasps entered figs on all four male recipient plants, but only three of the female recipients (Figure 5.14). Pollination rates of female var. *angustifolia* figs were lower than in males. Between 5-45% of the male figs were entered compared 0 to 10% of the figs on female trees (Figure 5.14). The crop sizes of male recipient var. *angustifolia* ranged from 11 to 2115 figs while in female recipient trees the range was from 175 to 1984 figs.

There was a negative relationship between crop size of the recipient male trees and the percentage of available figs entered (Figure 5.15). A similar relationship was present in female figs, though the proportion of figs pollinated was never high. These results reflect the small numbers of pollinators that were present. No figs on female plants were entered by pollinators emerging from donor FDA 1 but this tree had 25% of total number of entered figs in male tree. The highest number of entered figs comes from FDA 2 with 106 figs in the male tree. This compares with 34, 18 and 58 figs around donor trees FDA1, FDA3 and FDA5 respectively.

The number of entered figs varied from 5 to 106 figs on recipient male trees while on female trees the range was from zero to 58 figs (Figure 5.16). The male trees around donor trees FDA 1 and FDA 2 had a higher total number of figs entered than female trees, whereas more pollinators entered more female figs than male figs around donor trees FD3 and FD5 (Figure 5.16). However, the proportion of male figs entered was at least slightly higher than with female figs around all four donor trees (Figure 5.17). In terms of proportions of available figs, the figs entered on male trees ranged from 0.05 to 0.46 while on female trees, the range was from 0 to 0.11. The highest proportion of entered figs was around donor tree FDA 5, where 5 out of 11 male figs were entered. Chi square tests showed there was a significant difference between entered and not entered figs in male and female figs from donor tree FDA 1 ($\chi^2 = 420.19$, df = 1, $P < 0.05$), FDA 2 ($\chi^2 = 115.35$, df = 1, $P < 0.01$) and FDA 4 ($\chi^2 = 10.25$, df = 1, $P < 0.01$). Around donor tree FDA 3, the difference was not significant ($\chi^2 = 0.91$, df = 1, $P > 0.01$).

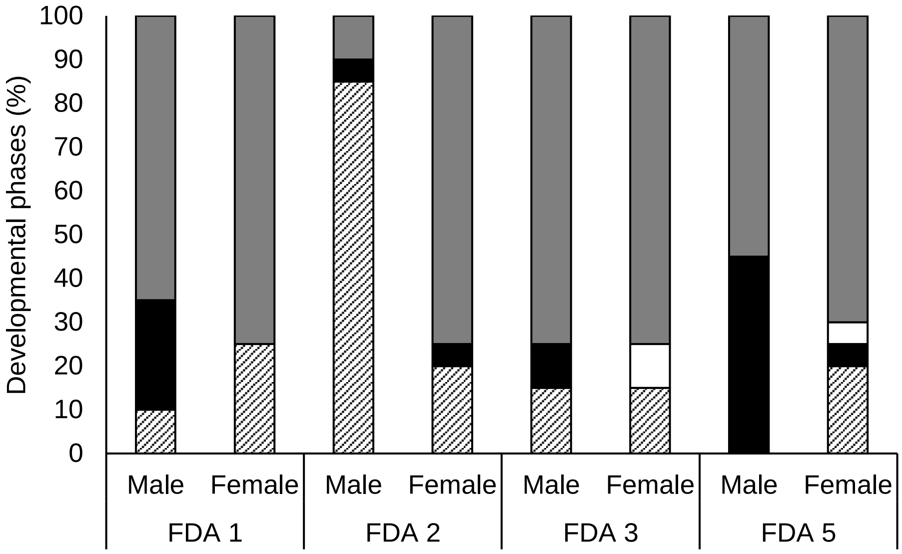


Figure 5.14 The developmental phases of figs on male and female var. *angustifolia* located around four different donor trees (FDA 1-3 & 5). Hashed bars = AB phase, black bars = C phase, white bars = DE phase, grey bars = aborting figs. Four male and four female plants were positioned around each donor tree.

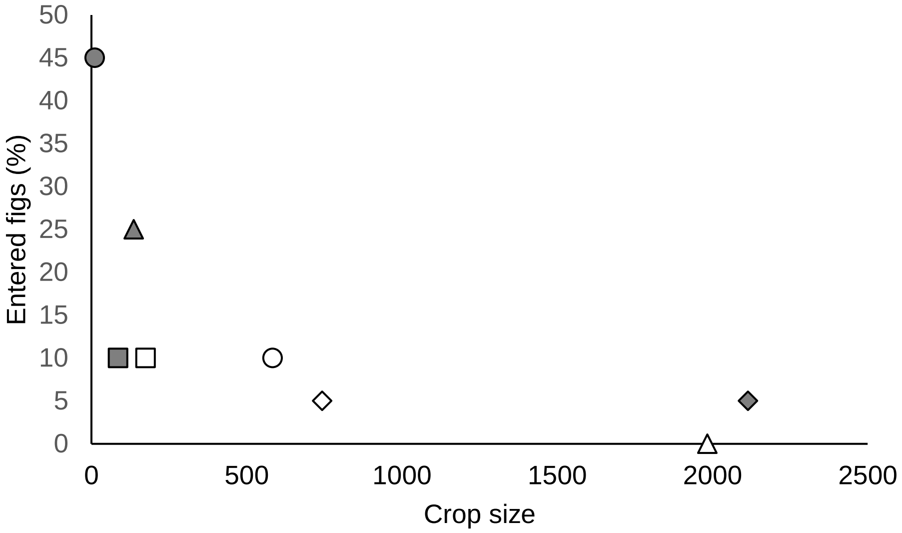


Figure 5.15. The relationship between recipient tree crop size and the percentage of entered figs from different donor trees. (Grey colour = male trees, white colour = female trees, (▲) = donor tree FDA 1, (♦) = FDA 2, (■) = FDA 3, (●) = FDA 5).

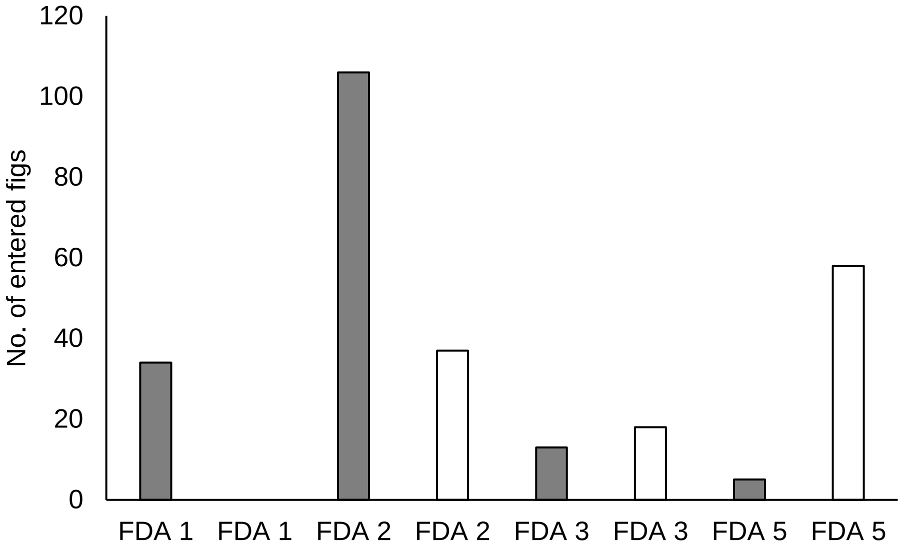


Figure 5.16. The numbers of entered male and female figs around different donor trees (Solid bars = male recipient trees, open bars = female recipient trees). No female figs were entered around tree FDA1.

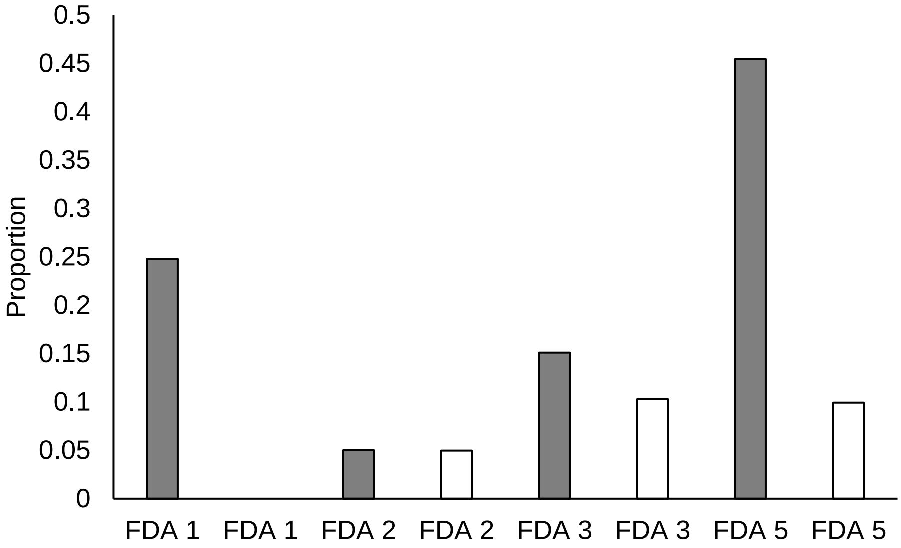


Figure 5.17 The relationship between the proportion of entered figs and different donor trees. (Solid bars = male trees, open bars = female trees).

Host choice by *Blastophaga* sp. (var. *deltoidea*)

From 9-18 receptive figs of var. *angustifolia* were in the fine mash bags and available for entry by the single *Blastophaga* sp. (var. *deltoidea*) female that was placed in each (Table 5.8). The figs were brought to the Lab. fourteen days later to check for wings and foundress bodies. None of the confined figs were entered by the introduced foundresses, with no sign of wings and wasp bodies in the confined figs. Similar experiments carried out using pollinators obtained from figs of var. *angustifolia* routinely resulted in pollinator entry (Chapter 7).

Table 5.8. Numbers of confined figs of var. *angustifolia* available for entry.

Tree	Sex	Bag	Figs		Figs with wings/wasps
			Receptive	Entered	
FD1	Female	1	12	0	0
		2	14	0	0
		3	13	0	0
		4	9	0	0
		5	15	0	0
		6	10	0	0
FD6	Male	1	14	0	0
		2	13	0	0
		3	11	0	0
		4	11	0	0
		5	13	0	0
FD7	Male	1	17	0	0
		2	18	0	0
		3	15	0	0
		4	13	0	0
		5	16	0	0
FD22	Male	1	11	0	0
		2	15	0	0
		3	15	0	0
		4	14	0	0
		5	11	0	0
FD40	Female	1	15	0	0
		2	18	0	0
		3	16	0	0
		4	14	0	0
		5	9	0	0
		6	11	0	0

5.5 Discussion

Ficus deltoidea often exhibits variable leaf and fig characters that have made delimitation of the varieties difficult. Because of intermediates in morphology, Berg and Corner (2005) considered all variation within *F. deltoidea* to be within a single species, but more recent morphological studies conducted by Mat et al., (2012) and Nur Fatihah et al. (2014) confirmed that varieties can vary greatly in their leaf morphology. Whether the varieties are actually distinct biological species is unclear. Whether varieties have different species of host specific associated pollinators provides an indication of likely barriers to gene flow between them. Among the factors that maintain specificity in the fig and fig wasp mutualism are fig wasp behavioural responses and morphological adaptations towards the structure of the figs (Liu et al., 2013). In closely related fig tree species, pollinator host specificity is maintained by combinations of long range cues from floral scent, short-range contact cues and physical matching between the fig wasp and its host (Wang et al., 2013). This host specificity between fig and fig wasps ensures that genetic integrity is maintained (van Noort, 2003).

5.5.1 Host choice by *Blastophaga* sp. (var. *angustifolia*)

5.5.1.1 Host preference between varieties

In Experiment one, all recipient trees of variety *angustifolia* had some figs where seeds or pollinator offspring were developing, but no similar development was recorded among figs of any other varieties. Dissections of figs showed there was no sign of fig wasp females entering the figs of these varieties, so their lack of development was the result of pollinators from var. *angustifolia* failing to enter, rather than an inability to gall flowers of male figs or to pollinate flowers in female figs. This result suggested that figs of different varieties may emit different attractant volatiles and that pollinator sharing by different varieties is likely to be rare, even when they are living sympatrically (Moe et al., 2011).

In the second experiment, competition for pollinators from receptive var. *angustifolia* figs was much lower than in experiment one, because far fewer receptive figs of the usual host were available for entry. However, far fewer pollinators were also being released in the area. Under these conditions, only male trees of var. *angustifolia* were pollinated, and no females of this variety or individuals of either sex belonging to other varieties were entered by pollinators. This suggests that pollinator specificity can be maintained away from natural conditions (Cornille et al., 2012), but the results were limited by the small number of pollinators that dispersed from their natal figs

Pollination rates among male var. *angustifolia* were higher among those plants that had fig wasps emerging from them and male FDA 9 was the only plant to acquire pollinators that had certainly flown from other male plants only a metre or so away. This result has parallels with a study by van Noort (2003) where he recorded that on an African tree with asynchronous fig development, the fig wasps tended to pollinate figs on their natal trees and that this reduced their flying costs as they only needed to fly a short distance.

Some fig wasps are able to move pollen between flowering fig trees that are separated by many kilometres (Harrison and Rasplus, 2006). Ahmed et al. (2009) showed that *Ceratosolen arabicus* Mayr females can reach trees with receptive figs160 km from their natal trees, even if the flight period of fig wasps can last for just a few hours (Liu et al., 2013). However, Duthie and Nason (2016), suggest that foundress density increases when tree connectivity is higher, because pollinator mortality will increase with distance travelled. The fig wasps from monoecious trees often have wider dispersal ranges compared to the fig wasps associated with dioecious fig tree species such as *F. deltoidea* (Borges 2016).

The entry of small numbers of fig wasps from var. *angustifolia* into var. *deltoidea* figs in experiment four showed that under these experimental conditions the fig wasps will enter figs of other varieties. Most of the pollinators did choose to enter figs of their typical host. It seems likely that emerging females enter the first receptive figs they encounter, which are usually the figs on their natal trees. However, in this experiment there were no receptive figs on the donor trees and this made the fig wasps need to fly to the other trees to find receptive figs. The figs of var. *deltoidea* are the most like var. *angustifolia* in their external appearance and size, compared to the other varieties, and this might be the reason why the fig wasps entered the figs of var. *deltoidea* rather than the other varieties (Chapter 4). Alternatively, the volatiles released by this variety may be the most similar to those of var. *angustifolia*. However, a conclusion cannot be made as the fig wasps might have entered this particular variety because its figs were at their peak attractiveness whereas other varieties may have had figs that were a little older or younger.

Regardless of why only figs of var. *deltoidea* were entered, this result showed that the var. *angustifolia* pollinator can enter male var. *deltoidea* figs, and that the ostiole does not present a physical barrier. The fig wasps were also able to gall the flowers. Galling is initiated at the time of oviposition, so eggs were probably laid in these figs, because the style lengths of the flowers are suitable for this pollinator (Ghana et al., 2015a) (Chapter 4). The initial formation of galls requires the injection of a secretion from the foundress into the ovules. The gall tissue later become the source of food for the developing larva (Martinson et al., 2014). However, even though galls developed, no sign of larval development were detected in any of the galls in the three figs of var. *deltoidea*. They had become empty galls (bladders) and clearly if eggs had been laid than any larvae were unable to develop successfully. This will generate a strong selection pressure to avoid male figs var. *deltoidea* in areas where the two varieties grow close together.

The specificity of the interaction between *Blastophaga* sp. and var. *angustifolia* is maintained because few pollinators were willing to enter var. *deltoidea* figs and no larvae successfully developed inside the figs of var. *deltoidea*. This in turn will make it also less likely that they will enter female figs of var. *deltoidea* and this will cut down the chances of hybrids being formed. None of the female figs of var. *deltoidea* were pollinated, so whether hybrid seeds are viable is unknown. Indications that the pollinator of var. *deltoidea* similarly avoids figs of var. *angustifolia* are provided after the following section.

5.5.1.2 Preferences between male and female plants

Because of the relative costs of seed and pollen production, in plants in general, male-functioning individuals are expected to allocate more resources to secondary floral structures and products than female individuals (Delph and Lively, 1992). In *Ficus*, the pollinator is not expected to be able to distinguish the sex to maintain the mutualism. Otherwise the pollinator could turn into a parasite that avoids female trees (Kjellberg et al., 1987).The conflict between what is best for the fig tree and what is best for the pollinators is thought to have led to inter-sexual mimicry, with figs on male and female plants producing the same volatiles and other cues to make it difficult for pollinators to avoid female figs (Soler et al., 2002; Hossaert-McKey et al., 2016). This mimicry is strengthened by vicariant selection, because it is in the interest of both male and female plants that the pollinators cannot distinguish between male and female figs (Grafen and Godfray, 1991).

Almost all the control plants (var. *angustifolia* with receptive figs and no emerging fig wasps) had at least some of their figs entered by pollinators. The frequency of pollination was lower on female plants. This suggests that the pollinator of var. *angustifolia* can detect differences between figs on male and female plants and prefers the cues provided by male figs. Entering a female fig guarantees that a foundress fig wasp will produce no offspring (Cook and Rasplus, 2003; Borges, 2016) but benefits the natal male tree from where the fig wasp emerged, because its pollen will be generating seeds. Conversely, a fig wasp that enters a male fig can reproduce but provides no benefits to its natal tree if it entered figs on another male tree. If it enters figs on its natal tree then there are nonetheless postponed potential benefits because one foundress can produce numerous offspring, each of which has a chance to fly away and enter female figs.

Several studies have found that male and female figs release similar volatiles and pollinators cannot distinguish between them (Hossaert-McKey 2016; Rodriguez et al. 2017). However, in *Ficus carica* L. the pollinators prefer to enter male figs rather than female figs (Anstett et al., 1998). It was suggested that in this species inter-sexual mimicry is not important, because very few receptive male figs are available at the time of year when female figs are receptive (Hossaert-McKey et. al., 2016). This is not the case with var. *angustifolia*, which produces figs asynchronously on both male and female trees, so this hypothesis fails to explain the preference shown by its pollinator for male figs. The mean diameter of receptive male and female figs is similar (Chapter 4) so the figs seem to offer similar visual cues. A difference in volatiles may be involved.

5.5.2 Host choice by *Blastophaga* sp. (var. *deltoidea*)

A molecular study of four varieties of *Ficus deltoidea* (var. *angustifolia*, var. *bilobata*, var. *trengganuensis* and var. *kunstleri*) suggested that there is gene flow among varieties of this species (Zimisuhara et al., 2015). Based on the behaviour of the pollinators of var. *angustifolia* and var. *deltoidea* any gene flow between these two varieties is likely be very limited if it occurs at all, because pollinators of both varieties were generally unwilling to enter figs of other varieties.

5.6 Conclusions

With its asynchronous crops, var. *angustifolia* often offers the chance for populations of its pollinators to cycle on a single male tree. Fig wasps tend to enter the first figs they have encountered regardless of the sexes and diameter of the figs (Moore et al., 2003). Taking time to make a choice of which fig to enter will cost them time and thus increase the possibility of reproductive failure (Moore et al., 2003). The pollinators of var. *angustifolia* are reluctant to fly away from their natal trees, even if receptive figs on other trees are only a metre or two away. If they do disperse from their natal trees they prefer to enter figs on male trees. This behaviour clearly benefits the insects, and has the consequence that pollinator limitation of seed production is very strong in natural populations (Chapter 6).

Chapter 6

Phenology of *F. deltoidea* var. *angustifolia* in a Malaysian oil palm plantation

6.1 Introduction

6.1.1 Phenology

The tracking of suitable environmental conditions through time is an essential adaptation for plants and animals (Diekmann, 1996). The word phenology is derived from Greek ‘phainein’ which means to show or appear (Fenner, 1998). Phenology is the study of the periodical (often seasonal) timing of life cycle events. In plants this commonly includes events such as flowering, fruiting, leaf production and leaf fall and when they occur in relation to biotic and abiotic factors (Harrison et al., 2000). Phenology can be considered within an individual and within a population (Elzinga et al., 2007). Phenology studies usually integrate variation within plants and populations with variations of climate (Visser et al., 2010). The timing of vegetative growth and reproduction is an important adaptation to seasonality and can be predictable from year to year but variation in temperature and other climatic variables such as rainfall can generate variation between years in flowering times (Inouye, 2008).

The study of phenology can deliver information on many aspects of plant biology, including development and patterns of plant growth and the extent of the influence of environment and selective pressures on flowering and fruiting behaviour (Zhang et al., 2009). Data on phenology can also be used in land-use planning, agricultural control, protection of species and notification of pollen release, which has implications for human health (Ruml and Vulic, 2005). Long term observations on phenology can also be used to discover patterns resulting from climate change.

Temperature and the availability of water and light are often the critical environmental factors that determine patterns of phenology (Ulian et al., 2013; Krishnan et al., 2014). Phenological patterns reflect both responses to environmental triggers and the selection pressures that have favoured these responses. These include both abiotic and biotic influences such as those generated by pollinators and other mutualists.

6.1.2 *Ficus* phenology

The phenological patterns of fig trees, particularly those involving fruiting and leaf production, are central to their pollination biology and broader ecological significance for animals. Fig trees (*Ficus*, Moraceae) are involved in a highly specific and often one-to-one relationship with pollinating fig wasps (Agaonidae) that breed in their figs. Consequently, their fruiting phenology has a critical influence on populations of their pollinators. Any mismatch in timing will then have a detrimental effect on seed production as well as their pollinators (Zhao et al, 2014). Comparative phenological studies can reveal how the mutualism functions and how it has evolved (Bronstein and McKey, 1989; Patel, 1996)

Most phenological studies have involved monoecious rather than dioecious fig tree species (Herre, 1989; Harrison et al., 2000). Monoecious fig trees are often characterised by having synchronous fruiting within individual trees and asynchrony between trees in a population (Herre et al., 1996). Dioecious fig trees respond differently to seasonal variability than monoecious figs due to the separation of seed and wasp production between trees (Kjellberg and Maurice, 1989). In dioecious species there are often phenological differences between male and female individuals, but there needs to be reproductive coordination between the sexes (Zhao et al, 2014).

Relationships between leaf and fruiting phenology are variable. Individuals of some species do not produce young leaves and figs at the same time (Compton, 1993) while others produce young leaves and figs simultaneously (Peng et al., 2009). Some trees have synchronous leaf production but asynchronous fruiting phenology. For example, *Ficus racemosa* Linn. in China can display synchronous flushing and senescence of leaves, but they produce new figs all year long (Zhang et al., 2006). Local environmental conditions can alter phonological patterns. An example is where drought can trigger fig trees to lose their leaves to minimize water loss (Compton, 1993).

The phenology of fig trees is likely to be different in seasonal and aseasonal habitats, with more synchrony in the former (Spencer et al., 1996). A common pattern in monoecious species is for figs to be produced throughout the year with seasonal peaks during the warmest periods and low numbers of tree fruiting during cold seasons (Kjellberg and Maurice, 1989). This allows populations of their pollinators to be maintained throughout the year. In dioecious species seasonal or dry environments should favour fig production by female trees to be concentrated during periods when fruit dispersal and seed germination and likely to be most successful (Patel, 1996). For example, (Patel and McKey, 1998) showed that female trees of *Ficus exasperata* Vahl. and *Ficus hispida* L.f. have more mature figs during the wet season when conditions are most suitable for germination.

Female trees have no role in maintaining pollinator populations, though they do act as traps for the pollinators that they attract (Suleman et al., 2011). The reproductive success of male trees depends on their production of pollen-carrying fig wasps that enter figs on female trees. Reflecting this, male trees of *F. hispida* produce more figs during the dry and early wet season and male *F. exasperata* release fig wasps at the time of peak production of receptive female figs (Patel and McKey, 1998). Limited out-of-peak-season fig production on male trees ensures that their pollinator populations are maintained throughout the year.

The effects of seasonal variation are usually less prominent close to the equator and this leads to more asynchrony in leafing and fruiting at individual and population levels (Galil and Eisikowitch, 1968; Zhang et al., 2006). This results in temporal overlap between the sexes in dioecious species (Patel, 1996) with mature and receptive figs produced continuously throughout the year (Suleman et al., 2011). The need to maintain pollinator populations throughout the year helps makes monoecious fig trees keystone in tropical areas as they provide food to frugivores at times when the other plants are not fruiting (Shanahan et al., 2001). Male dioecious fig trees must also maintain fig wasp populations, but their figs are not normally eaten by vertebrates. Figs of female dioecious fig trees are attractive to vertebrate frugivores, but their greater seasonality may reduce their significance as keystone resources.

6.1.3 *Ficus deltoidea*

Ficus deltoidea is a small dioecious fig tree from SE Asia (Corner, 1969). It is highly variable and numerous varieties have been described, some of which are likely to be different biological species. It is unusual within its genus because its female figs contain an unusually small number of flowers that generate atypically large seeds. It is also unusual because some varieties grow as true epiphytes. *Ficus deltoidea* also displays extreme variation in leaf forms between varieties and can also produce leaves that differ in shape on young and mature individuals (Mat et al., 2012).

Hetrophylly is defined as being variable leaf morphology within a single species. It is often exhibited in response to varying environmental conditions (Kuwabara et al., 2003). Variable environmental factors that promote heterophylly include light intensity and quality, ambient temperatures and water availability (Nakayama et al., 2017). Plant hormones involved in heterophylly are ethylene that influences leaf elongation (Kuwabara and Nagata, 2006), gibberellins that cause expansion of the leaf lamina (Sun, 2010) and auxins that generate outgrowths of the leaf lamina (Scarpella et al., 2010).

In *F. deltoidea,* heterophylly is reflected in the production of leaves of different size or shape. Elongate and deltoid leaves are produced. Juvenile individuals tend to have elongate leaves while mature trees tend to have deltoid leaves or a mixture of the two (S.G. Compton personal communication). Reasons for the change in leaf form with maturity are unknown but having fewer leaves with more surface area may help small trees to capture light and carbon (Pearcy et al., 2004). Plants with only juvenile leaves rarely produce figs under field conditions but will do so in containers (personal observations of var. *angustifolia*). Plants grown from cuttings do not revert to elongate leaves (S. G. Compton personal communication).

Corner (1969) recorded elongate juvenile leaves in several varieties of *F. deltoidea* (var. *angustifolia*, var. *deltoidea*, var. *intermedia* and var. *motleyana*). Mat et al. (2012) similarly recorded elongated juvenile leaves in var. *angustifolia*, var. *bilobata*, var. *intermedia* and var. *trengganuensis*. No other study of heterophylly was conducted after that.

6.2 Objectives

The phenology of any variety of *F. deltoidea* has not been recorded previously. We (1) examined the leaf and fruiting phenology of var. *angustifolia*, a variety that exhibits heterophylly, when growing as an epiphyte in an oil palm plantation in Peninsular Malaysia. (2) Examined the extent of within and between individual asynchrony in fig production and how crop and fig wasp population sizes responded to seasonal changes. (3) Asked whether the phenology of male and female plants differs and (4) recorded the extent to which seed production is limited by pollinator availability and whether more isolated female trees were more likely to suffer from a shortage of pollinators.

6.3 Methods

6.3.1 Study sites and sampling methods

In this study 39 dioecious *F. deltoidea* var. *angustifolia* individuals were monitored every two weeks for 15 months from 13th June 2016 until 21st August 2017. This provided a total of 34 dates when 21 male fig trees and 18 female fig trees were observed to determine their leafing and fruiting phenology. All the plants were epiphytes in an oil palm plantation at Banting in Selangor, Peninsular Malaysia. They were mature trees producing figs and were growing less than 2.5 meters from the ground, which provided easy access. The rest of the epiphytes either did not produce fruit or were too high to be assessed. The sexes of the plants were determined by opening a few figs on each and observing the number of female florets.

At the beginning of the study, the *F. deltoidea* var. *angustifolia* were mapped, located using GPS and tagged. The plants have a multi-stemmed, shrubby growth form that makes size estimates difficult. Measurements of the diameters of the thickest stems on each plant were made using callipers to provide an indication of plant size. The height of the basal part of each tree was recorded using an adjustable pole and measuring tape. Climate data (minimum and maximum temperatures, monthly rainfall totals) were obtained from the nearest meteorological station (KLIA) located 19 km from the site. Details were provided in Chapter 2. The observations were done visually. Visual assessments of the leaves and figs on the plants were made during each visit.

In some cases, the leafing and fruiting phenology of two plants growing on the same trunk was recorded.

6.3.2 Leaf phenology

During each visit the presence or absence of elongate (juvenile) and adult leaves was recorded (Figure 6.1) and leaf condition was scored as new, mature or senescent. Elongate leaves are produced by seedlings and young plants and are gradually replaced by smaller, more rounded leaves as plants get older. The proportion of leaves that were juvenile was assessed by visual inspection. The stage of development of the juvenile and adult leaves (combined) was also scored by visual inspection. New leaves were smaller and paler green in colour. Mature leaves were larger and darker green. Senescent leaves were yellow. If less than ten senescent leaves were present this state was recorded as ‘rare’. Bare stems were also recorded. Juvenile leaves also scored as young, mature or senescing.

6.3.3 Fruiting phenology

The approximate numbers of figs on each tree were counted and the developmental phases were recorded. *F. deltoidea* produces two figs at the axil of each other. The sizes of fig crops were scored as being one of the followings <10, 11- 50, 51-100, 101-200, 201-500, 501-1000, 1001-2000 and more than 2001. Because of their relatively small crop sizes and the usually continuous presence of figs on the trees, large scale destructive sampling of figs to record their developmental stages was not considered appropriate. Estimates of the development of the figs were therefore based mainly on non-destructive visual assessments.

Before the start of routine monitoring, figs that covered the whole range of sizes were collected from male and female plants. After noting their external appearance (size, thickness, colour) the figs were opened, and their developmental phases were compared with their appearance. This preliminary study was used in building knowledge that allowed visual assessments to be made of the development of the figs without opening them.

Fig developmental phases were recorded based on Galil and Eisikowitch (1968). The details for fig developmental phases in male and female figs were described in Chapter 2. In both male and female figs, the colours were recorded as bright green, yellowish green and yellow. A bright green colour was associated with young figs at AB phase, yellowish green was associated with C phase while yellow usually indicated that the figs were aborting. Size can also give an indication of a fig's developmental phase. AB phase figs of var. *angustifolia* usually lie within the range of 1-4 mm, figs with a range of 4-6 mm can belong to any phase, but figs with a diameter more than 6mm are usually either C or DE phase. AB phase figs are usually soft when squeezed, as are aborting figs. During C and DE phases, the texture is typically hard.

6.3.4 Fig development cycle

At the start of the study, ten random A phase figs on each tree were marked using a permanent fine marker. The diameter of each fig was then measured fortnightly and the developmental stage of the fig was recorded based on its appearance. For figs that failed to be pollinated, the duration they remained on the tree before being aborted was also recorded.

6.3.5 Statistical methods

The relationship between the crop size categories and the thickest stem diameter for both plants sexes were correlated using Spearman rank test. Similar tests were also used for the correlation between crop size categories with the basal height from the ground. Between sexes comparisons used Mann-Whitney U tests for proportional data and Chi-squared for binary data. All tests were performed in SPSS Statistics 20.

Figure 6.1 Leaf developmental phases and juvenile leaves of *F. deltoidea* var. *angustifolia*. From left, young, mature, senescent and elongate (juvenile) leaves. The size of senescent leaves was variable.

6.4 Results

One hundred and thirteen *F. deltoidea* var. *angustifolia* were present as epiphytes on the trunks of the oil palms, with 83 producing figs during the period of the study. Among the 113 trees, only 8 produced only juvenile leaves and none of these produced any figs. A further 105 trees had a mixture of juvenile and adult leaves or only adult leaves. The exact number of trees with only adult leaves was not recorded as some of the trees were too high to assess the overall crown. The sexes of 51 trees with accessible figs were determined. There were 27 male trees and 24 female trees. From these, the leaf and fruiting phenologies of 21 males and 18 females were recorded. The sexes, diameter of the thickest stem and the height of the basal parts of these var. *angustifolia* are recorded in Table 6.1. Most of the tress survived throughout the period of observations. However, before the last three observation dates, 9 trees (7 male and 2 female) were cut off by the farmer.

6.4.1 Leaf phenology

Among the 21 male trees, 9 consistently had a mixture of juvenile and adult leaves, 7 intermittently had adult leaves in combination with juvenile leaves and 5 trees had only adult leaves throughout (Table 6.2). The juvenile leaves on a tree ranged between 0 to 60% of the total (Table 6.3). Among female trees, 10 out of 18 consistently had a mixture of juvenile and adult leaves and 5 intermittently had this mixture while only three of them never had any elongate leaves. The juvenile leaves on the trees again ranged from 0-40% of the total leaves. Male and female trees had the same frequencies of leaf types (mixed, intermittent adult only and adult only) (Chi square, $\chi^2 = 0.66$, df = 2, $P > 0.05$). On plants with a mixture of leaves, the location of juvenile leaves was usually at the tips of new branches, but on older branches they were usually near the base of the branch (Figure 6.2). In both sexes, nearly mature leaves started to show golden spots. Leaves facing the sun also tended to be have some yellowish colour. This was distinct from the yellow colour of the senescing leaves.

Figure 6.2. The locations of juvenile and mature leaves on an epiphytic *F. deltoidea* var. *angustifolia* tree. The yellow arrow indicates a juvenile leaf, and the red arrow a mature leaf.

Table 6.1 Sexes, diameter of the thickest stem and the height of the basal parts of trees of *F. deltoidea* var. *angustifolia* in Banting oil palm plantation.

No.	Sex	Diameter (cm)	Height (m)	No.	Sex	Diameter (cm)	Height (m)
1	Female	1.7	2.02	21	Female	0.6	1.45
2	Female	2.1	1.93	22	Male	1.7	2.07
3	Male	1.2	2.27	23	Female	1.0	4.17
4	Female	2.1	2.58	24	Male	0.8	3.65
5	Female	0.9	2.88	25	Female	0.5	1.60
6	Male	0.8	2.30	26	Male	1.0	2.77
7	Male	2.0	1.46	27	Female	1.8	3.45
8	Male	1.6	2.20	28	Male	1.0	1.12
9	Female	0.7	1.62	29	Female	0.7	0.99
10	Male	1.5	2.44	30	Male	1.3	1.68
11	Female	0.7	2.57	31	Male	1.4	1.33
12	Male	1.0	1.22	32	Male	1.2	0.70
13	Male	1.7	1.35	33	Female	0.8	1.10
14	Female	1.0	0.11	34	Male	1.0	0.73
15	Female	0.2	1.20	35	Male	0.9	2.47
16	Female	1.0	0.43	36	Female	1.0	2.32
17	Female	1.3	2.08	37	Male	2.1	1.85
18	Male	0.8	0.29	38	Female	1.2	1.68
19	Male	0.8	1.56	39	Male	0.5	0.48
20	Male	0.7	2.96				

Table 6.2. Types of leaf on the 39 accessible var. *angustifolia* fig trees.

	Consistent mixture of juvenile and mature leaves	Intermittent mixture of juvenile and mature leaves	Only mature leaves
Male	9	7	5
Female	10	5	3

Table 6.3 Juvenile leaves on var. *angustifolia* in Banting plantation as a proportion of total leaves based on 34 observations at approximately 14 day intervals.

Tree no.	Sex	Juvenile leaves (%)		Tree no.	Sex	Juvenile leaves (%)	
		(Mean ± SD)	Range			Mean ± SD	Range
1	Female	8.09 ± 2.47	5-10	21	Female	23.68 ± 5.68	10-30
2	Female	3.97 ± 2.05	0-5	22	Male	0	0
3	Male	2.21 ± 2.52	0-5	23	Female	1.03 ± 3.85	0-20
4	Female	0	0	24	Male	0	0
5	Female	7.5 ± 2.54	5-10	25	Female	0	0
6	Male	27.94 ± 4.10	20-30	26	Male	0.88 ± 2.60	10-20
7	Male	5.00 ± 0.00	5	27	Female	0	0
8	Male	3.82 2.15	0-5	28	Male	30.97 ± 5.95	20-60
9	Female	9.18 ± 4.35	5-20	29	Female	13.87 ± 11.88	5-30
10	Male	0	0	30	Male	4.41 ± 2.39	0-10
11	Female	7.36 ± 2.53	5-10	31	Male	2.42 ± 2.54	0-5
12	Male	12.10 ± 4.96	5-20	32	Male	3.71 ± 2.22	0-5
13	Male	2.35 ± 2.53	0-5	33	Female	26.29 ± 8.75	10-40
14	Female	8.68 ± 5.81	5-30	34	Male	27.10 ± 10.39	10-60
15	Female	2.79 ± 3.73	0-15	35	Male	0	0
16	Female	8.38 ± 2.37	5-10	36	Female	9.85 ± 6.90	0-30
17	Female	20.00 ± 6.63	10-30	37	Male	7.79 ± 2.51	5-10
18	Male	9.19 ± 1.87	5-10	38	Female	0.74 ± 1.80	0-5
19	Male	3.87 ± 2.13	0-5	39	Male	22.06 ± 4.10	20-30
20	Male	0	0				

During the 15 months of observation individual plants often had a mixture of different leaf states at the same time (combinations of bare stems, young, mature and senescing leaves). In January 2017 the farmers at Banting sprayed the ground vegetation in the plantation. The main purpose of the spraying was to eliminate weeds and the other plants around the bases of the palms. The farmers did not deliberately want to spray the fig trees but some were damaged, especially those growing low down on the trunks. The effects of the sprays are shown clearly by the number of male plants that had one or more bare stems (Figure 6.3).

Among the male trees, 19 had new leaves present every time and the remaining tree (D10) had only one time (25.05.2016) when new leaves were not present. Mature leaves were present on each male tree throughout the period. Senescent leaves were present on at least some male trees throughout the year, but there were more male trees with senescent leaves in the period around January 2017, when the spraying took place, and fewer plants with senescing leaves at the beginning and end of the survey period (Figure 6.4).

Leaf production on the female trees was similar to that of the males. Young and mature leaves were present on all 18 female trees throughout the period of observations. The numbers of female plants with bare stems increased after the spraying activities on January 2017 (Figure 6.5). The numbers of senescing leaves kept going up and down throughout the year, with no clear pattern (Figure 6.6).

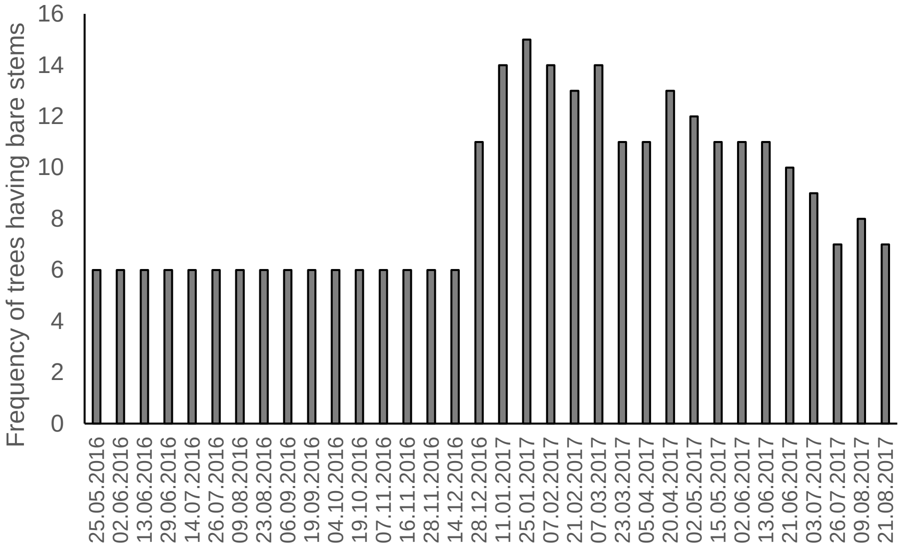


Figure 6.3 Changes in the frequency of male trees having one or more bare stems. (A total of 20 male trees were observed each time). Spaying activities occurred in January 2016.

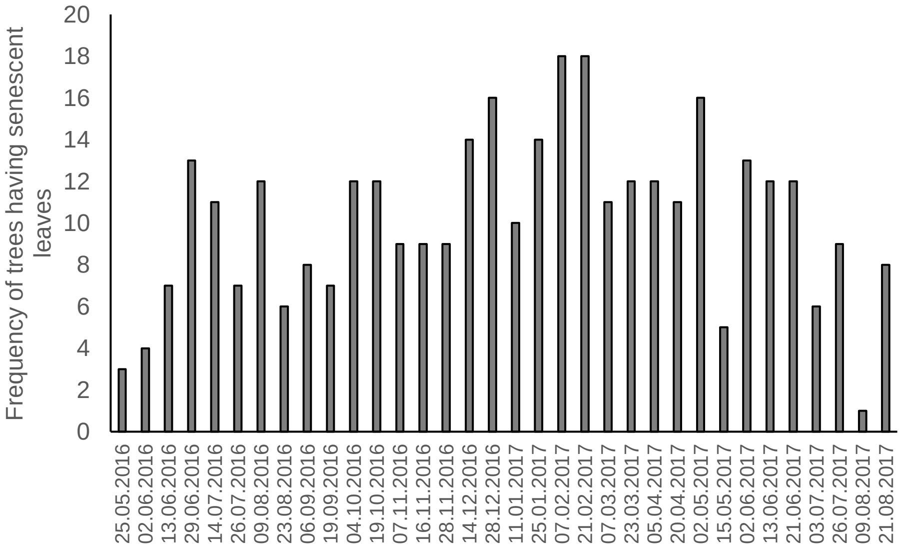


Figure 6.4 Changes in the frequency of male trees having senescent leaves. (A total of 20 male trees were observed each time).

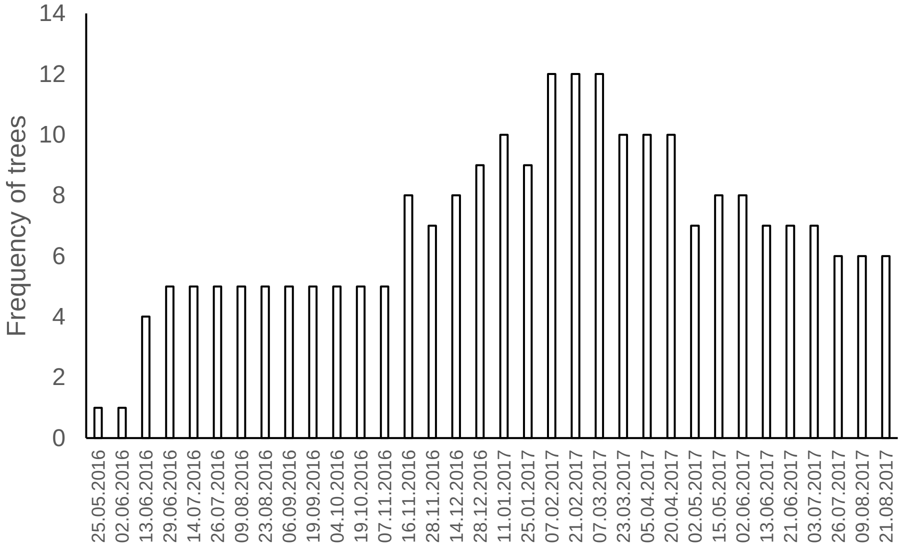


Figure 6.5 Changes in the frequency of female trees having bare stems. (A total of 18 female trees were observed each time).

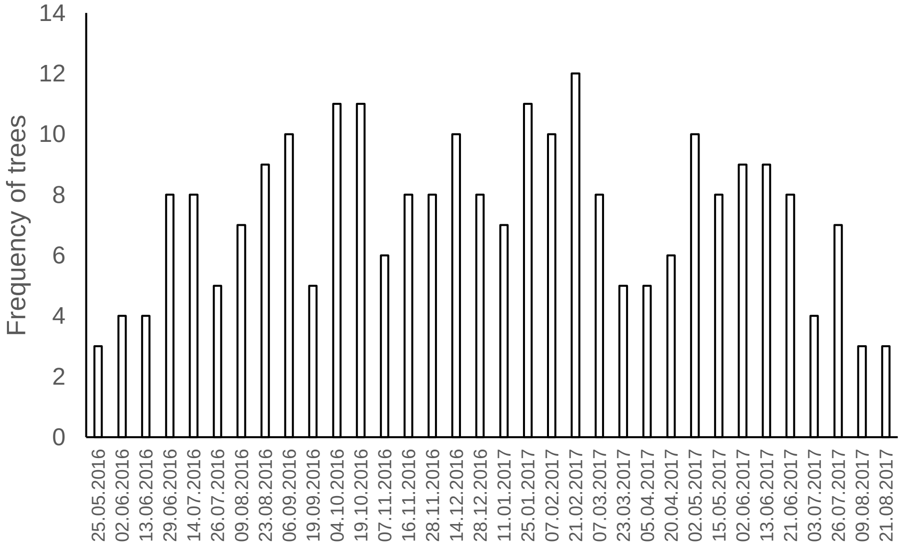


Figure 6.6 Changes in the frequency of female trees having senescent leaves. (A total of 18 female trees were observed each time).

6.4.2 Fruiting phenology

6.4.2.1 Male plants

Figs were present all the time on almost all the male trees. The single male tree that did not bear figs all the time (D39) had figs present on about half of the sample dates. This plant was small, with about 30% juvenile leaves. Crop sample sizes (the number of figs present on a tree) on male trees ranged from less than ten to over 2000. Most crops on male trees numbered between 200-500 and 500-1000 figs (Figure 6.7).

No more than two trees had less than 10 figs or more than 2000 figs at any one time. Numbers of figs on the trees increased from September 2016 until November 2016 and from May 2017 until July 2017, when a few trees were cut off by the farmers. There was no evidence that larger plants produced larger crops because there was no significant relationship between the thickest stem diameter of a plant (a measure of plant size) and crop size categories (Spearman rank correlation, $r_s = 0.39$, $n = 21$, $P > 0.05$). Crop sizes were larger on male plants that originated higher on the trunks of the oil palms (Spearman rank correlation, $r_s = 0.54$, $n = 21$, $P < 0.05$).

The production of new figs by male trees was all year around and all the male trees were producing new figs from mid-July to mid Sept-16 (Figure 6.8 and Figure 6.9). The development of the figs on most male trees was asynchronous. The D and E phase figs were routinely still on the trees when young A phase figs appeared, so emerging fig wasps could often enter figs on the same trees.

Only a few male individuals produced synchronised crops with only one or two phases present at any one time. The release of pollinators (as shown by the presence of D+E phase figs) occurred throughout the year with a peak in February and March 2017. The sudden decrease in the numbers of figs in all developmental phases during the last three observations was due to the cutting of plants by the farmers. Seven male trees were affected. Tree D22 was the only plant that had DE phase present on every visit (Figure 6.10). This was the largest male plant with a crop size of more than 2000 figs all the time. DE phase figs were only recorded on single occasions on trees D28, D34 and D39. They had only small numbers of figs throughout. The peak of fig wasp emergence was in February 2017 when 16 of the 21 male trees had DE phase figs. This was about six months after the peak in production of new figs in August 2016.

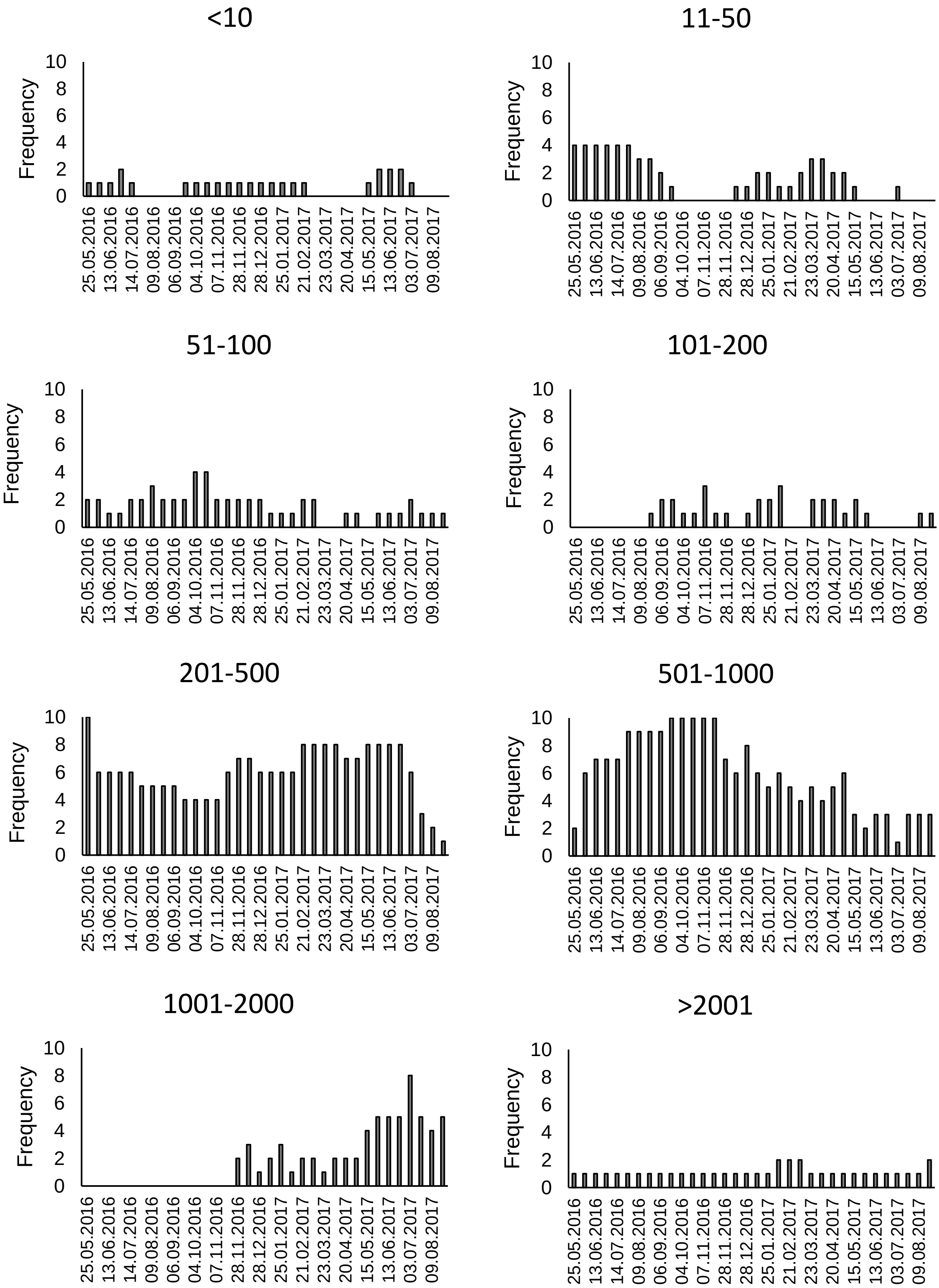


Figure 6.7 Changes over time in crop sizes on 21 male trees.

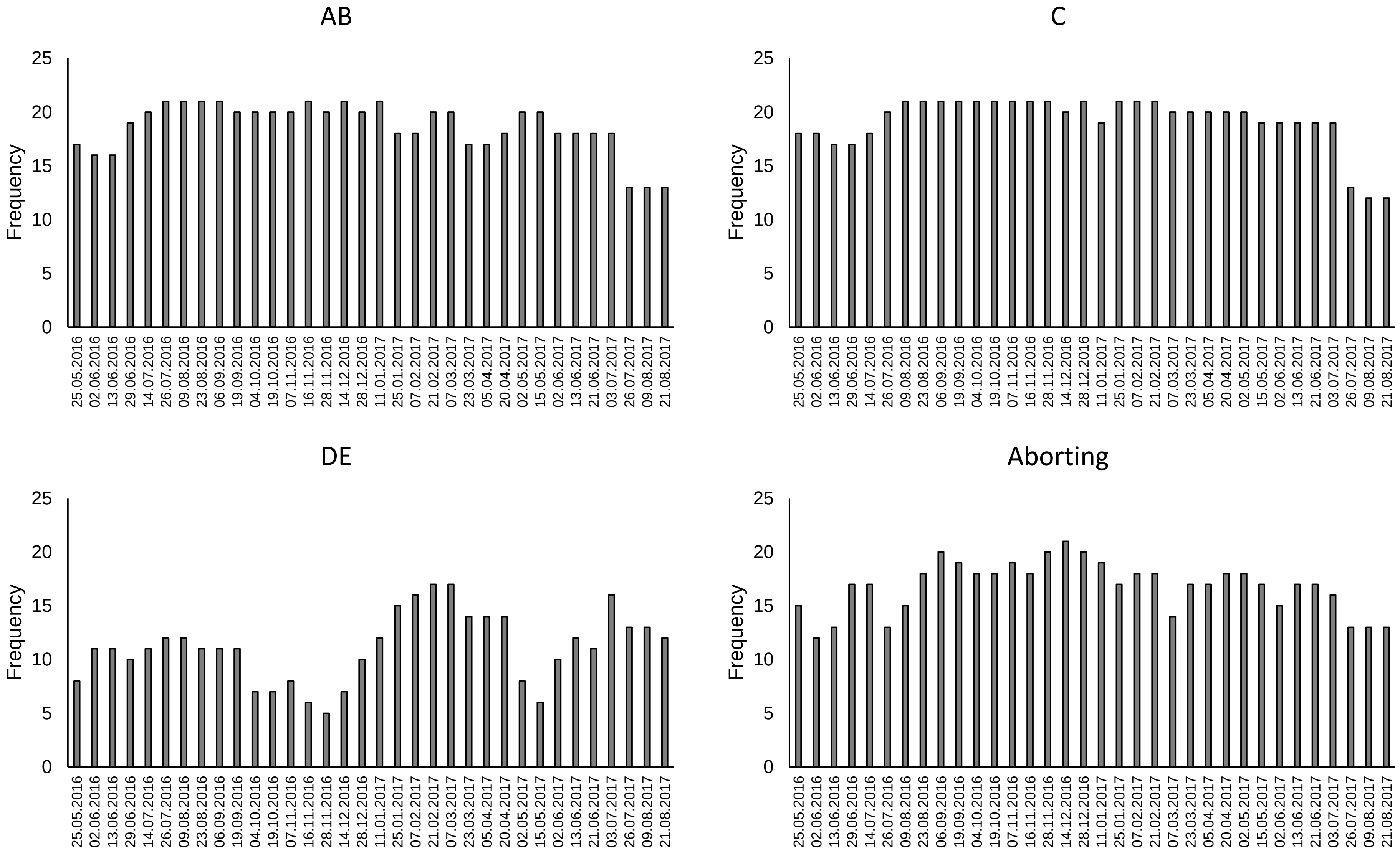
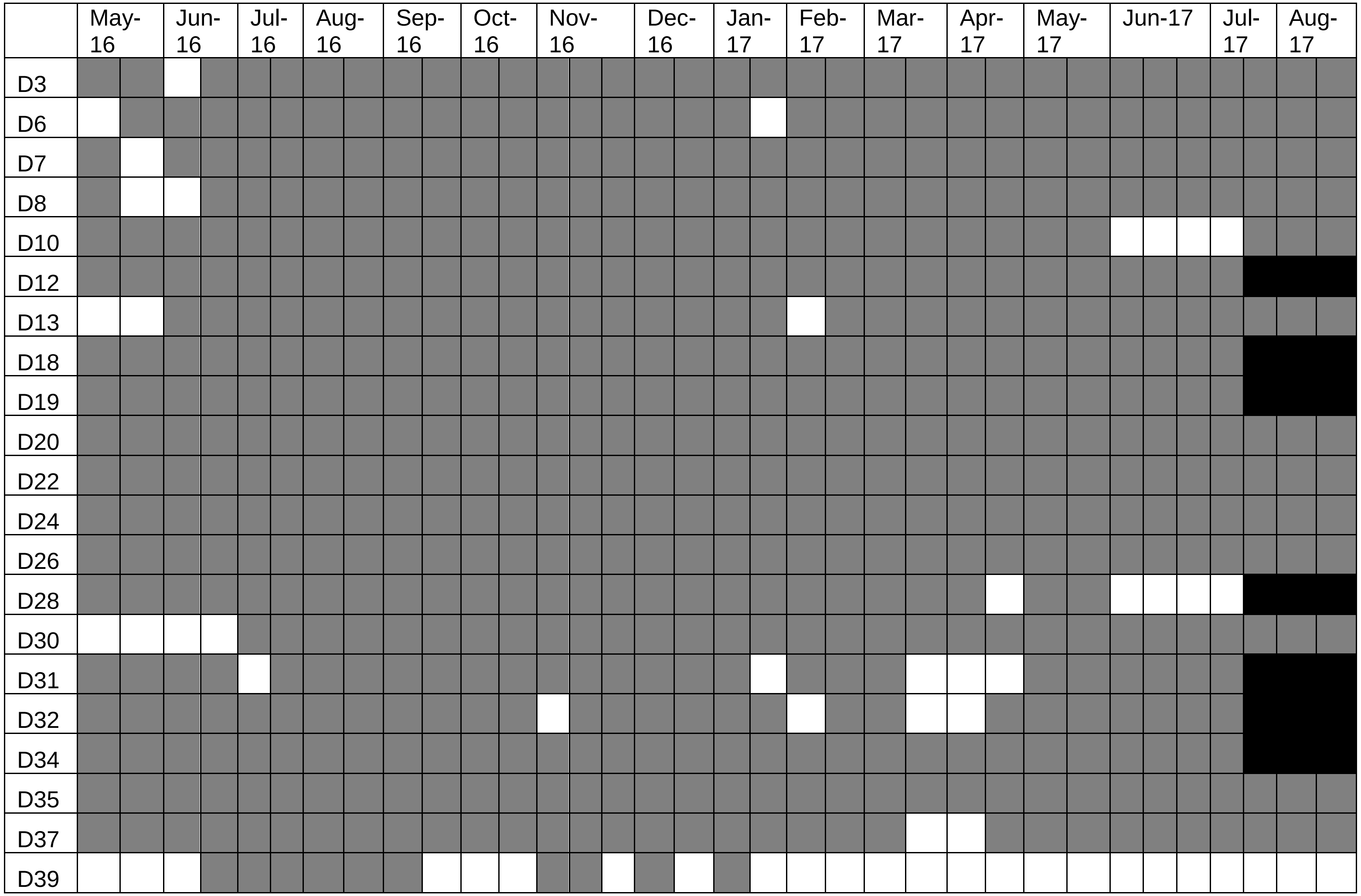


Figure 6.8 Changes over time in the fig developmental phases on 21 male fig trees.



	May-16		Jun-16		Jul-16		Aug-16		Sep-16		Oct-16		Nov-16			Dec-16		Jan-17		Feb-17		Mar-17		Apr-17		May-17		Jun-17			Jul-17		Aug-17	
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D22	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
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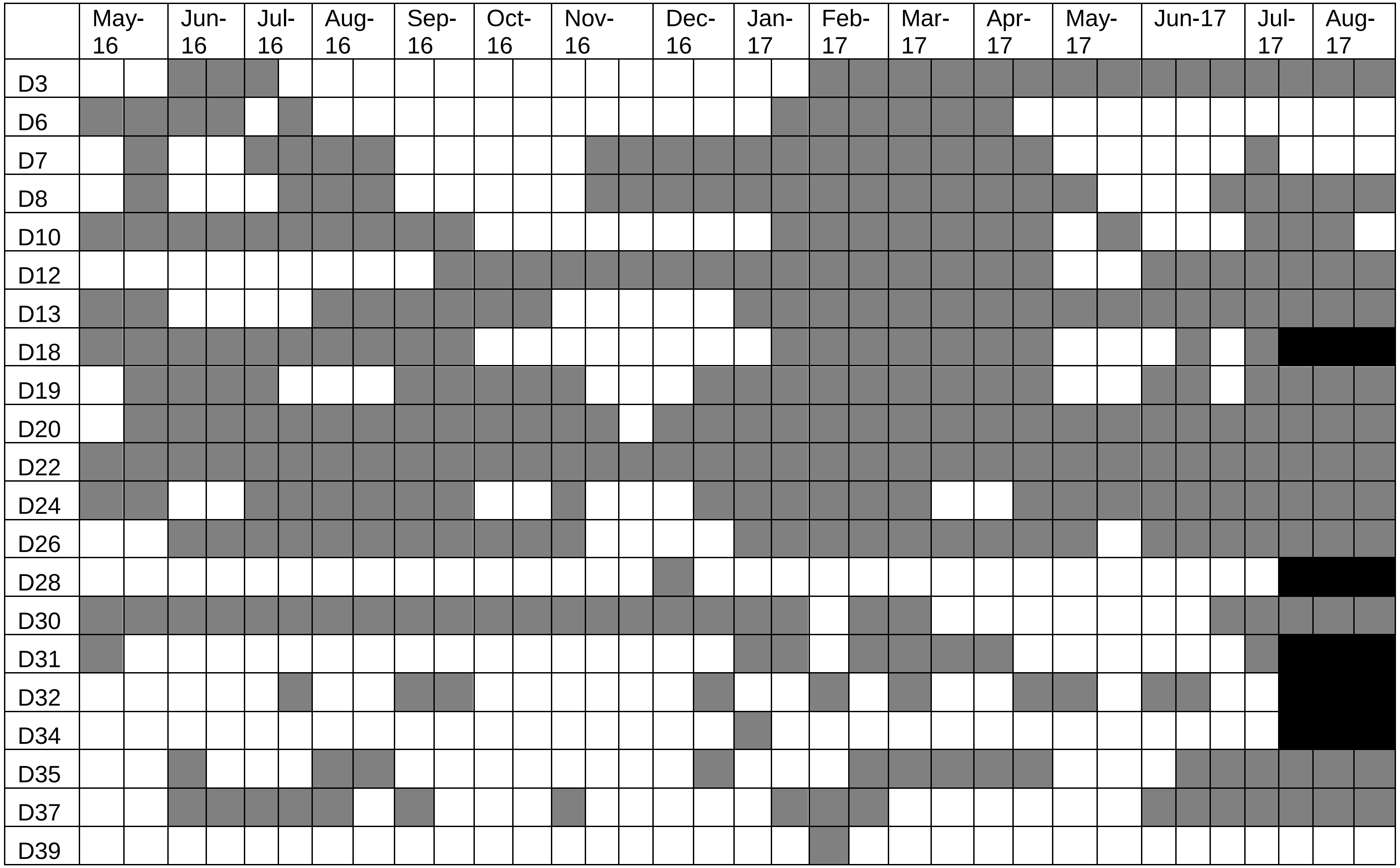
D37	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	☐	☐	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
D39	☐	☐	☐	▨	▨	▨	▨	▨	▨	☐	☐	☐	▨	▨	☐	▨	☐	▨	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Figure 6.9 The presence of young AB phase figs on male trees of *F. deltoidea* var. *angustifolia* in Banting oil palm plantation in each month between May 2016 and August 2017. (Grey bars = Figs were present, white bars = figs were absent and black bars = when the trees were cut off).



	May-16		Jun-16		Jul-16		Aug-16		Sep-16		Oct-16		Nov-16			Dec-16		Jan-17		Feb-17		Mar-17		Apr-17		May-17		Jun-17			Jul-17		Aug-17	
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D39																				▨														

Figure 6.10 The presence of DE phase figs on male trees of *F. deltoidea* var. *angustifolia* in Banting oil palm plantation in each month between May 2016 and August 2017. (Grey bars = Figs were present, white bars = figs were absent and black bars = when the trees were cut off).

6.4.2.2 Female plants

The development of figs on female trees was also asynchronous. The highest frequency of crop sample size categories on female trees were 10-100 and 100-500 figs, so smaller than on the male plants (Figure 6.11). Few trees had less than 10 figs present at any one time. The trees that had 500-1000 figs had this number consistently, whereas trees with larger crops were consistent in the beginning of the study and then their crop sizes increased in March 2017 and April 2017. Almost all the 18 female fig trees produced new figs throughout the year (Figure 6.12). The numbers of plants with C phase figs showed ups and downs with the lowest numbers in December 2016 when only 8 trees showed signs having had any of their figs pollinated. The period between July and September showed the highest number of trees bearing pollinated figs in both years, with 15 trees having pollinated figs. Different to the male plants, a significant positive correlation was found between crop size and the thickest diameter of the stems (Spearman rank correlation, $r_s = 0.56$, $n = 18$, $P < 0.05$). As in the male plants, females established higher on the oil palms had larger crops (Spearman rank correlation, $r_s = 0.59$, $n = 18$, $P < 0.05$).

Half the female trees had AB young figs present all the time while the rest had new figs more than 50% of the time (Figure 6.13 and Figure 6.14). The absence of new figs on trees D14 and D15, which were located close to the ground was due to spraying activities by the farmers. As in male trees, some female trees in the last three observation periods were removed by the farmers. Tree D25 produced very low numbers of figs (less than 50) whereas tree D27 consistently had very large crops of more than 2000 figs. Three female trees that consistently had ripe figs were D1, D2 and D4 (Figure 6.14). These trees had seeds available for dispersal all the time. The highest frequency of trees with seeds available to be dispersed was in December 2016.

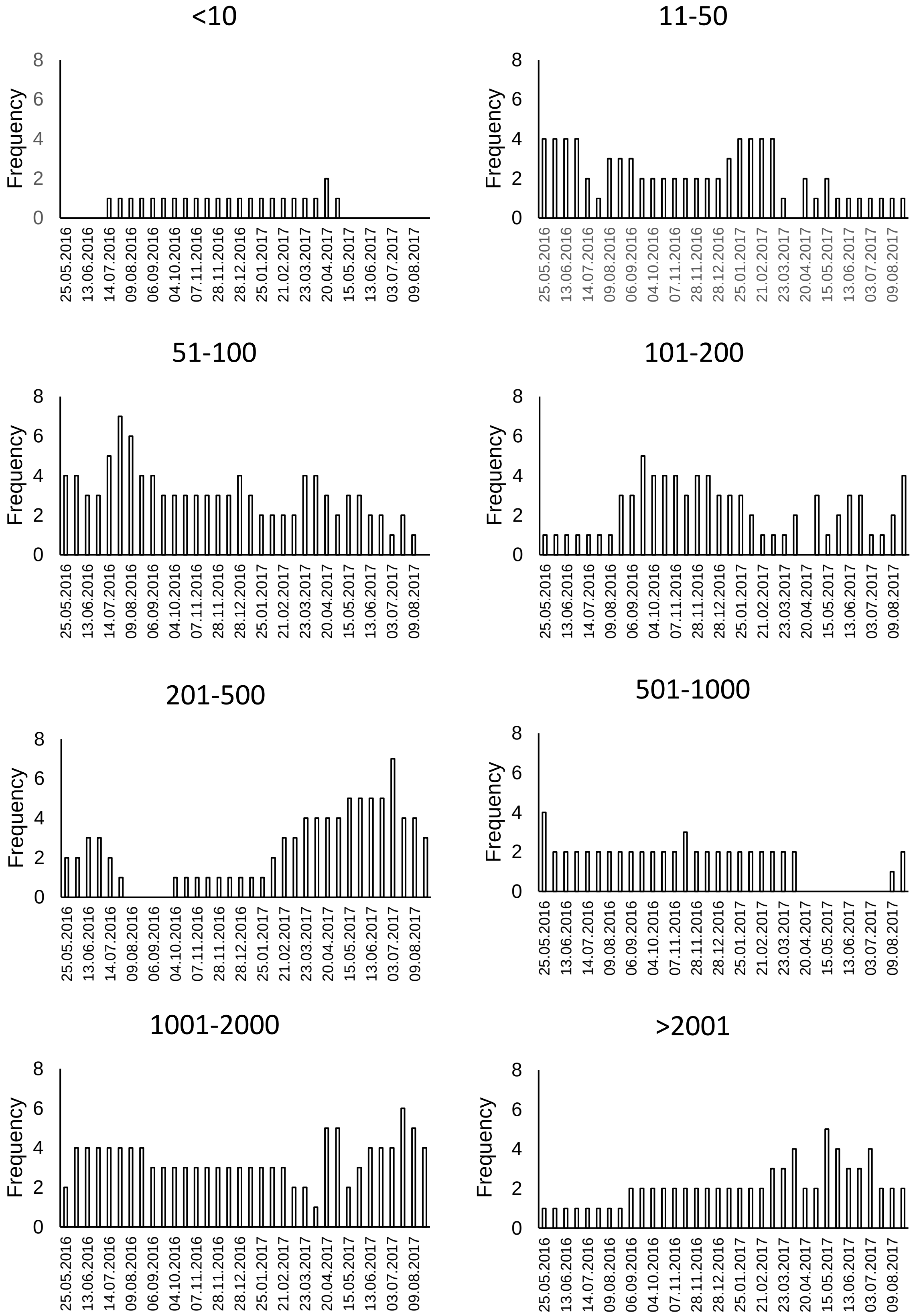


Figure 6.11 The crop sizes of figs on female trees.

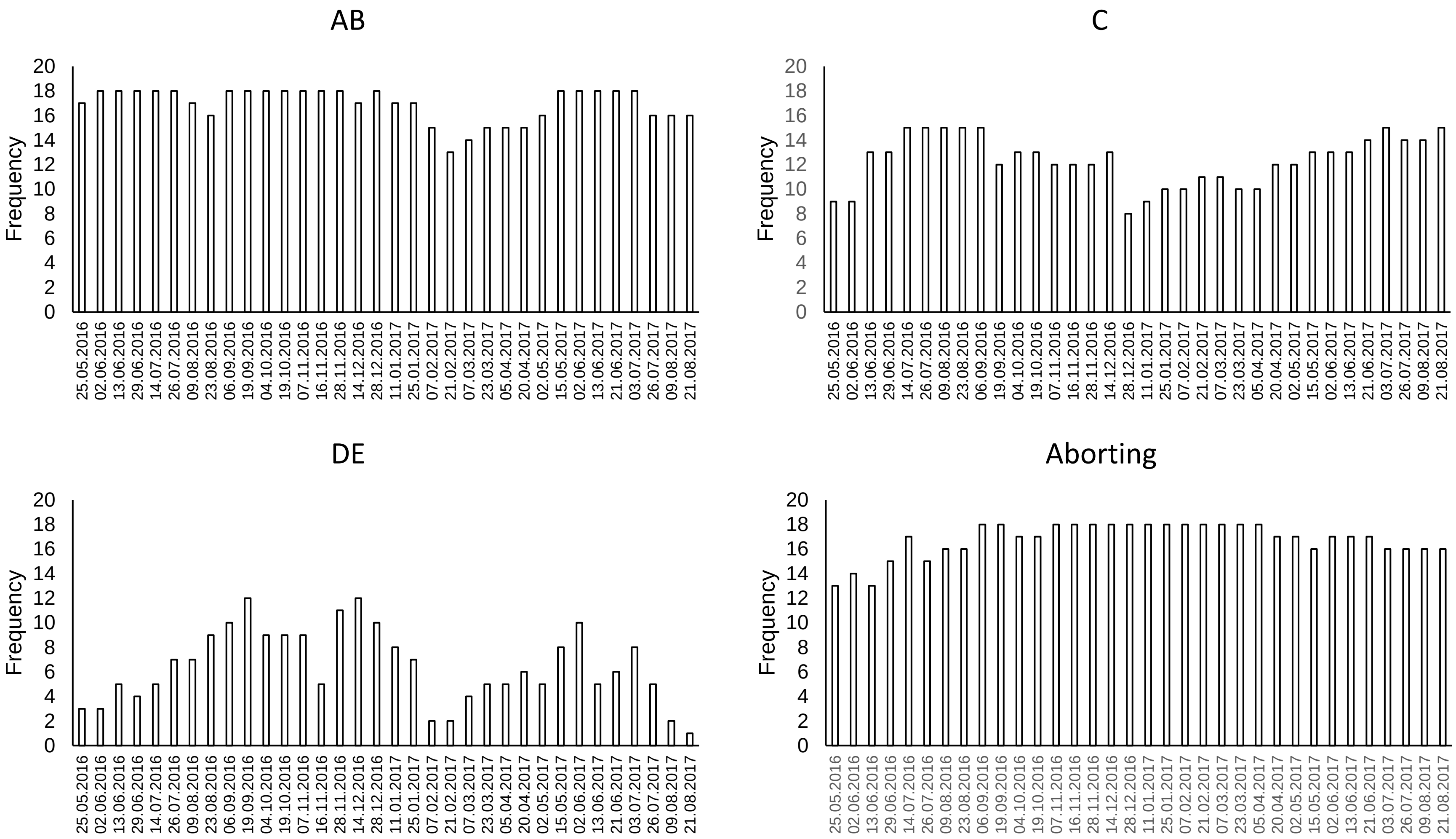
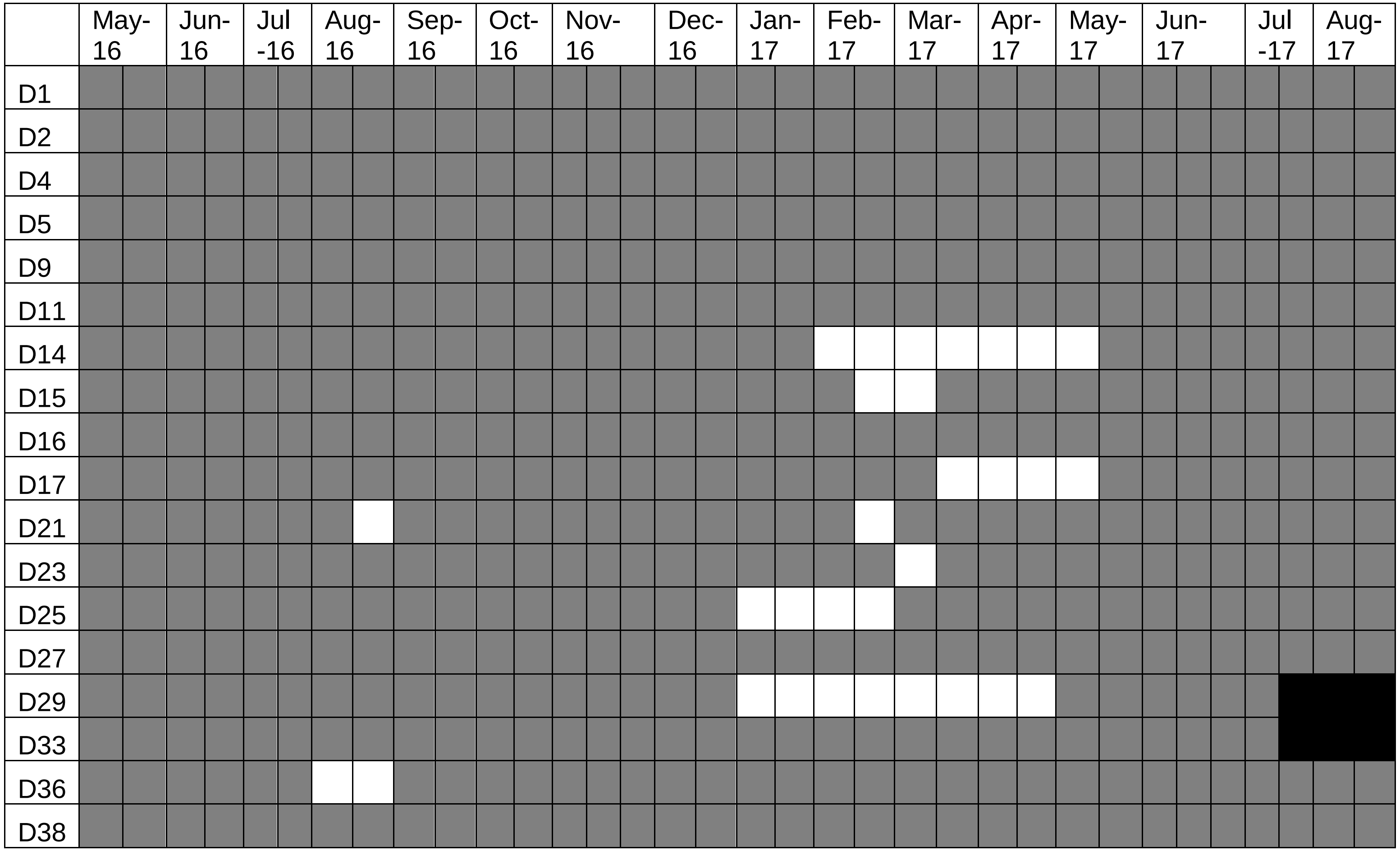
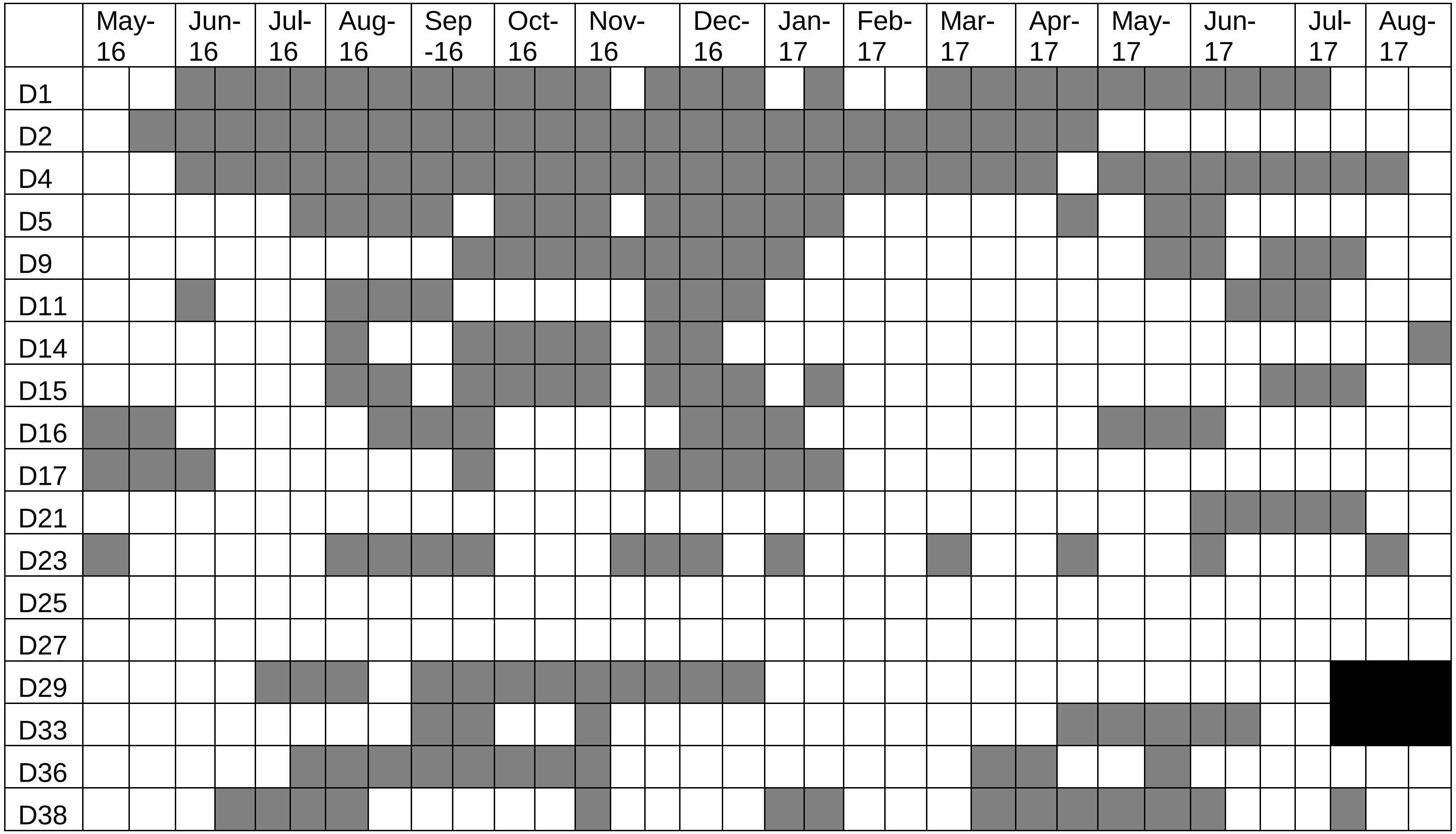


Figure 6.12 Frequency of the fig developmental phases on 18 female fig trees.



	May-16		Jun-16		Jul -16		Aug-16		Sep-16		Oct-16		Nov-16			Dec-16		Jan-17		Feb-17		Mar-17		Apr-17		May-17		Jun-17			Jul -17		Aug-17	
D1	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
D2	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
D4	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
D5	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
D9	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
D11	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
D14	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	□	□	□	□	□	□	□	▨	▨	▨	▨	▨	▨	▨	▨
D15	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	□	□	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
D16	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
D17	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	□	□	□	□	▨	▨	▨	▨	▨	▨	▨	▨
D21	▨	▨	▨	▨	▨	▨	▨	□	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	□	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
D23	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	□	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
D25	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	□	□	□	□	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
D27	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
D29	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	□	□	□	□	□	□	□	□	▨	▨	▨	▨	▨	▨	■	■	■
D33	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	■	■	■
D36	▨	▨	▨	▨	▨	▨	□	□	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
D38	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨

Figure 6.13 The presence of young AB phase figs on female trees of *F. deltoidea* var. *angustifolia* in Banting oil palm plantation in each month between May 2016 and August 2017. (Grey bars = figs were present, white bars = figs were absent and black bars = when the trees were cut off).



	May-16		Jun-16		Jul-16		Aug-16		Sep-16		Oct-16		Nov-16			Dec-16		Jan-17		Feb-17		Mar-17		Apr-17		May-17		Jun-17			Jul-17		Aug-17	
D1			▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨		▨	▨	▨		▨			▨	▨	▨	▨	▨	▨	▨	▨	▨	▨			
D2		▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨									
D4			▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨		▨	▨	▨	▨	▨	▨	▨	▨	
D5						▨	▨	▨	▨		▨	▨	▨		▨	▨	▨	▨	▨						▨		▨	▨						
D9										▨	▨	▨	▨	▨	▨	▨	▨	▨									▨	▨		▨	▨	▨		
D11			▨				▨	▨	▨						▨	▨	▨												▨	▨	▨			
D14							▨			▨	▨	▨	▨		▨	▨																		▨
D15							▨	▨		▨	▨	▨	▨		▨	▨	▨		▨											▨	▨	▨		
D16	▨	▨						▨	▨	▨						▨	▨	▨								▨	▨	▨						
D17	▨	▨	▨							▨					▨	▨	▨	▨	▨															
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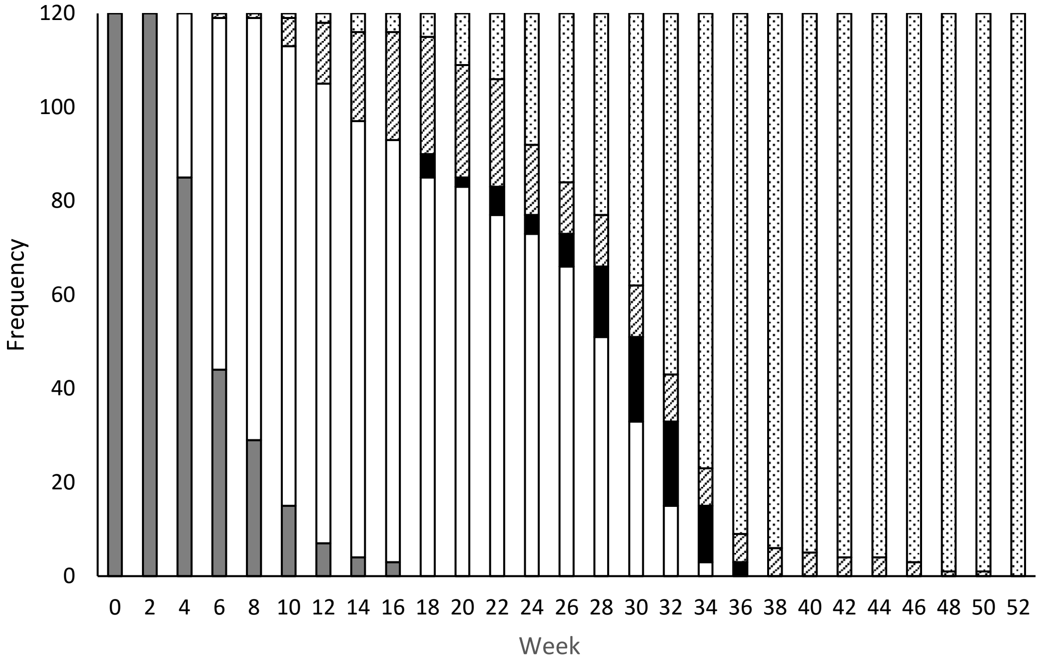
D33									▨	▨			▨												▨	▨	▨	▨	▨			■	■	■
D36						▨	▨	▨	▨	▨	▨	▨	▨										▨	▨			▨							
D38				▨	▨	▨	▨						▨					▨	▨				▨	▨	▨	▨	▨	▨				▨		

Figure 6.14 The presence of mature phase figs on female trees of *F. deltoidea* var. *angustifolia* in Banting oil palm plantation in each month between May 2016 and August 2017. (Grey bars = figs were present, white bars = figs were absent and black bars = when the trees were cut off).

6.4.3 Fig development

One hundred and twenty four randomly selected A phase male figs on 21 trees were marked and observed until they were no longer on the trees. Ninety one of these figs were entered by pollinators and developed normally, whereas the remaining figs eventually aborted. The A phase figs were entered as early as the fourth week after being marked. The fastest maturation from A phase until E phase was 17 weeks while the slowest was 36 weeks (**Error! Reference source not found.**). The average duration from A phase until the emergence of pollinators was 28.36 ± 4.58 weeks (Mean ± SD). DE phase male figs mostly stayed on the trees for two weeks before falling. Figs that had not been entered began to show signs of the changes associated with future abortion after 16 weeks. Un-entered figs stayed on the trees for up to 50 weeks before being aborted and falling from the trees.

After the figs had been exposed for 16 weeks, all the figs had either been pollinated or where showing indications that they were going to abort (Figure 6.15). Some figs showed external signs of being pollinated rapidly, within four weeks (Figure 6.16) and after 36 weeks all the pollinated figs were starting to release pollinator offspring (Figure 6.17). The first figs releasing the next generation of pollinators were on week 18, suggesting that the generation time was very short (only about 18 weeks) (Figure 6.17). No figs remained receptive after week 16 (by that day they were either pollinated or starting to abort). No C phase figs remained after week 36, which was 18 weeks later, again suggesting that the generation time was less than five months. Some figs showed signs that they were going to abort by week 6 (Figure 6.18), but presumably they had already been waiting to be pollinated by one or more days when the bags were removed. The aborting figs remained longer on the trees than those that were pollinated (Figure 6.19).



Summary of the frequency of developmental phases of figs on male trees. 124 un-pollinated A phase figs were marked in week zero. Grey bars = AB phase, white bars = C phase, black bars = DE phase, hashed bars = aborting figs and spotted bars = absent figs.

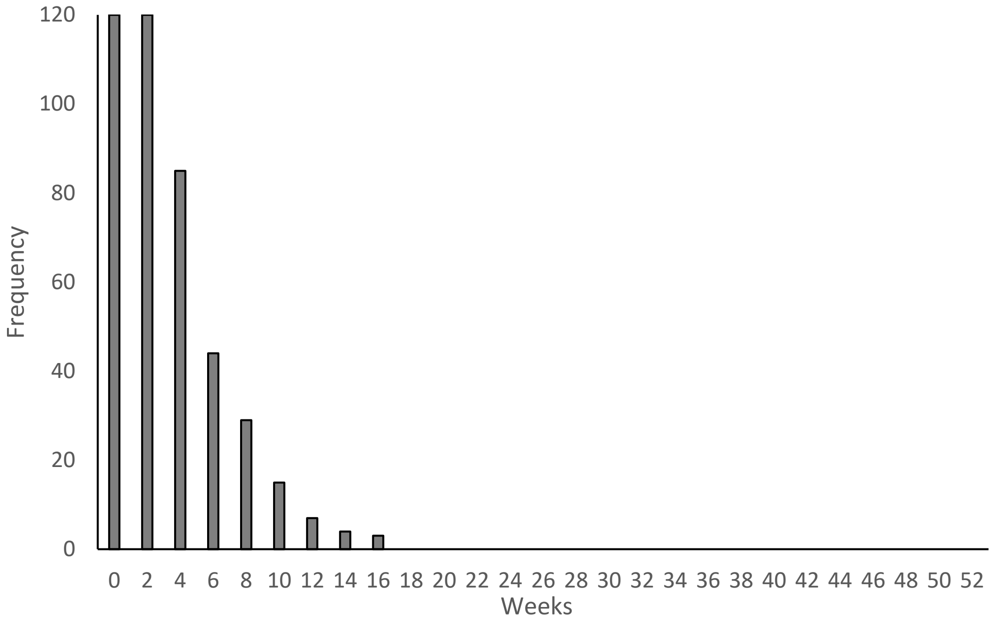


Figure 6.15. The frequency of AB phases of figs on male trees. 124 un-pollinated A phase figs were marked in week zero.

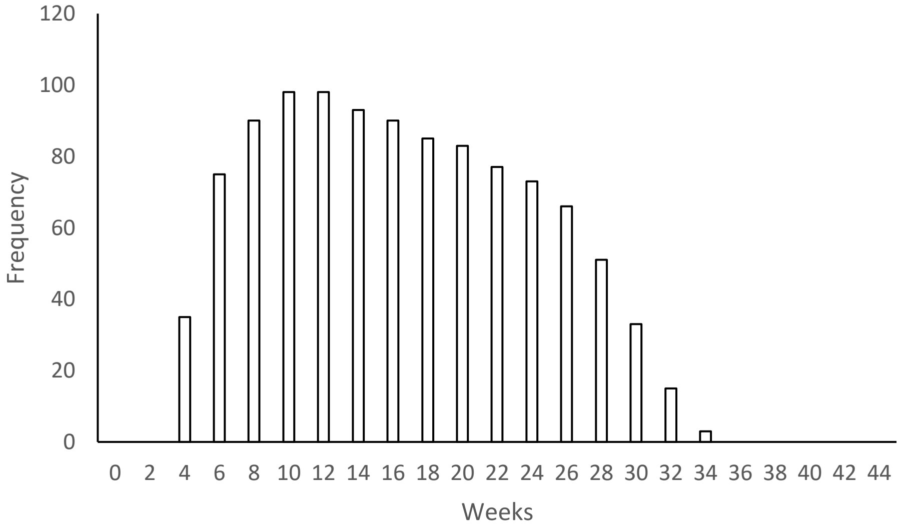


Figure 6.16. The frequency of C phases of figs on male trees. 124 un-pollinated A phase figs were marked in week zero.

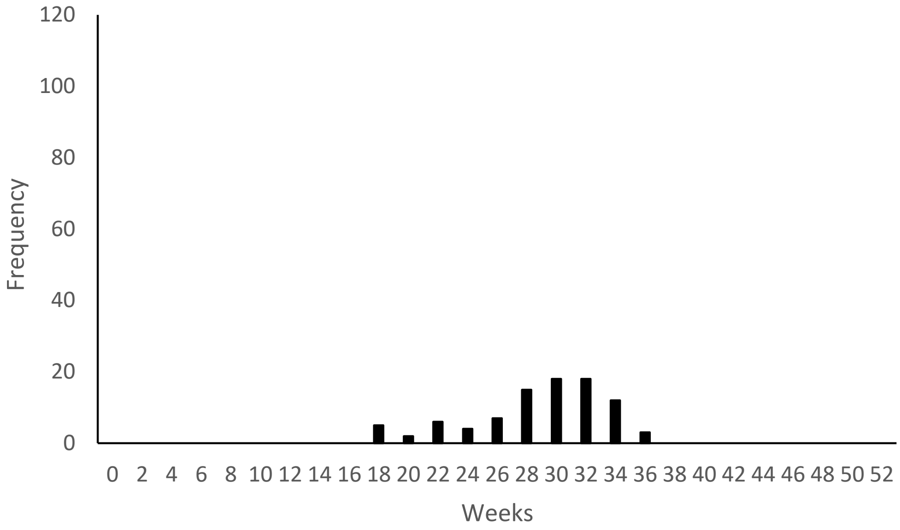


Figure 6.17. The frequency of DE phases of figs on male trees. 124 un-pollinated A phase figs were marked in week zero.

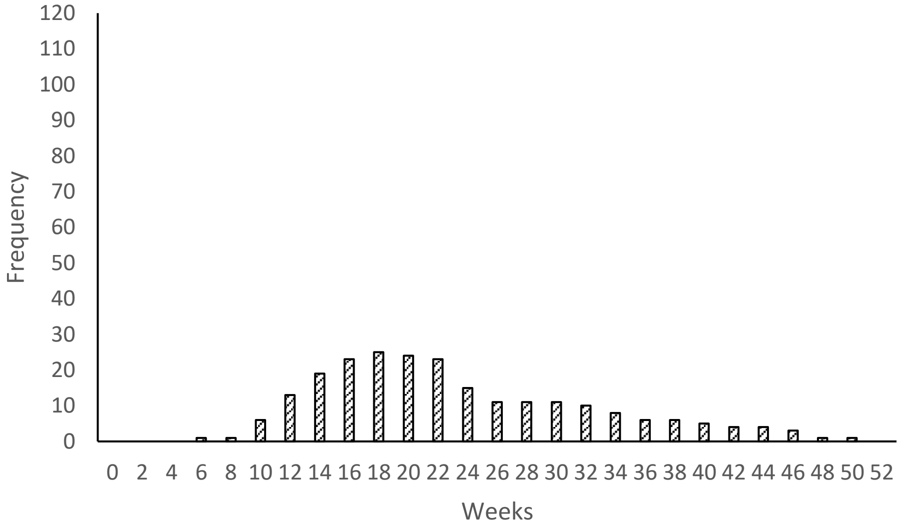
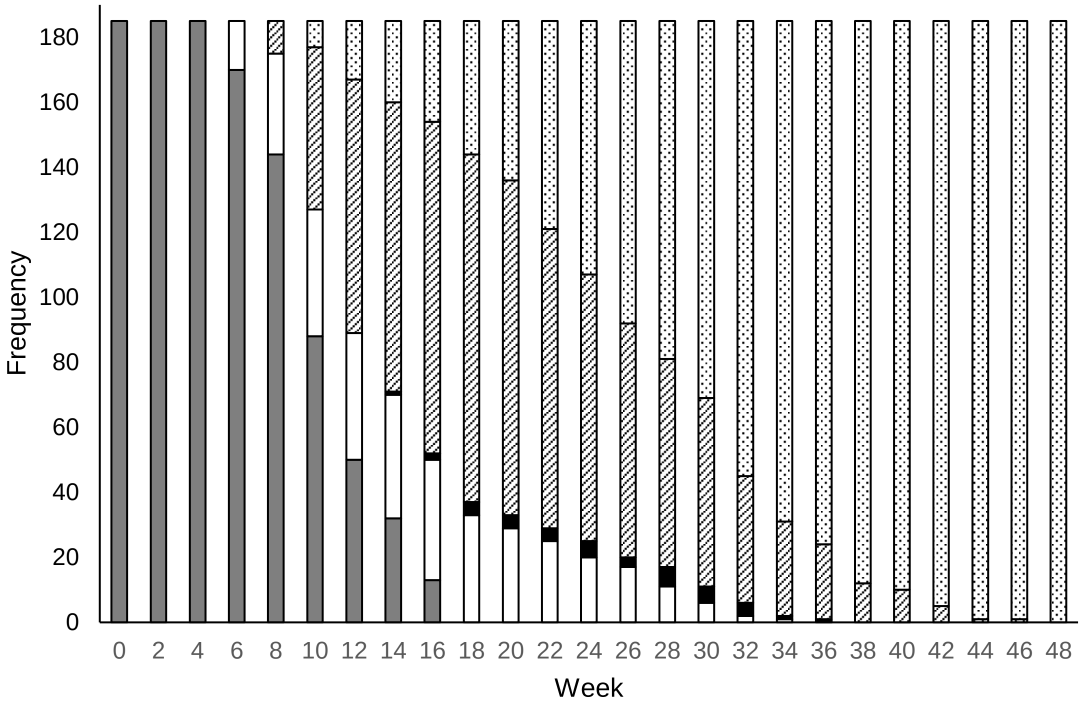


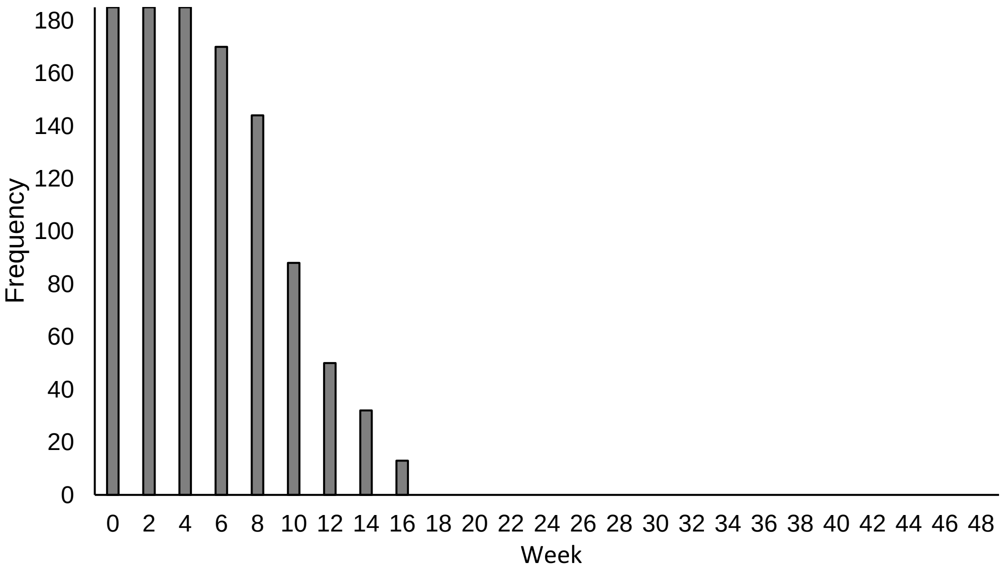
Figure 6.18. The frequency of aborting phases of figs on male trees. 124 un-pollinated A phase figs were marked in week zero.

The 185 randomly-selected A phase female figs on 18 trees showed signs of being pollinated within as soon as 6 weeks (**Error! Reference source not found.**). Thirty nine of these figs were entered by pollinators and developed normally, whereas the remaining figs eventually aborted. The complete cycle from A phase to E phase varied from 16 to 34 weeks with a mean of 26.21 ± 5.89 weeks. Un-pollinated female figs were only detectable visually after 17 weeks and could last on the trees for 46 weeks before falling.

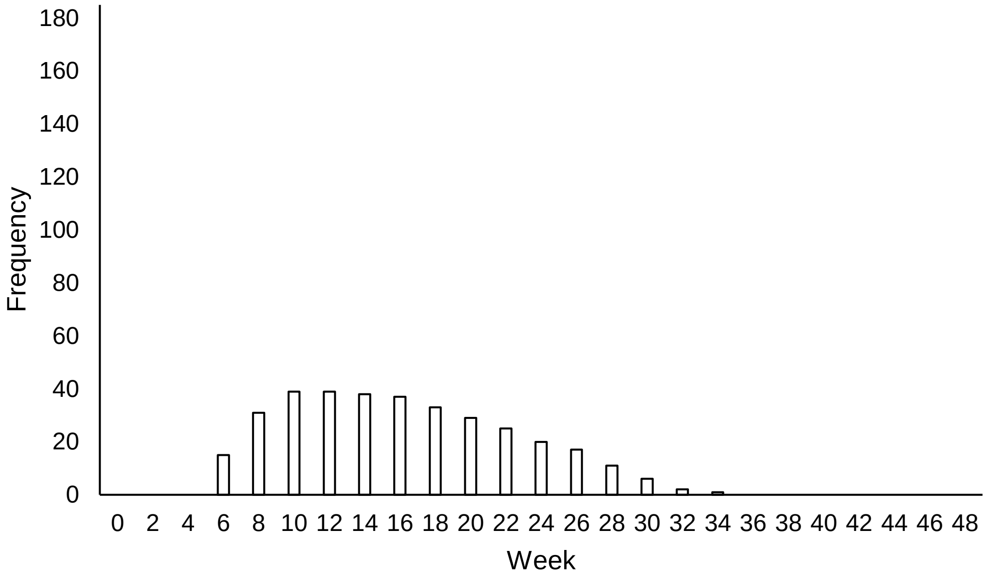
After the figs had been exposed for 16 weeks, all the figs had either been pollinated or where showing indications that they were going to abort (Figure 6.15). Some figs showed external signs of being pollinated rapidly, within sixr weeks (Figure 6.16) and after 36 weeks all the pollinated figs were starting to release pollinator offspring (Figure 6.17). The first figs ripen were on week 14 and ready to be consumed (Figure 6.17). No figs remained receptive after week 16 (by that day they were either pollinated or starting to abort). No C phase figs remained after week 36, which was 18 weeks later, again suggesting that the full life cycle of female fig was less than five months. Some figs showed signs that they were going to abort by week 8 (Figure 6.18), but presumably they had already been waiting to be pollinated by one or more days when the bags were removed. The aborting figs remained longer on the trees than those that were pollinated (Figure 6.19).



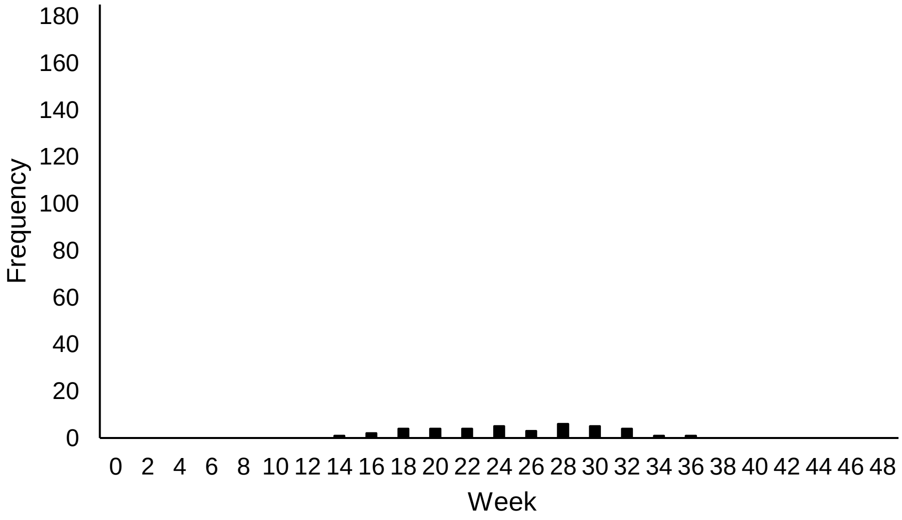
Summary of the frequency of developmental phases of figs on female trees. 185 un-pollinated A phase figs were marked in week zero. Grey bars = AB phase, white bars = C phase, black bars = DE phase, hashed bars = aborting figs and spotted bars = absent figs.



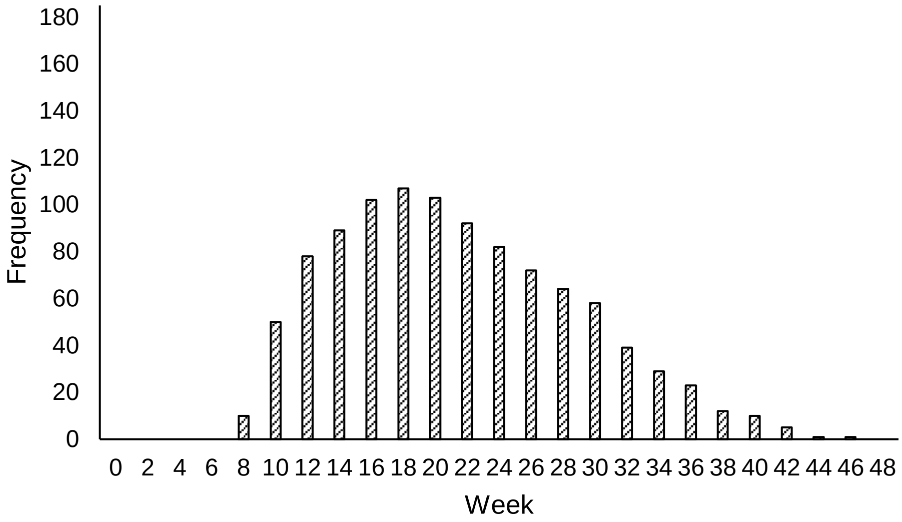
The frequency of AB phases of figs on female trees. 185 un-pollinated A phase figs were marked in week zero.



The frequency of C phases of figs on female trees. 185 un-pollinated A phase figs were marked in week zero.



The frequency of DE phases of figs on female trees. 185 un-pollinated A phase figs were marked in week zero



The frequency of aborting phases of figs on female trees. 185 un-pollinated A phase figs were marked in week zero

The average development times from pollination to wasp emergence or female fig ripening (the lengths of C phases) were 20 weeks in the male figs and 18 weeks in the female figs (**Error! Not a valid bookmark self-reference.**). The durations of earlier fig developmental phases on male and female trees were similar, but DE phase male figs fell from the trees more quickly than E phase female figs. (**Error! Not a valid bookmark self-reference.**). Despite this, the figs were present for slightly longer on the male trees (**Error! Not a valid bookmark self-reference.**).

Figs that were not entered by pollinators stayed on the plants for about six months (Table 6.5). Un-entered male and female figs took the same length of time before showing signs that they were aborting, and once they started to abort were retained by the trees for the same length of time, though the times that aborting figs stayed on the trees was very variable Table 6.5).

Table 6.4. The numbers of weeks (means with range in brackets) of different phases during development of marked figs. Pairwise comparisons between sexes used Mann-Whitney U-tests.

	N (figs)	AB	C	DE	Total
Male	91	5.46 (2-10)	20.91 (12-28)	2.04 (2-4)	28.36 (17-36)

Female	39	5.51 (4-8)	18.64 (10-30)	2.20 (2-4)	26.21 (16-34)
M-W U		1700.0	2207.5	1631.5	1378.0
P value		0.69	0.08	0.05	0.04

Table 6.5. The numbers of weeks (means with range in brackets) that un-pollinated figs remained as AB phase in appearance and stayed on the trees after being recorded as starting to abort. Pairwise comparisons between sexes used Mann-Whitney U-tests.

	N (figs)	AB	Aborting	Time to abortion
Male	33	10.67 (6-16)	16.73 (2-38)	27.39 (8-52)
Female	146	10.69 (6-16)	13.56 (2-34)	24.25 (8-48)
M-W U		2379.5	2026.0	2086.5
P value		0.91	0.15	0.23

6.4.4 Pollinator limitation

Across the 15 month period of study there was considerable variation in the maximum numbers of figs recorded on different trees, but the range of maximum crop sizes on male and female trees was similar (Figure 6.15).

Aborting figs were present on most of the trees most of the time (Figure 6.8 and Figure 6.12), but the chance of pollinator entry was lower for figs on female trees. All the male trees had at least some of their figs pollinated, but the continuous absence of DE figs showed that female trees D25 and D27 did not have any pollinated figs throughout the whole sampling period (Figure 6.14). Few male trees had more un-entered than entered figs on any of the sampling dates, whereas un-pollinated figs routinely outnumbered pollinated figs on female trees (Table 6.6).

The proportion of figs on each tree that had failed to be entered and were starting to abort was calculated as the mean percentage of aborting figs divided by percentage the figs that had developed to the stage where entry by pollinators was possible (C phase + DE phased + aborting figs). The proportion of un-entered figs on male trees ranged from 0.05 to 0.57 (Figure 6.16). D28 had the highest rate of non-entry among the male trees. This tree produced few figs (the mean crop size was less than 50) and only had DE (entered) figs once throughout the 15 months of observations. A high proportion of un-entered figs were present on every female tree throughout the sampling period (Figure 6.17), ranging from half to all of the figs. Female tree D25 never showed any sign of pollination even though it produced large crops of more than 2000 figs. There was a significant difference in the proportion of un-entered figs on male and female trees (ANOVA, F = 141.20, df = 1, 37, P < 0.001).

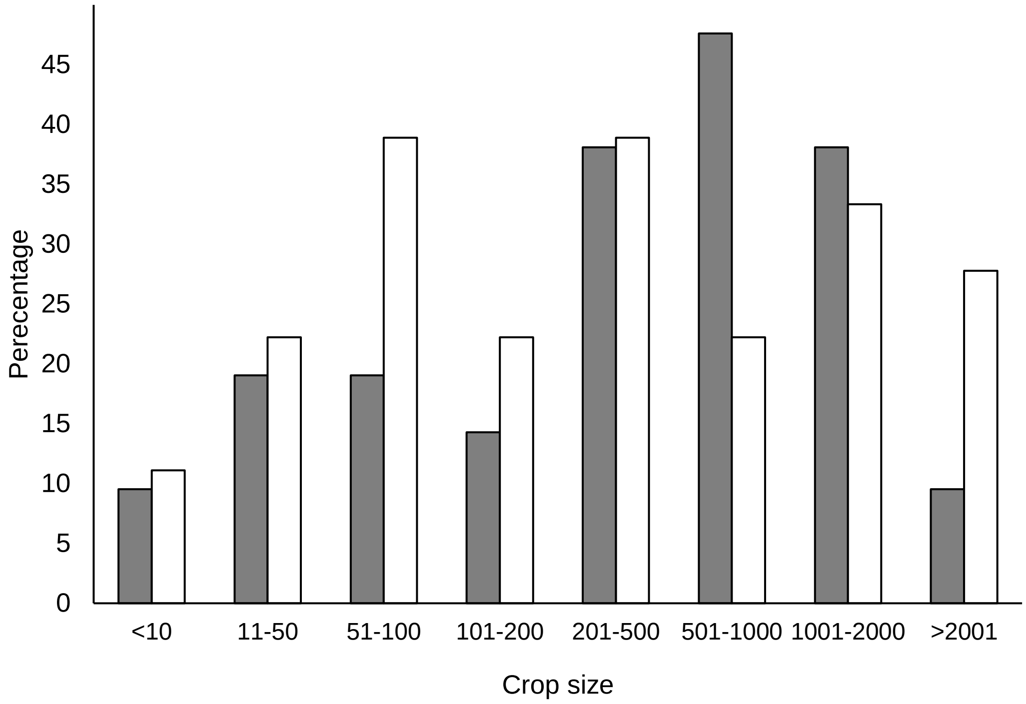


Figure 6.15 The maximum crop sizes on 21male and 18 female trees during 15 months of observations. Solid bars = male trees and open bars = female trees.

Table 6.6. The relative numbers of pollinated and non-pollinated figs on male and female fig trees (P = pollinated, NP = non-pollinated).

Sampling date	Male trees			Female trees		
	P>NP	NP>P	NP=P	P>NP	NP>P	NP=P
25.05.2016	19	2	0	2	15	1
02.06.2016	19	2	0	3	13	2
13.06.2016	16	4	1	4	11	3
29.06.2016	16	4	1	4	11	3
14.07.2016	16	4	1	6	9	3
26.07.2016	18	2	1	10	6	2
09.08.2016	20	1	0	8	6	4
23.08.2016	17	3	1	7	9	2
06.09.2016	18	2	1	5	11	2
19.09.2016	19	2	0	2	13	3
04.10.2016	17	3	1	2	12	4
19.10.2016	17	3	1	2	12	4
07.11.2016	19	1	1	4	13	1
16.11.2016	18	3	0	3	13	2
28.11.2016	20	1	0	3	14	1
14.12.2016	18	3	0	2	15	1
28.12.2016	17	4	0	0	17	1
11.01.2017	19	1	1	0	18	0
25.01.2017	20	1	0	0	18	0
07.02.2017	20	1	0	0	18	0
21.02.2017	20	1	0	0	18	0
07.03.2017	20	0	1	0	18	0
23.03.2017	17	3	1	0	16	2
05.04.2017	17	3	1	0	14	4
20.04.2017	19	1	1	1	15	2
02.05.2017	17	2	1	2	13	3
15.05.2017	20	0	0	4	13	1
02.06.2017	19	1	0	1	16	1
13.06.2017	19	1	0	0	18	0
21.06.2017	19	1	0	0	18	0
03.07.2017	19	1	0	3	15	0

26.07.2017	13	0	0	4	12	0
09.08.2017	13	0	0	3	12	1
21.08.2017	13	0	0	3	13	0

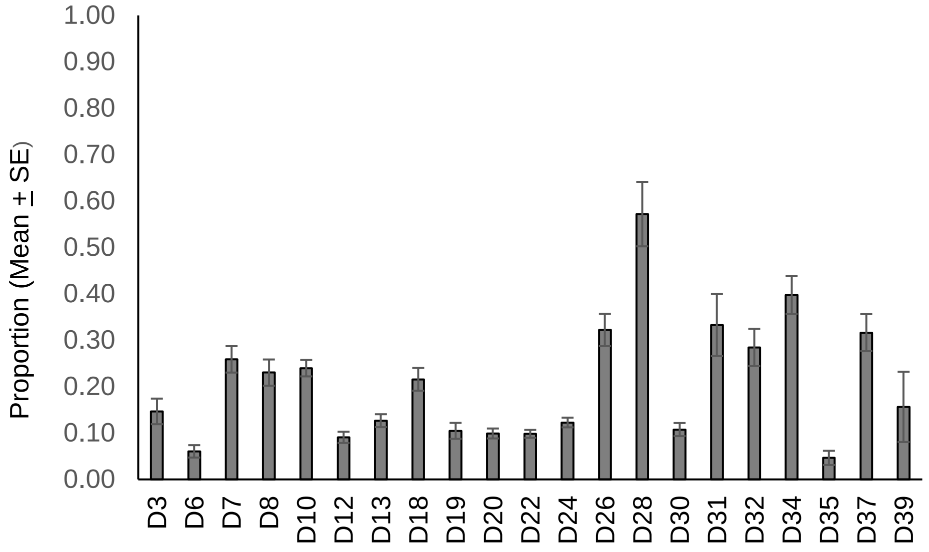


Figure 6.16 The proportion of figs on 21 male trees that were aborting during the 15 months of observation (young AB figs were excluded when calculating proportions).

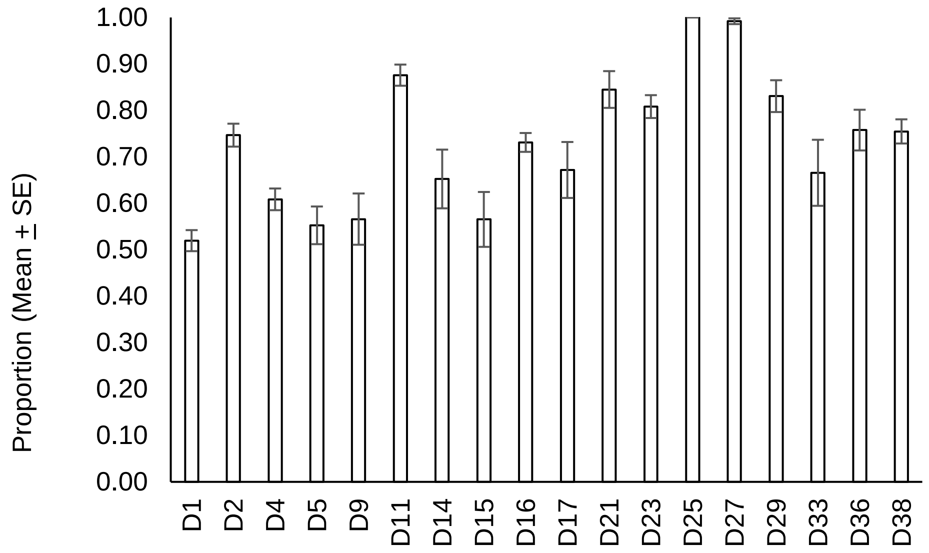


Figure 6.17 The proportion of figs on 18 female trees that were aborting during the 15 months of observation (young AB figs were excluded when calculating proportions).

Evidence given in Chapter 5 indicates that the pollinators of var. *angustifolia* are reluctant to disperse far from their natal figs and that dispersal distances away from natal trees are limited. This suggests that female plants that are further from male trees are likely to suffer greater pollinator limitation and that entry rates on male trees will be independent of the number of other male trees around them.

All the male var. *angustifolia* trees in the Banting plantation had at least some of their figs entered by pollinators. There was a significant positive correlation between the estimated numbers of figs releasing fig wasps on a given tree over the 15 months sampling period and the percentage of its figs that were entered by pollinators over that period (Spearman correlation, $r_s = 0.45$, df = 20, $P < 0.05$). Almost all the female trees had mature male plants growing within 10 metres (measured from the base of the trunks of their host oil palms) (Figure 6.18). There was a regular increase in numbers of mature male trees only within increasing distances from the focal female trees (Figure 6.21).

A mean of about 16% pollination was recorded if there was a male tree within 5 m (11 female trees) and 84% if the female trees were more isolated (7 female trees). Having a mature male plant within 5 m did not increase pollination rates (Figure 6.22). There was only one female tree with the nearest mature male than 30 m away from it. The percentage pollination of this tree was 12%. However, any relationship with distance could be obscured by the size of a female tree. The very small female tree D25 was never pollinated, yet the nearest male tree was less than 5m away. Male trees were also releasing highly variable numbers of pollinators. The likelihood of a female fig being pollinated was not related to distance to the nearest mature male tree (Pearson correlation, $r = -0.11$, df = 17, $P > 0.05$). However, pollination rates did increase in relation to the number of mature male trees within 50 m of a focal female tree (Figure 6.23) (Pearson correlation, $r = 0.52$, df = 17, $P < 0.05$).

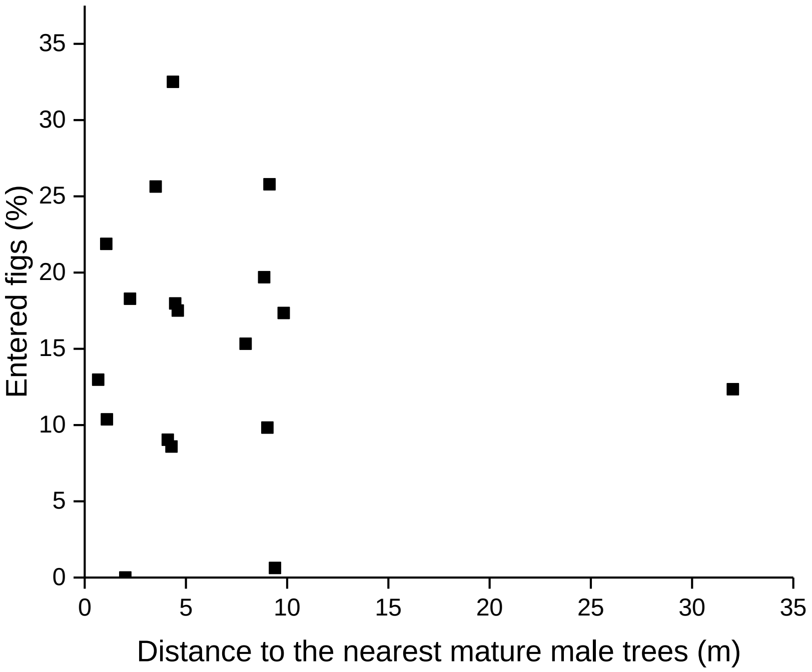


Figure 6.18 The relationship between the percentage of entered figs in 18 female trees and the distance to the nearest adjacent mature male tree.

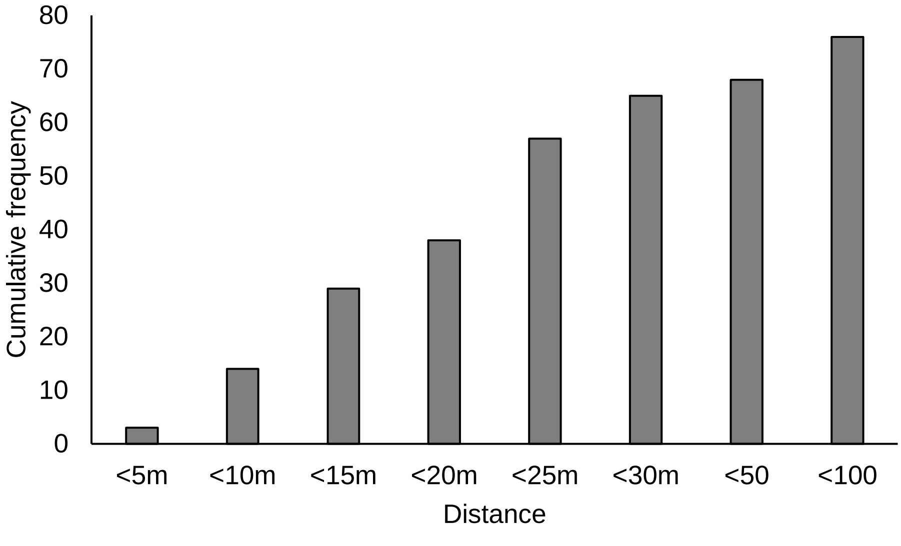


Figure 6.21 The cumulative frequencies of mature male trees only within varying distances from 18 focal female trees.

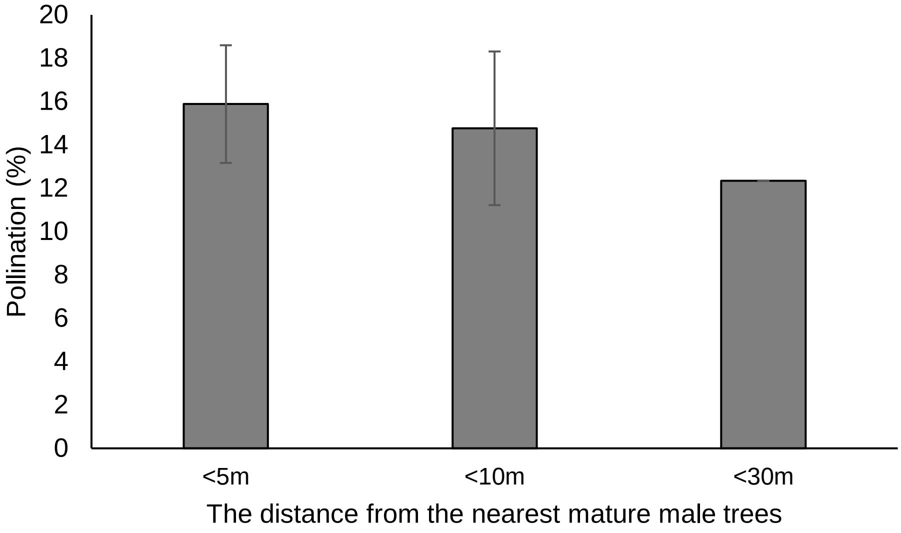


Figure 6.22 The relationship of pollination rates with the distance of the nearest mature male trees from focal female trees. (n = 18 female trees).

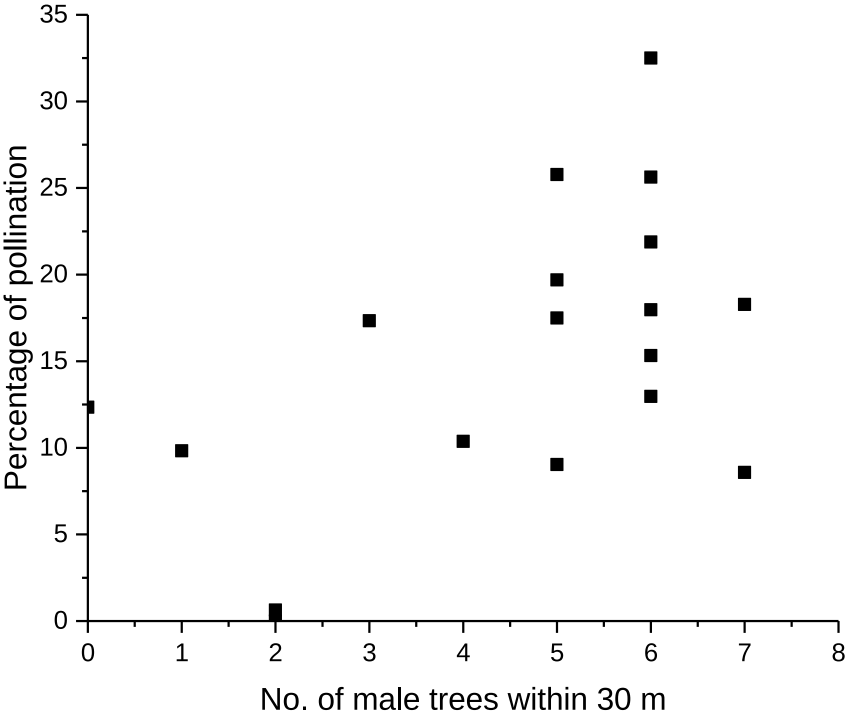


Figure 6.23 The relationship between pollination rates on 18 female trees and the number of mature male trees within a distance of 30 m.

6.5 Discussion

The phenology of 39 *F. deltoidea* trees growing as epiphytes in an oil palm plantation was observed over a period of 15 months to record within-tree and within-population patterns of leafing and fruiting. The phenology of this species is typical of a species living in an aseasonal environment (Harrison, 2000; Corlett, 1993), with production of new leaves and figs occuring throughout the year. This is in contrast to the leaf and fruiting phenologies of fig trees growing in more seasonal environments, and of most monoecious species (Krishnan et al., 2014). Lin et al. (2015) showed that tropical climatic factors (temperature and rainfall) did not shape the phenology of *Ficus benguetensis* Merr. in Northern Taiwan even though male and female trees showed different phenologies. A similar limited impact of climatic factors is seen with the phenology of the *F. deltoidea* in the oil palm plantation.

6.5.1 Leaf phenology

Most of the mature individuals had some elongate juvenile leaves as well as the smaller more rounded leaves that are seen as typical of older plants. New leaves and senescing leaves were present throughout the year. Suleman et al., (2011) found rather similar leafing phenology in the dioecious *Ficus montana* Burm. f. when the plants were kept under a controlled laboratory environment. However, in some years special events such as drought might stimulate more synchronised leaf senescence in *F. deltoidea*, as in *Ficus fulva* Reinw. ex Blume in Lambir Hills, Sarawak (Harrison et al., 2000). Stress caused by accidental spraying had this effect in the plantation and is an example of how control activities are likely to reduce biodiversity (Suzanti et al., 2016).

6.5.2 Fig phenology

In nursery pollination mutualisms, synchronisation between the development times of fruit and the pollinators is critical to ensure the survival of both parties (Sakai, 2002). At the population level the mutualism between dioecious fig trees and fig wasps is maintained by compatible fruiting phenology of the two plant sexes to allow seed production at the same time as maintaining pollinator populations. If there is a particularly favourable season for fig seed germination and establishment then female trees can concentrate seed production at this appropriate time.

The availability of receptive male figs at the times when pollinators emerge is critical to ensure the survival of fig wasp populations (Zhao et al., 2014). Estimating the size of var. *angustifolia* plants is difficult because of the shrubby growth form that can be compact or loose. The measure of tree size we used showed that larger female plants had more figs, but no relationship was confirmed in male plants. In both sexes the plants that had established higher up the trunks of the oil palms produced more figs. This may be because they experience higher light levels or other environmental conditions are more favourable further from the ground. Crop sizes of male and female trees did not differ significantly. A study of a dioecious fig tree under controlled conditions recorded the same crop size results (Suleman et al., 2011), whereas another dioecious species in Sarawak had males that produced more figs than the female trees (Harrison et al., 2000).

An asynchronous fruiting phenology usually occurs in *Ficus* species that occupy wet and aseasonal areas where the availability of water and ability for photosynthesis are consistent throughout the year (Patel, 1996). Asynchronous fruiting patterns, at the population and single tree levels, allow the production of the fig wasps and seeds all year and allow fig wasp populations to be maintained without the need for females to disperse long distances to find figs at a suitable stage for entry and oviposition (Patel et al., 1993; Patel, 1996; Hossaert-McKey and Bronstein, 2001). Having small numbers of mature female figs present all year-round can benefit local resident populations of frugivores (Patel, 1996) and may benefit the plants because their seed is being dispersed continuously (Mackay et al., 2018). Because only a small number of figs are mature at any one time it means that birds such as hornbills which disperse widely in search of large crops will not be attracted and this is likely to mean that long distance seed dispersal is rare. A disadvantage of crop asynchrony is that it provides a larger window for non-pollinator fig wasps to oviposit and they should become more abundant (Harrison and Yamamura, 2003). However, *F. deltoidea* in Peninsular Malaysia supports no known species of non-pollinators so this is not a problem for the plant and its pollinator.

Among dioecious *Ficus* species where receptive male and female figs are receptive at the same time the sexes typically emit similar attractant volatiles to ensure the visitation to female trees and ensure seed development (Hossaert-McKey et al., 2016). This is the case in *F. deltoidea*, where receptive figs are present on male and female trees almost continuously. When male and female figs are receptive at different times the selection for mutual mimicry is relaxed and the volatiles released by receptive male and female figs can be different (Chen et al., 2009). This suggests that the receptive figs of male and female *F. deltoidea* are likely to emit similar volatiles, but experimental results suggest this is not the case (Chapter 5).

In monoecious fig trees the development of seeds and pollinator offspring is in the same figs and their development times are similar. In most dioecious fig trees, the development of figs is faster on male trees (Harrison et al., 2000; Patel and McKey, 1998; Suleman et al., 2011). Male fig trees also tend to have figs with more female flowers and produce figs more frequently than female fig trees (Mizuki et al., 2005). This may be because female fig trees need to allocate more resources to produce seeds than male trees provide to support pollinator offspring (Leigh and Nicotra, 2003). Short development times benefit the fig wasps as there is less time to be attacked by parasitoid fig wasps (Harrison and Yamamura, 2003), but this is not a factor in *F. deltoidea*. Assessing the exact timing of fig receptivity is really difficult without using fig wasp choice experiments (Harrison et al., 2000) but fig development after pollinator entry appears to be about the same in female and male var. *angustifolia*.

The period of receptivity varies among different plant species and reflects a balance between the benefits of a greater possibility pollinator visitation and the greater maintenance cost of the flowers (Hossaert-McKey et al., 1994; Rathcke, 2003). Non-pollinated figs that remain receptive for longer give extra time for the pollinators to enter them, but older figs may produce less seed and fig wasp offspring (Liu et al., 2013). A study of *Ficus montana* showed that in this species the ostiole can also remain open for at least three days after it is first entered (Suleman et al., 2011). Like *F. montana*, pollinator foundresses of *F. deltoidea* can each enter several figs to lay their eggs and pollinate (Chapter 7), so an extended period of receptivity after entry of the first pollinator may also be present in this species.

6.5.3 Pollination limitation

Many figs of *F. deltoidea* failed to be entered by figs wasps and were eventually aborted. Figs on female plants were particularly likely to fail to be pollinated, despite each foundress being capable of pollinating up to six figs (Chapter 7). This routine failure of pollination in female figs might be due to the fig wasps preferring to enter male figs (Chapter) or might occur due to the poor dispersal ability of the fig wasps

(Chapter 5). Successful pollen flow in flowering plants involves (i) both maternal and paternal plants producing receptive female flowers and source of pollen at the same time, (ii) a distance component between pollen donor and receptive flower locations; and (iii) a pollinator that is able to disperse the pollen across the distances required (Jordano, 2017).

In this study, all three aspects appear to be optimum but still the number of pollinated figs was very low. Insect mediated pollination depends on the insect’s behaviour and the spacing of the plants (García and Chacoff, 2007). The nature of fig wasps, which are small, short lived, delicate and weak flyers might be the reason spatial limitation might lead to pollinator limitation of isolated fig trees, but the pollinators of some fig trees can travel over very long distances between trees (Compton, 2002; Ahmed et al., 2009). Although the pollinators of some monoecious *Ficus* are able to fly over hundreds of kilometres (Harrison and Rasplus, 2006; Ahmed et al., 2009), more restricted dispersion seems to be typical of pollinators of dioecious species, including var. *angustifolia*. The observed reduction in pollination rates as local densities of male trees declined in the oil palm plantation are in agreement with the results of the experiments described in Chapter 5 and suggest that the *Blastophaga* sp. has very limited dispersal.

Due to continuous production of figs by both sexes of *F. deltoidea*, the temporal overlap within trees was high with receptive figs and DE phase figs often present at the same time. This permits entry of the pollinators into figs on their natal trees (Patel, 1996) and reduces the energetic costs and dangers involved when they need to fly to figs on other trees (Chapter 7). In particular, entering figs on natal male trees ensures that the figs are suitable for oviposition, whereas dispersal to other trees can lead to entry into female figs, where they cannot reproduce, and cannot fly to other trees because their wings are lost during entry through the ostiole. Selection to avoid female trees is less prominent in seasonal environments where pollinators may be released at times when very few receptive male figs are available (Patel, 1996). In var. *angustifolia* the production of figs was all year-round, generating strong selection for pollinators to differentiate between male and female figs (Patel, 1996; Hossaert-McKey et al., 2016; Chapter 5).

6.6 Conclusions

In response to the stability of its Malaysian climate *F. deltoidea* produces new leaves and figs throughout the year. Plants with some elongate juvenile leaves can produce figs. The asynchrony of fig production within and between trees permits continuous production of fig wasps and seeds by male and female plants, which should allow fig wasp populations to cycle on the same plant. Despite the high density of plants within the oil palm plantation, many female figs failed to be pollinated and this suggests that its pollinator usually disperses very short distances from its natal figs.

Chapter 7

The pollination biology of *Ficus deltoidea* var. *angustifolia*, an epiphytic fig tree with very few flowers in its seed-producing figs

7.1 Introduction

Inflorescence design influences the relationship between plants and their pollinators and in particular the behaviour of the pollinators. Selection acts on inflorescence traits (and floral traits) to increase reproductive outcomes (Iwata et al., 2012). The number of flowers, inflorescence architecture, the number of flowers opening at one time (also known as display size) and the separation of sexual roles in time and space within an inflorescence are among the floral traits that influence pollinator behaviour (Harder et al., 2004). These floral traits evolve to maximize pollinator visitation rates, pollen removal and pollen transfer. Inflorescence design is also influenced by post-pollination factors such as diaspore size and its relationship to likelihood of dispersal.

7.1.1 Fig tree pollination

Fig trees (*Ficus*) are unique in having an urn-shaped inflorescence with its inner surface lined by tiny male and uni-ovulate female flowers. The inflorescences are called figs (syconia). As well as developing into seeds, these flowers provide development sites for the plants' fig wasp pollinators (Suleman et al., 2011). All *Ficus* species are pollinated by either a single or several species of fig wasps (Family Agaonidae, Hymenoptera) that are very rarely associated with other *Ficus* species (Cornille et al., 2012). Although most fig wasps are host specific, hybrids can occur if compatible fig trees are pollinated by fig wasps that developed in the figs of other species (Ghana et al., 2017). The specificity of the relationship is maintained by physical structure of the figs (van Noort and Compton, 1996) and differences in the volatile cues emitted from figs (Hossaert-McKey et al., 2016).

The access of pollinator foundresses to the stigmas of the flowers is controlled by a narrow ostiole, through which pollinators must crawl if seeds are to be produced. In *Ficus*, floral traits such as the number of male and female flowers in each inflorescence are easily altered to influence and respond to pollinator behaviour (Cook et al., 2004). Figs vary greatly in the numbers of flowers they contain, but inflorescence design in *Ficus* is relatively conservative and has remained unchanged for tens of millions of years. Figs vary in size and location, but this variety appears to be related to the different vertebrates that are responsible for seed dispersal, rather than differences in pollination biology (Compton et al., 2010).

One of the more variable aspects of inflorescence design in *Ficus* species is the relative numbers of male and female flowers that are present. Pollination of some species is active, with specific behaviours and pollen-carrying structures present in the fig wasps. This type of pollination is usually associated with a small number of male flowers in the figs (Kjellberg et al., 2001). Pollination can also be passive, where no specific pollen deposition or collection behaviour occurs. Figs with passive pollinators have higher ratios of anthers to female flowers and produce more pollen per fig (Kjellberg et al., 2001). To facilitate passive collection by their pollinators, the mature anthers in these figs burst open spontaneously (Jousselin et al., 2003).

A floral trait that controls pollinator behaviour in *Ficus* involves segregation of the sexual roles in their inflorescences. There are two types of breeding system associated with *Ficus* species. The figs of Monoecious species each have both male reproductive function (producing pollen and the fig wasp’s offspring to transport the pollen) and female function (produce seed) in a single fig while in dioecious species, the male and female reproductive functions are separated on different trees (Nefdt and Compton, 1996). In dioecious figs, the figs on male trees support only pollinator offspring, while in female figs only seeds are produced (Raja et al., 2008a). Oviposition is prevented in female figs by having flowers with longer styles than in male figs with the surface of the stigmas difficult to penetrate by pollinator ovipositors (Tarachai et al., 2008). This floral trait ensures that only pollination occurs in the female figs. Pollination also occurs in the male figs, but they are not capable of developing seeds.

7.1.2 Re-emergence of foundresses

Foundress fig wasp females enter the fig cavity through the ostiole on their way to lay their eggs in the female flowers. From one to tens of foundresses may enter a single fig. When passing through the ostiole they lose their wings and parts of their antennae (Kjellberg et al., 2005b). It used to be thought that a foundress will always be trapped in the first fig they enter and die inside. Some re-emergence of fig wasps from figs of *Ficus carica* L. was nonetheless suggested by Grandi (1920). He found a lot of dissected figs had foundresses with their heads pointing out of the ostioles and he also found developing figs with no foundresses inside. Later discoveries have showed that females of some species of fig wasps regularly re-emerge and successfully enter and oviposit into subsequent figs (Gibernau et al., 1996; Suleman et al., 2013b).

Fig wasps that have entered a monoecious fig or a male dioecious fig can then walk to another fig and lay more eggs. However, if the fig wasp had first entered a female fig, re-emergence will still mean they are unable to reproduce as they can only walk to other female figs on the same tree. They still continue to pollinate flowers inside female figs, so re-emergence can benefit the host plant by allowing several figs to be pollinated by a single foundress fig wasp (Tarachai et al., 2008). Because such females can never reproduce, natural selection can only shape the behaviour of females that have entered male figs, so re-emergence from male figs needs to be of benefit to foundresses if the same behaviour will be selected for among females that entered female figs (Raja et al., 2008a).

Rates of re-emergence vary between species (Gibernau et al., 1996) and may be more common in pollinators of dioecious than monoecious fig species (Moore et al., 2003). The factors selecting for re-emergence behaviour will vary between species and reflect their population dynamics and environmental factors (Gibernau et al., 1996). Re-emergence is said to happen due to the limited number of oviposition sites in a single fig (Moore et al., 2003) and that females that entering more than one fig can produce more offspring (Gibernau et al., 1996). Only one offspring can develop in a single flower (Jousselin et al., 2001) and if the combined eggs loads of the foundresses that enter a fig are more that the total number of flowers the oviposition site limitation is inevitable. This means that if the egg load of a single female pollinator is larger than the number of female flowers present then re-emergence would always be favoured.

7.1.3 Pollinator sex ratio variation

Organisms typically produce equal numbers of male and female offspring (Fisher, 1958). However, Hamilton (1967) stated that in some situations where brothers and sisters mate with each other a female-biased sex ratio is optimal because this results in a greater number of grandchildren than if equal numbers of sons and daughters were produced. Hamilton (1967) proposed that organisms in isolated sub-populations (in a colony with limited dispersal where sib-mating is common) could adjust their offspring to a female-biased sex ratio because of this inbreeding and Local Mate Competition (LMC). In *Ficus*, where LMC conditions are typical inside the figs, a female-biased offspring sex ratio is expected to be produced and has frequently been observed (Martel et al., 2016). As foundress numbers entering a single fig increases a less female-biased offspring sex ratio becomes optimal and is often observed (Peng et al., 2014). In some species, at least, this results from an oviposition strategy where mostly male eggs are laid first, and where total clutch sizes decline with the increased competition for oviposition sites as more foundresses enter a fig (Raja et al. 2008).

The sex ratio of their offspring also often varies according to the number of foundresses that entered a fig. Hymenopterans are able to control the sex of their offspring, because male offspring result from a lack of fertilization and are haploid, whereas fertilized eggs produce diploid females. The ability to control the sex ratio by a foundress allows natural selection to influence female behaviour to produce sex ratios adjusted to local conditions (Kinoshita et al., 2002). Moore et al. (2005) and Raja et al. (2008b) showed that clutch size variation allows foundresses of one species to adjust their sex ratios because they lay most of their male eggs at the start of an oviposition sequence. The more eggs that are laid, the more female-biased the sex ratio becomes and more eggs are laid in figs where fewer foundresses are present because of competition for oviposition sites. Foundresses of the parasitoid wasp *Nasonia vitripennis* assess potential LMC by the presence of other females (Shuker and West, 2004) and numbers of foundresses can also directly influence offspring sex ratio in some fig wasps (Greeff and Newman, 2011). Because only female fig wasps carry pollen the sex ratio influences the proportion of fig wasp offspring that are valuable to their natal plant.

7.1.4 *Ficus deltoidea* reproduction

Ficus deltoidea is exceptional among *Ficus* species in that the figs produced by female plants contain only small numbers of flowers, sometimes just a single flower (Corner, 1969). Each pollinator foundress carries multiple pollen grains, and given that seed production in *Ficus* is often pollinator limited, a situation where each foundress can only generate a very limited number of seeds is likely to be sub-optimal and inefficient use of the foundresses. Given the apparent selective advantage for *F. deltoidea* of having figs that contain small numbers of large seeds (Corner, 1969), a feature that may be associated with the unusual, true epiphytic biology of many varieties of *F. deltoidea*, having female figs that allow foundresses to re-emerge from receptive figs and go on to pollinate further figs, would clearly be advantageous.

Foundresses that re-emerge have lost their wings (Suleman et al., 2013b). This prevents them from flying off in search of figs on male trees, but also means that they must walk between figs. This clearly limits their mobility and further requires that the plant produces figs that grow close together, and are at similar developmental stages. To be effective, this suite of characters necessarily requires foundresses to be willing to re-emerge from the first fig they enter, despite the risks that they will be making themselves more vulnerable to predation.

Every foundress that enters a female fig has no chance of reproducing. Because selection can only operate via male figs, where fig wasps do reproduce (Raja et al., 2008b), plants of this sex need to offer conditions that provide a selective advantage for those foundresses that enter more than one fig. Specifically, they need to produce more offspring than foundresses that opt to lay all their eggs in a single fig. This vicariant selection (Grafen and Godfray, 1991) might take the form of male figs having only small numbers of flowers, in combination with an ostiole structure that facilitates easy emergence from the figs.

7.2 Objectives

The questions addressed by the experiments described in this chapter were 1) How many foundress fig wasps enter a single fig of *F. deltoidea* var. *angustifolia*? 2) Does the number of foundresses vary between male and female figs? 3) How often do foundresses re-emerge from figs and does this differ between male and female figs? 4) How many figs can a single foundress enter? 5) How many eggs do foundresses lay in their first and subsequent figs? What is the relationship between egg loads and the number of female flowers in male figs? 6) How many flowers do they pollinate in female figs? And 6) Do their offspring sex ratios vary between the first and subsequent figs that they enter?

7.3 Methods

7.3.1 Study varieties

The experiments were made using epiphytic *Ficus deltoidea* var. *angustifolia* in oil palm plantations and its pollinator *Blastophaga* sp. This variety has asynchronous leafing and fig phenology patterns on individual plants of both sexes and figs are produced more or less continuously throughout the year (Chapter 6). This results in typically several receptive figs being present on each tree at any one time. The anatomy of the figs is described in Chapter 4. Each male fig contains about 143 female flowers and each female fig contains only 4 female flowers. The figs of this variety have a long pedicel.

The only known pollinator for *Ficus deltoidea* was *Blastophaga quadrupes* which was probably reared from var. *lutescens* in 1993 collected in Java and Sumatra (Wiebes, 1993). Whether this pollinator species pollinates some other varieties of *F. deltoidea* is unknown, but it is likely that pollinator of var. *angustifolia* is restricted to this one variety (Chapter 5). The anatomy of the wasps and the large numbers of male flowers in male figs suggest that pollination by this species is passive (Chapter 4).

7.3.2 Study sites and experimental methods

The study was located in Banting oil palm plantation. Details of this site were provided in Chapter 2. To get an indication of the number of female fig wasps that can enter one particular fig, and how many re-emerge 200 male and 200 female figs were taken randomly from 21 male and 10 female trees during their early C phase (Galil and Eisikowitch, 1968). How the developmental phase of the figs was determined can be found in Chapter 2. Records of wings and bodies inside the figs provide only a minimum estimate of how many foundresses entered each fig because foundresses that lacked wings on entry could not be accounted for. The presence of wings indicates that the fig was the first one to be entered by one or more females. During early C phase, the wings can be seen either at the entrance to the ostiole or in the inner ostiole using a stereomicroscope.

The presence of a female fig wasp body also can be seen clearly in this phase as they have not decayed. If a developing fig did not contain any wings or foundress inside, then it indicates that a foundress that had previously entered another fig was responsible for pollination or galling the ovules. When a fig wasp body was present, the counts recorded their head position. If the body were found in the fig cavity, or pointing outwards through the ostiole, it shows they were potentially able to lay some eggs or pollinate the ovules. However, if the head were positioned in the ostiole facing towards the cavity, it means they died before reaching the female flowers.

To determine how many figs one foundress can enter, groups of 4 to 15 adjacent A phase figs were selected. These pre-receptive figs were confined in a fine mesh bags to prevent any entry of pollinators. One to two weeks later, mature male figs were collected just before fig wasp release and placed in mesh-covered containers to let the foundresses emerge naturally. After the foundresses emerged, they were brought to the plantation and a single foundress were placed in the bags using a fine brush (Figure 7.1). After the foundress was placed in the bag, it was closed again to prevent any entry from other pollinators. A total of 30 bags on 6 male trees and 38 bags on 8 female trees were included in this experiment.

Six week later, the bags were opened to record how many figs were in C phase, and count the numbers of fig wasp offspring and the numbers of seeds. The first fig entered by each foundress was found by looking for the presence of the wings under a stereoscopic microscope. The number of galls were counted in the male figs and the number of seeds were counted in the female figs. These counts allowed the size of clutches laid by the single foundress in their first and subsequent figs to be compared. However, the precise sequence of entry by the wingless foundress into the subsequent figs could not be determined.

7.3.3 Statistical methods

The number of foundresses remaining inside the figs, the differences in the head position of the foundress inside the figs and the frequency of figs entered by a single foundress were analysed using Fisher’s Exact Test as some of the expected outcomes were less than 5. For differences in the number of winged and wingless foundress in a fig, Chi square test were used. Pearson correlations were examined between empty visited fig percentage and the mean number of dead foundresses within male and female trees.

The total brood size for foundresses that entered different numbers of figs, total brood size of first entered and subsequent male figs, differences in total brood size between experimental and wild figs, and total seed production in the first and subsequent female figs entered were analysed using generalized linear models (GLM) with Poisson error distribution. If overdispersion occurred, quasipoisson errors were used for those count data.

The offspring sex ratios in emerged and non-emerged foundresses were analysed using generalized linear models (GLM) with a gaussian error. The offspring sex ratios in first and subsequently entered figs and comparisons of sex ratios between wild and experimental figs were analysed using generalized linear models (GLM) with a quasibinomial error distribution and logit link. Spearman rank test correlation were used when examined the relationship between brood size and sex ratio in natural and experimental figs. The tests were all performed in R studio (1.0.153) and SPSS Statistics 20.

7.4 Results

7.4.1 The number of fig wasps entering a fig

The minimum number of fig wasps entering a fig in male and female trees can be estimated from the number of remaining fig wasp bodies in the figs. Based on these, the range of fig wasps entering the male trees was from 1 to 6 while for female trees the range was from 1 to 4 fig wasps (Figure 7.2). These values do not include pollinators trapped while attempting to enter the figs. The plant sexes showed rather similar patterns, with empty developing figs without any foundress bodies being the most common and the frequency of figs with more foundresses present declining (Figure 7.2). Fisher’s Exact test nonetheless found a significant difference between male and female trees in the number of remaining foundresses inside the figs (df = 6, $P < 0.05$). Developing figs without any foundresses inside were frequently observed in both sexes. In male trees 81 out of 200 of the figs did not have the foundresses’ body inside the fig while in the female figs, the numbers without foundresses’ bodies were 110 out of 200. The number of visited figs without a foundress body varied from 14.3% to 66.7% in male fig trees (Table 7.1) and from 25.0% to 100% in female trees (Table 7.2).

Figure 7.1 The process of inserting a single foundress in mesh bags containing receptive figs.

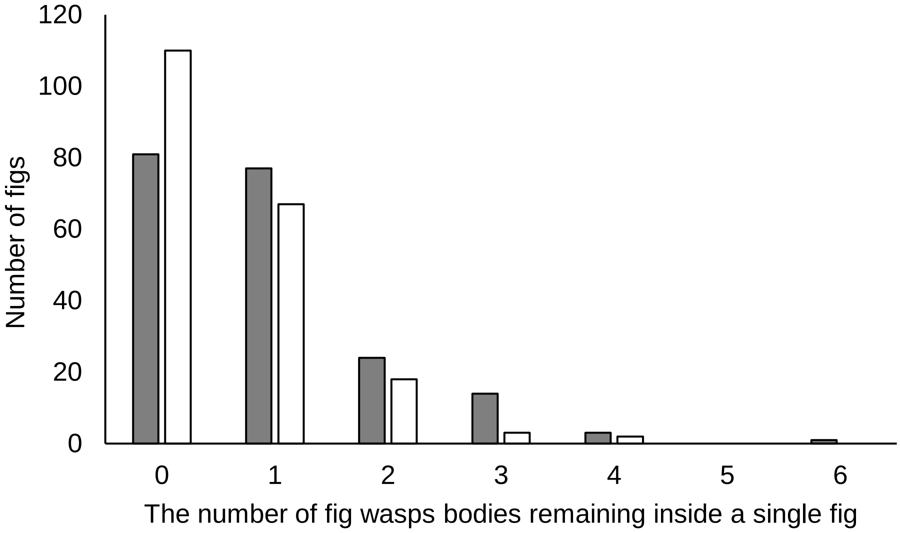


Figure 7.2. The number of foundresses remaining inside figs of var. *angustifolia* that had seeds or galls developing. Solid bars = male trees, open bars = female trees.

Table 7.1 The numbers of naturally-visited male figs with foundress bodies either in the fig cavity or with the body stuck head-outwards in the ostiole. Means do not include figs with no foundress corpses.

Tree	n figs	Figs with foundresses (%)	Figs without foundresses (%)	No. of foundress corpses per fig		
				Mean	SD	Range
1	21	80.95	19.05	1.76	1.30	1-6
2	11	45.45	54.55	1.73	1.10	1-4
3	5	80.00	20.00	1.40	0.89	1-3
4	6	83.33	16.67	1.67	0.82	1-3
5	5	40.00	60.00	1.00	0.00	1
6	5	60.00	40.00	1.20	0.45	1-2
7	5	40.00	60.00	1.00	0.00	1
8	17	64.71	35.29	1.29	0.69	1-3
9	5	20.00	80.00	1.00	0.00	1-3
10	6	66.67	33.33	1.00	0.00	1
11	7	57.14	42.86	1.00	0.00	1
12	13	69.23	30.77	1.38	0.65	1-3
13	7	71.43	28.57	2.00	0.82	1-3
14	5	40.00	60.00	1.00	0.00	1
15	5	40.00	60.00	1.00	0.00	1
16	9	33.33	66.67	1.11	0.33	1-2
17	21	57.14	42.86	1.24	0.44	1-2
18	10	50.00	50.00	1.60	1.07	1-4
19	9	66.67	33.33	1.22	0.44	1-4
20	7	85.71	14.29	1.57	0.77	1-3
21	21	76.19	23.81	1.10	0.44	1-3
Total	200	62.00	38.00	1.57	0.49	1-6

Table 7.2 The numbers of naturally-visited female figs with foundress bodies either in the fig cavity or with the body stuck head-outwards in the ostiole. Means do not include figs with no foundress corpses.

Tree	n figs	Figs with foundresses (%)	Figs without foundresses (%)	No. of foundress corpses per fig		
				Mean	SD	Range
1	53	50.94	49.06	1.04	0.19	1-3
2	8	75.00	25.00	1.63	0.92	1-2
3	13	69.23	30.77	1.00	0.00	1
4	45	44.44	55.56	1.13	0.40	1-3
5	33	60.61	39.39	1.33	0.69	1-3
6	13	30.77	69.23	1.23	0.44	1-2
7	10	30.00	70.00	1.50	0.97	1-4
8	12	66.67	33.33	1.33	0.49	1-2
9	8	37.50	62.50	1.00	0.00	1
10	5	0.00	100.00	0.00	0.00	0
Total	200	50.00	50.00	1.30	0.41	0-3

From a total of 324 foundress bodies found in the 400 dissected figs, 286 bodies were situated with heads towards the outside, 28 were present inside the fig cavity and only 10 had died while attempting entry through the ostiole (Figure 7.3). More foundress bodies were found facing the ostiole in the male trees while more foundress body were found in the cavity in the female figs. There was a significant difference in the location of the foundress in the figs between male and female trees using Fishers Exact Test (df = 2, $P < 0.001$).

The wings counts in developing figs also give an estimate of the number of figs that were entered by winged foundresses (and possibly also additional foundresses without wings) and those entered only by females that already had lost their wings. The likelihood of having wings at the ostiole was quite similar in male and female figs (Table 7.3) and did not differ significantly between figs on male and female trees (Chi square, $\chi^2 = 0.28$, df = 1, $P > 0.05$).

In male figs the percentage of wing-free visited figs on a tree and the mean number of dead foundresses in figs where foundresses were present was negatively correlated (Pearson correlation, $r = -0.55$, $n = 18$, df = 17, $P < 0.001$) (Figure 7.4). The relationship was also negatively correlated in female figs (Figure 7.5, but not significant (Pearson correlation, $r = -0.37$, $n = 10$, df = 9, $P > 0.05$). This suggests that crops where more of the figs were entered only by foundresses that had already entered other figs had fewer foundresses that remained in the figs. Variation in foundress densities on different crops may be an explanation.

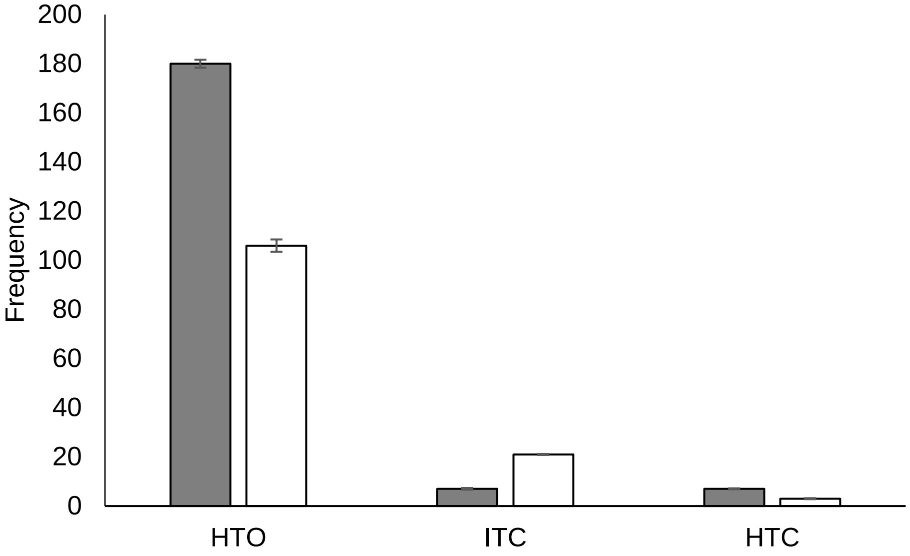


Figure 7.3 The positions of foundresses in the figs. HTO = head towards ostiole, ITC = body in the cavity, HTC = Head towards cavity. Solid bars = male trees, open bars = female trees.

Table 7.3. Wings counts in figs from male and female trees.

Sex	n (trees)	n (figs)	No. of figs with wings present	No. of figs with wings absent	Percentage with wings	Frequency of 1 winged foundress (Range)	Frequency of >1 winged foundress (Range)	Percentage of >1 winged
Male	21	200	136	64	62.35	120 (2-17)	16 (0-4)	6.24
Female	10	200	131	69	63.96	103 (3-43)	28 (0-19)	11.46

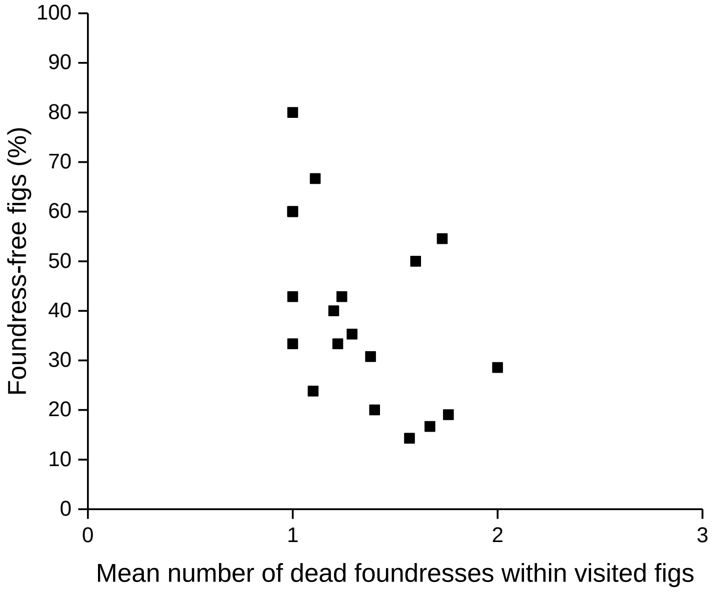


Figure 7.4 The relationship between the percentage of developing male figs with no foundress present and the mean number of foundresses in the figs where one or more foundresses were present. Each data point indicates one crop of male figs.

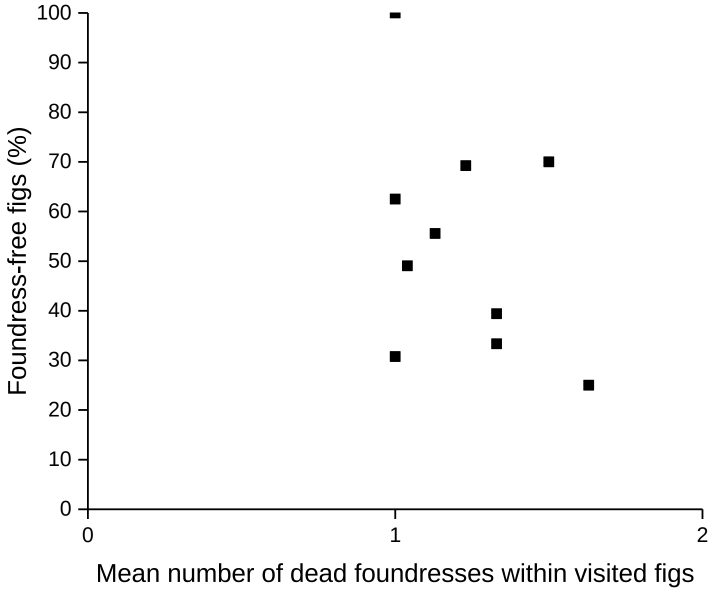


Figure 7.5 The relationship between the percentage of developing female figs with no foundress present and the mean number of foundresses in the figs where one or more foundresses were present. Each data point indicates one crop of female figs.

7.4.2 Emergence rates from experimental figs

In the bags where a single foundress was introduced, 50 of the 68 foundresses (73.53%) entered more than one fig with a maximum of four male figs and six female figs entered (Figure 7.6). The likelihood of re-emergence from the first fig was not significantly higher from female figs (79.0%) compared to male trees (66.7%) (Chi square, $\chi^2 = 1.30$, df = 1, $P > 0.05$), and the difference in the number of figs entered was not significant (Chi square, $\chi^2 = 4.48$, df = 3, $P > 0.05$).

7.4.3 Brood size in re-emergence and non-emergence foundresses

There was a large increase in the total brood sizes achieved among foundresses that re-emerged and entered additional figs. The brood size of non re-emerging females ranged from 42 to 137 while in the re-emerging females the brood size ranged from 26-236 (Table 7.4). There was a significant difference in the brood size of emerging and non re-emerging foundresses (GLM, $\chi^2 = 211.84$, df = 1, $P < 0.001$). Females that only oviposited in one fig had a mean brood size of 80.7, whereas foundresses with offspring in three figs averaged more than twice this number of offspring (198.2) (Figure 7.7). The number of offspring did not increase for foundresses that entered four rather than three figs (Figure 7.7). There was a significant difference in the total brood size depending on the number of figs they entered (GLM, $\chi^2 = 348.35$, df = 3, $P < 0.001$).

7.4.4 Brood sizes in the first and subsequent figs entered

The increased offspring numbers among females that re-emerged may be a reflection of their vigour, rather than having the opportunity to access more oviposition sites. Brood sizes were higher in the first figs entered by foundresses compared to the subsequent figs they entered (Figure 7.8) (GLM, $\chi^2 = 243.27$, df = 1, $P < 0.001$). However, there was also a significant difference in the brood sizes in the first fig entered between re-emerging and non re-emerging foundresess (GLM, $\chi^2 = 0.22$, df = 1, $P < 0.001$) where non re-emerging foundress laid fewer eggs than re-emerging foundresses.A comparison of brood sizes between the experimental and randomly taken naturally-pollinated figs (Figure 7.9) suggested that they were similar (means of 60.53 and 59.94 respectively) (GLM, $\chi^2 = 0.16$, df = 1, $P > 0.05$).

Table 7.4 The differences in re-emergence and non-emergence foundress

Foundress	No. of figs entered	Re-emerged	Female offspring	Male offspring	Total brood size
1	1	No	77	9	86
2	1	No	80	14	94
3	1	No	110	27	137
4	1	No	40	6	46
5	1	No	57	22	79
6	1	No	37	5	42
7	2	Yes	101	60	161
8	2	Yes	88	41	129
9	2	Yes	106	22	128
10	2	Yes	77	38	115
11	2	Yes	20	6	26
12	3	Yes	184	33	217
13	3	Yes	113	64	177
14	3	Yes	184	52	236
15	3	Yes	140	23	163
16	4	Yes	182	26	208
17	4	Yes	99	63	162
18	4	Yes	147	68	215

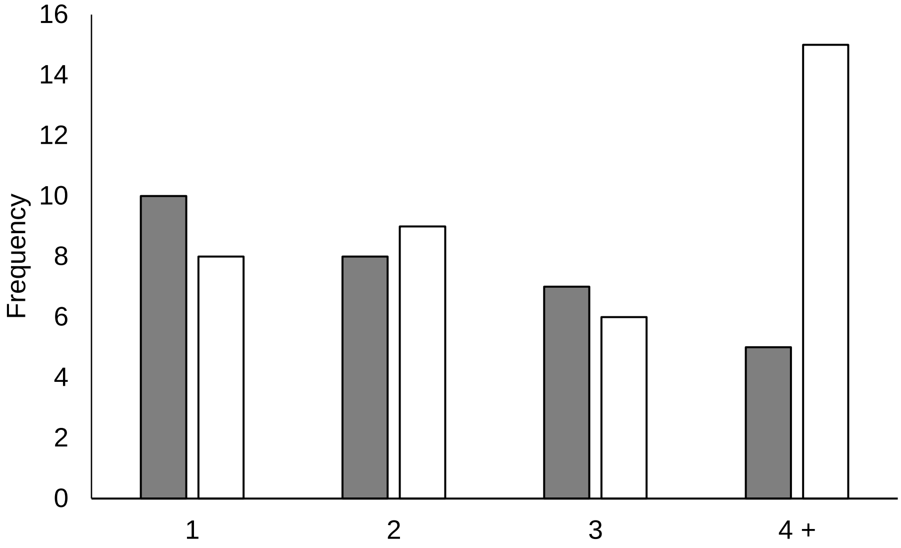


Figure 7.6 The number of figs entered by single foundresses. Solid bars = figs on male trees, open bars = figs on female trees.

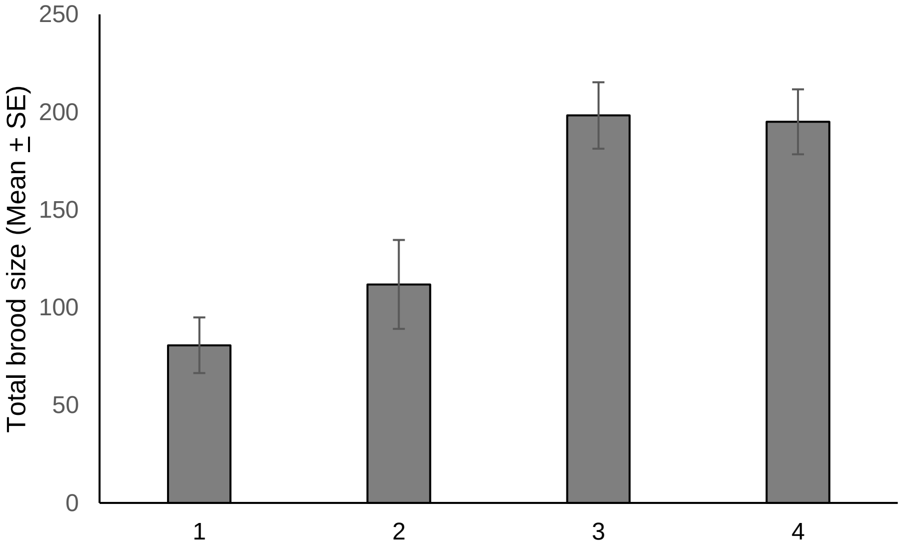


Figure 7.7. Total brood sizes for single *Blastophaga* sp. foundresses that entered different numbers of figs on male trees.

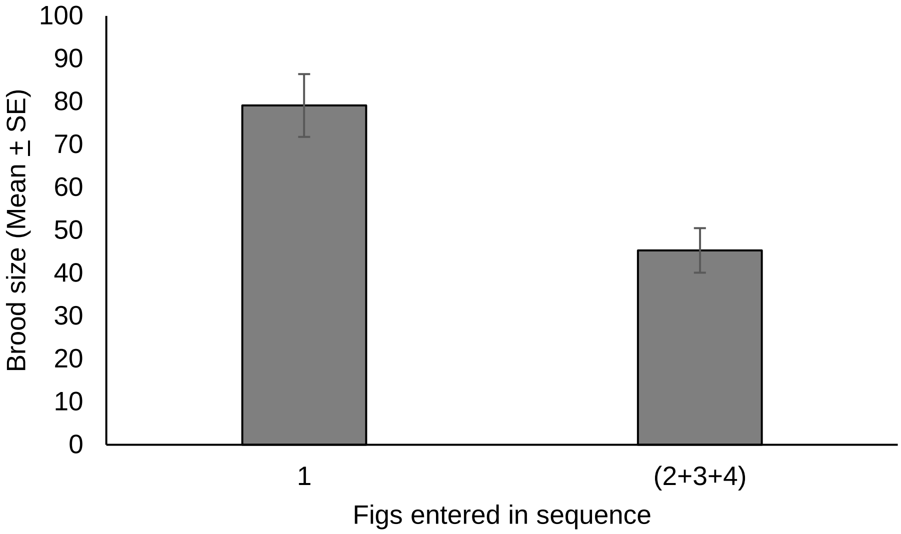


Figure 7.8. The numbers of pollinator offspring in the first figs entered by foundresses and the subsequent figs they entered.

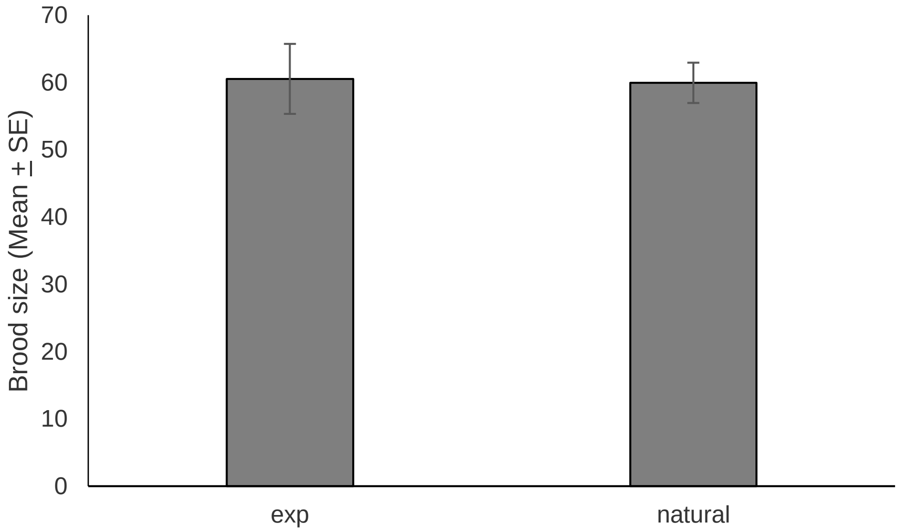


Figure 7.9 Pollinator brood sizes in the experimental figs and naturally-pollinated figs.

7.4.5 Pollination by foundresses that entered one or more female figs

In figs on female trees, pollinator foundresses also routinely re-emerged and pollinated flowers in up to six figs. In the first figs that the foundresses entered (where wings were present), all the flowers were successfully pollinated. This number decreased slightly in subsequent figs (Figure 7.10). However, each fig contained very few flowers to be pollinated and a GLM (with Poisson error), showed there was no significant difference in the number of flowers pollinated by the foundresses in the first and the subsequent figs ($\chi^2 = 0.38$, df = 1, $P > 0.05$). In figs of both sexes, those entered by only wingless foundresses were usually located very close to the first fig the pollinator had entered (Figure 7.11).

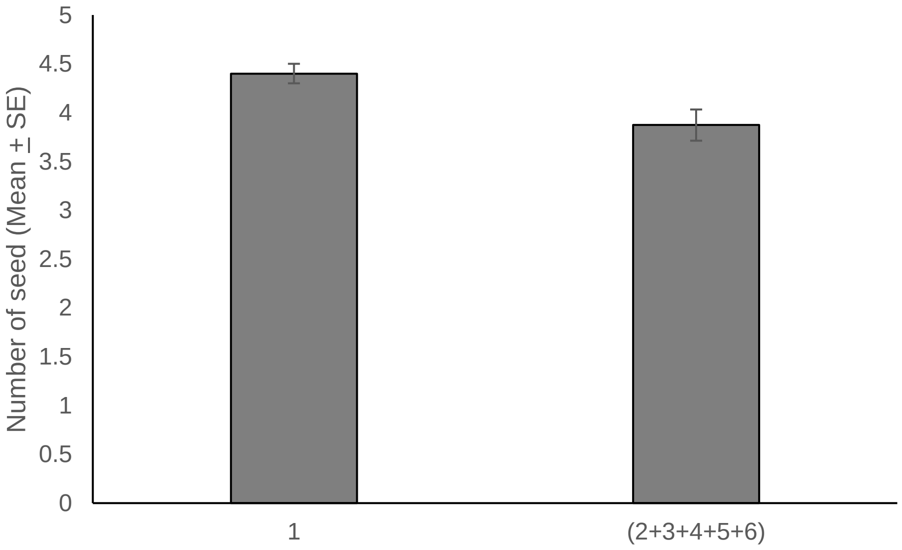


Figure 7.10. The number of seeds produced in the first figs entered and the subsequent figs.

Figure 7.11. The developing male figs pollinated by a single foundress.

7.4.6 Variation in offspring sex ratios in figs entered by a single foundress

The sex ratio of *Blastophaga* sp. offspring was usually strongly female biased (Table 7.4 and Table 7.5). The offspring sex ratio (proportion of male offspring) was higher in the foundresses that re-emerged than those that did not re-emerge (Figure 7.12) (GLM, $\chi^2 = 199.32$, df = 1, $P < 0.001$). This was strongly influenced by a small number of subsequent-entry figs where most of the offspring were male (Table 7.5).

The sex ratio also appeared to be higher in the subsequent figs entered by the foundresses than in first figs (Figure 7.13 but this reflected the small number of foundresses that had mainly male offspring in subsequent figs and there was no significant difference (GLM, $\chi^2 = 1.79$, df = 1, $P > 0.05$). There was also no significant relationship between offspring sex ratio and brood size (Spearman correlation, $r_s = 0.01$, df = 30, $P > 0.05$, Figure 7.14, Figure 7.15). From the twelve re-emerged foundresses, only four foundresses showed a significant difference between the offspring sexes in the first and subsequent figs and three of these had more male than female offspring in subsequent figs (Table 7.6).

A comparison of sex ratios between the experimental and naturally-pollinated figs showed that the experimental figs contained a slightly higher proportion of male offspring (0.25 and 0.18 males respectively, Figure 7.16), but the difference was again not significant (GLM, $\chi^2 = 3.70$, df = 1, $P > 0.05$). As in the experimental figs, offspring sex ratios in naturally-pollinated figs were not correlated with the number of offspring present (Spearman correlation, $r_s = 0.02$, df = 99, $P > 0.05$, Figure 7.17). Unfertilised foundresses produce only male offspring. Six of the broods in the naturally-pollinated figs consisted of more male offspring than female offspring (Figure 7.17), but only one fig contained only male offspring. This fig may have been entered by one un-mated foundress.

Table 7.4 The offspring sex ratio ratios (number of male offspring/total offspring) in the first fig entered by a *Blastophaga* sp. foundress.

Fig no.	Female	Male	Total offspring	Sex ratio
1	77	9	86	0.10
2	80	14	94	0.15
3	110	27	137	0.20
4	40	6	46	0.13
5	57	22	79	0.28
6	37	5	42	0.12
7	35	27	62	0.44
8	62	29	91	0.32
9	58	11	69	0.16
10	61	14	75	0.19
11	18	6	24	0.25
12	106	23	129	0.18
13	90	30	120	0.25
14	114	37	151	0.25
15	61	12	73	0.16
16	58	5	63	0.08
17	61	7	68	0.10
18	53	16	69	0.23
Total	1178	300	1478	0.20

Table 7.5 The offspring sex ratio (number of male offspring/total offspring) in the subsequent figs entered by a *Blastophaga* sp. foundress.

Fig no.	Female	Male	Total offspring	Sex ratio
1	66	33	99	0.33
2	26	12	38	0.32
3	48	11	59	0.19
4	16	24	40	0.60
5	2	0	2	0.00
6	50	6	56	0.11
7	18	5	23	0.22
8	44	8	52	0.15
9	48	8	56	0.14
10	44	8	52	0.15
11	5	44	49	0.90
12	21	13	34	0.38
13	28	4	32	0.13
14	5	29	34	0.85
15	26	7	33	0.21
16	31	3	34	0.09
17	34	2	36	0.06
18	29	6	35	0.17
19	2	27	29	0.93
20	46	11	57	0.19
21	52	11	63	0.17
22	23	7	30	0.23
Total	664	279	943	0.30

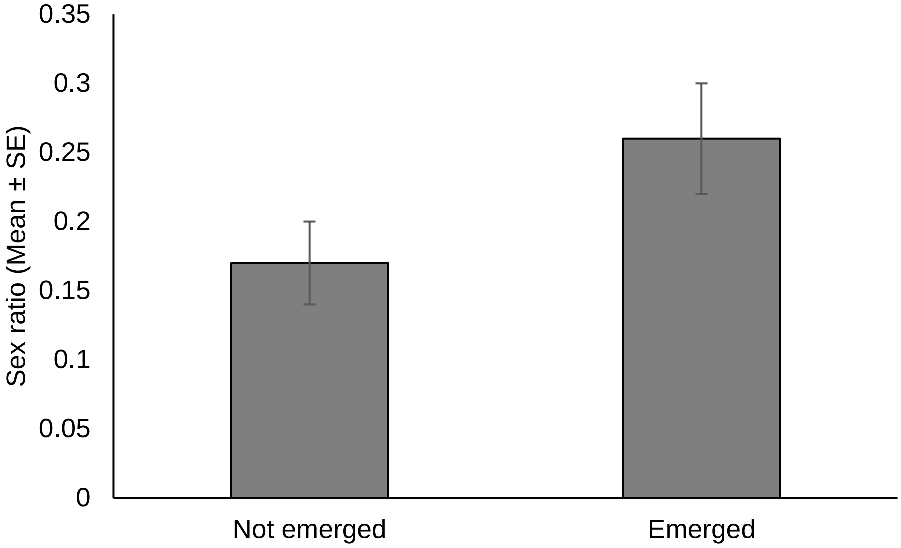


Figure 7.12 ***Blastophaga*** **sp. offspring sex ratios (number of male offspring/total offspring) in relation to emerging and not emerging behaviour.**

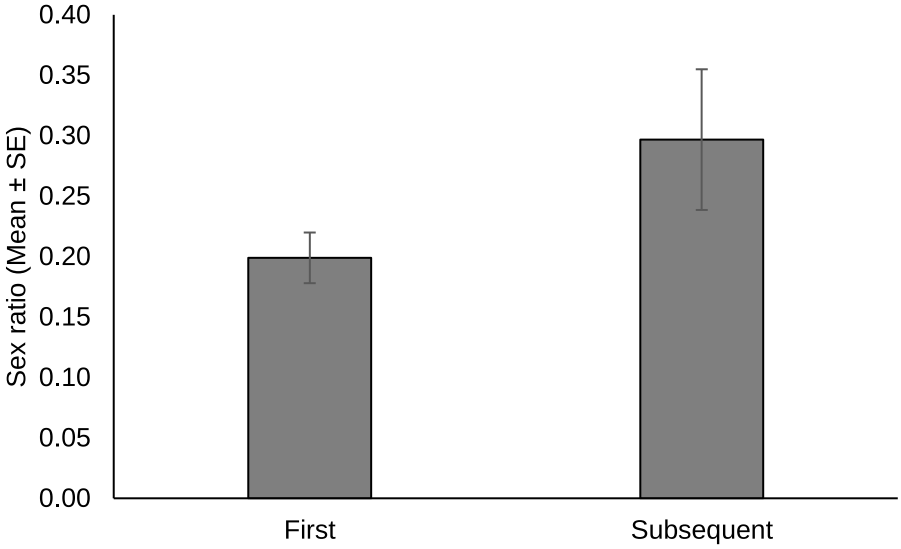


Figure 7.13 ***Blastophaga*** **sp. offspring sex ratios (number of male offspring/total offspring) in relation to oviposition sequence.**

Table 7.6 Offspring sex ratios in first and subsequently entered figs

Foundress no.	First		Subsequent		χ^2	P value
	Female	Male	Female	Male		
1	35	27	66	33	0.44	0.51
2	62	29	26	12	0.001	1.09
3	58	11	48	11	0.16	0.69
4	61	14	16	24	20.14	< 0.001
5	18	6	2	0	0.65	0.42
6	106	23	78	10	1.70	0.19
7	90	30	23	34	20.1	<0.001
8	114	37	70	15	1.49	0.22
9	61	12	79	11	0.59	0.44
10	58	5	124	21	1.72	0.19
11	61	7	86	61	20.93	<0.001
12	53	16	46	47	12.47	<0.001
Total	777	217	664	279	15.28	<0.001

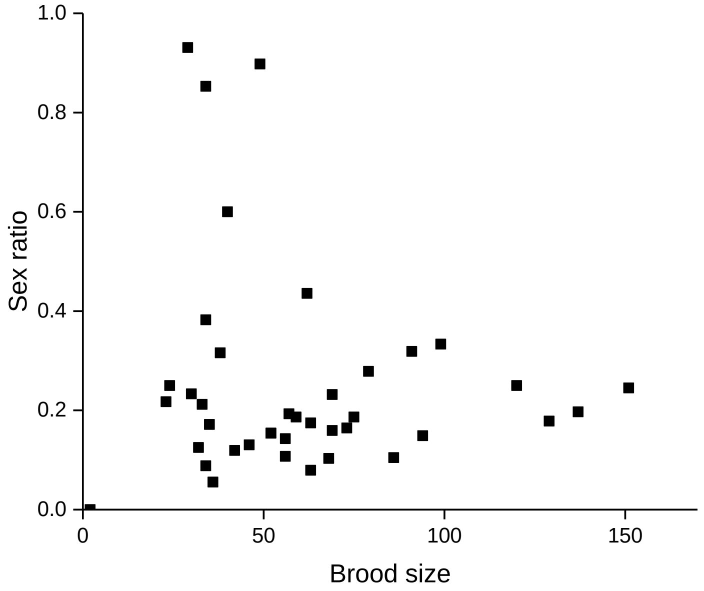


Figure 7.14 The relationship between the sex ratio and brood size in experimental figs entered by a single foundress. Each point indicates the contents of one fig. One foundress generated 1- 4 data points.

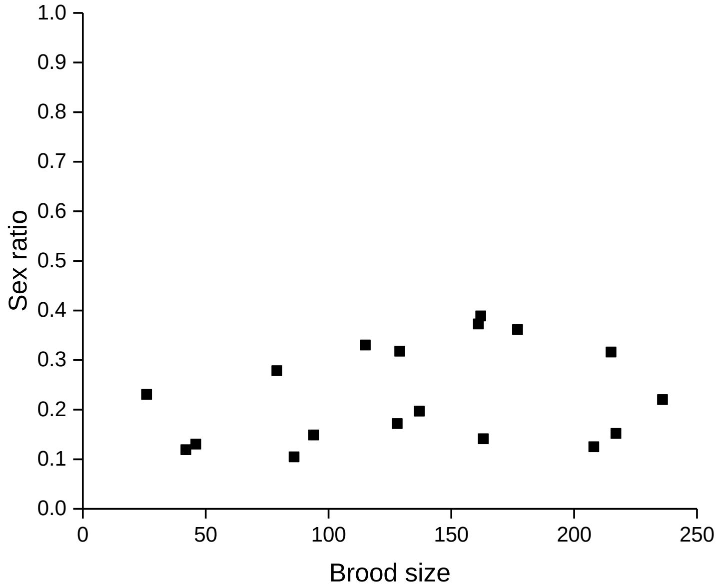


Figure 7.15 The relationship between the total offspring sex ratio and total brood size in experimental figs entered by a single foundress. Each point indicates the total brood size for one foundress, and includes broods spread across two or more figs.

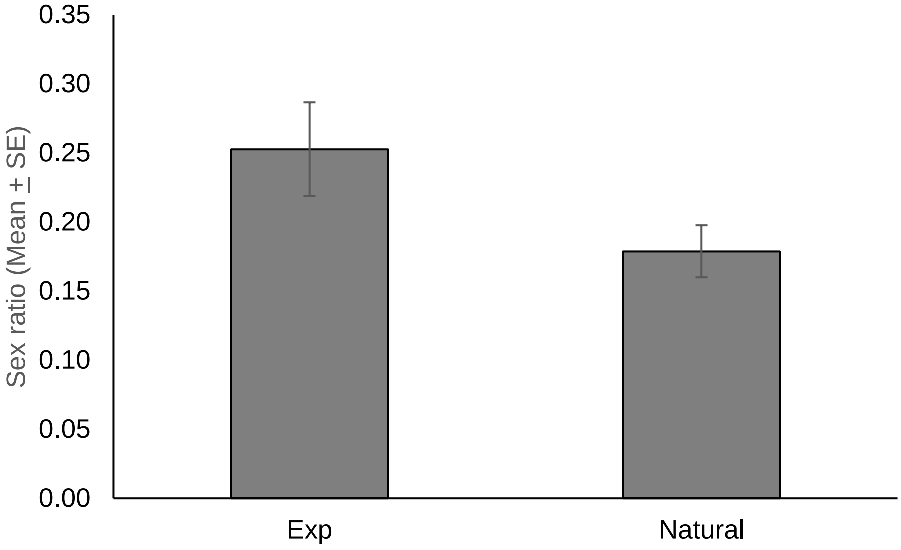


Figure 7.16 Pollinator offspring sex ratios in experimental and naturally-pollinated figs.

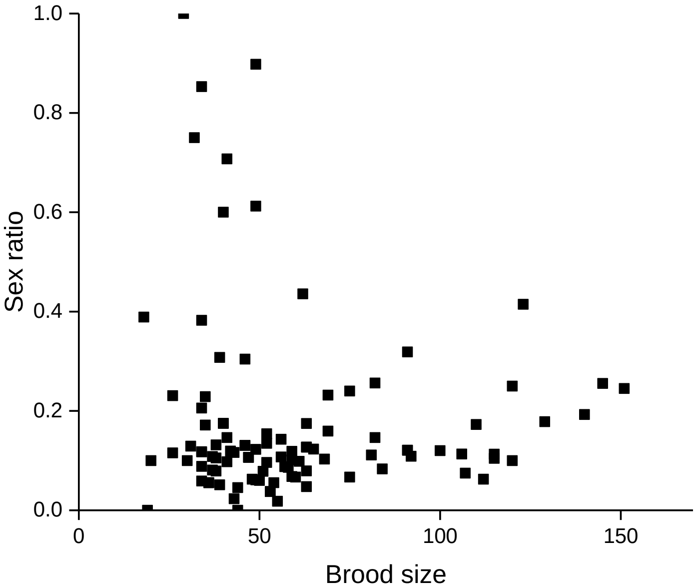


Figure 7.17 The relationship between the brood size and sex ratio in naturally-pollinated figs. Each point indicates the contents of one fig. Several foundresses could have contributed offspring to each fig.

7.5 Discussion

The results presented in this chapter have clarified several aspects of the pollination biology of var. *angustifolia* and the biology of its pollinator *Blastophaga* sp. The figs of this species are small, and its female figs have very few flowers. Despite the small size of the figs they can be entered by multiple pollinators, in part because foundress females routinely enter more than one fig. This behaviour is present on both male and female plants and although figs on female plants are more likely to fail to be entered by pollinators (Chapter 6) female figs entered by pollinators have almost all their flowers pollinated. Foundresses that lay eggs in more than one fig produce more offspring, so re-emergence from figs is likely to be to their advantage. The sex ratios of pollinator offspring are sometimes different in the first and subsequent figs they enter where the male ratio was higher in the subsequent figs.

7.5.1 Multiple foundresses

The number of foundresses that enter a fig varies greatly between species and might be expected to be higher in species with bigger figs, because they have more flowers to be pollinated and offer more sites for pollinator oviposition (Tayou, 1991; Patel et al., 1995; Chen et al., 2001; Yang et al., 2012). In var. *angustifolia* the number of flowers in female figs is very small and a single foundress would normally be sufficient to pollinate all of them, but large numbers of dead foundresses were recorded in some naturally-entered var. *angustifolia* figs.

7.5.2 Walking rates

According to Grafen and Godfray (1991) once a female fig wasp enters the fig cavity, they never leave and re-emergence is not possible. This view is now out-dated and many pollinators are known to be able to re-emerge from figs (Herre et al., 2008). The occurrence of figs showing the production of galls and seeds without any dead foundress inside confirms this (Pereira and Prado, 2006). Even though female wasps lose their wings in the first fig, they still able to leave the first fig and enter other figs by walking (Suleman et al., 2013a). A study of *Kradibia tentacularis* (Grandi) showed the fig wasps were able to re-emerge and oviposit in up to four receptive *Ficus montana* Burm. f. figs and that they tend to enter the first receptive fig they encounter (Suleman et al., 2013a). Most of the foundresses re-emerge from female and male figs and go to the other figs to lay further eggs (Tarachai et al., 2008). Not being able to re-emerge would prevent these foundresses from laying all their eggs (Raja et al., 2014).

Plant traits control whether foundress re-emergence is possible or not. In dioecious fig trees, allowing the foundress to re-emerge from male figs allows higher production of the next fig wasp generation (Moore et al., 2003). However, allowing re-emergence may result in the foundresses producing more male progeny and these do not give direct benefits to the plants (Anstett et al., 1998). In *F. deltoidea*, re-emergence gives benefits to both sexes of the plant. In the male trees, higher numbers of offspring are produced and some of these may carry pollen to female trees, and in female trees, re-emergence causes higher seed production. In *F. montana*, re-emergence rates increased with foundress density and this led to a more equal distribution of eggs/pollen across the figs (Moore et al., 2003).

The rates of emergence in this study were not significantly higher in female figs trees than male fig trees. Moore (2001) also found that rates of emergence were not dependent on plant sex. When foundresses enter female figs, they cannot lay eggs due to the long styles of the female flowers. This does not seem to make them more likely to re-emerge. The limitation of oviposition sites is one reason why fig wasps may re-emerge (Raja et al., 2008a) A foundress also wants to avoid losing all its offspring if something happens to one fig (fig abortion, parasitism and pathogens) so spreading the risk by laying in several figs is an advantage (Gibernau et al. 1996; Suleman et al., 2013b).

7.5.3 Brood size comparisons

Blastophaga sp., foundresses that re-emerged had more offspring than those that remained inside the first fig they entered. The total egg loads of fig wasps that enter a fig might be greater or smaller than the number of female flowers available for oviposition in monoecious or male figs (Nefdt and Compton, 1996). In *F. montana*, having more eggs than the number of female flowers in one fig promotes regular re-emergence (Ghana et al., 2012). The number of female flowers available for oviposition is highly variable in var. *angustifolia* male figs between 94 and 259 female flowers with a mean of 162.22 (Chapter 4) but most male figs of var. *angustifolia* have fewer female flowers than the egg loads of a single pollinator. Single foundresses were able to produce up to about 200 offspring if they were able to enter three or four figs to lay their eggs. This provides a selective advantage to re-emerge from male figs, especially as the figs may also have eggs laid by other foundresses so fewer sites will be available for oviposition. Female trees benefit because foundresses have the same behaviour in female figs (Raja et al., 2008b). Weaker females may be un-able to re-emerge and these individuals laid fewer eggs in the first figs they entered (Raja et al., 2014).

7.5.4 Seed production

In var. *angustifolia* the very small number of female flowers in female figs guaranteed extensive pollination even if entered by a single fig wasp. All the first figs entered were 100% pollinated. However, the second and subsequent figs entered figs did show a slightly lower number of seed produced. A study of female *F. montana* figs, where many more flowers are present than in var. *angustifolia*, showed there were slightly more overall seeds produced when foundresses re-emerged rather than staying in a single fig, with the first and second figs having equal numbers of seeds (Moore et al., 2003).

7.5.5 Sex ratios

Because brood sizes in the first figs entered were larger than in subsequent figs, there was an expectation that brood sizes in the first figs would be more female biased than in subsequent figs (Kjellberg et al., 2005a), if this species employs the male eggs first oviposition strategy as documented in *Kradibia tentacularis* (Suleman et al., 2013b). This was found not to be the case as most females had similar offspring sex ratios in the first and subsequent figs they entered. Having a higher proportion of female offspring is better from the plant’s perspective as more pollen can be dispersed. Some subsequently-entered figs had more male than female offspring. This was not present in any of the first figs that were entered. If these foundresses had shared a fig with another foundress that had laid mainly fertilised (female) eggs, then having many male offspring could be beneficial (Suleman et al., 2013b).

7.6 Conclusions

The foundress *Blastophaga* sp. routinely re-emerged from both sexes of the figs they entered. Re-emergence from male figs resulted in more overall offspring, though the first figs entered contained more offspring than subsequent figs. The effect of re-emergence from female figs was that more seeds were pollinated by each female. Re-emergence is favoured by the relatively small number of oviposition sites in male figs, which is often less than the egg loads of a single foundress.

Chapter 8

General discussion and Conclusions

8.1 Epiphytes in oil palm plantations

Oil palms housed a high number of epiphytes on their trunks. The epiphytes can partially mitigate the adverse effects caused by the conversion of natural habitat to oil palms, which generates a high degree of biodiversity loss (Foster et al. 2001). This is because epiphytes can provide habitats and food for other organisms such as insects (Suzanti et al., 2016). In Peninsular Malaysia, oil palms are often grown in areas that were once rubber plantations. The extent of epiphytes on both trees is strongly dependent on management intensity, but the architecture of the oil palm trunk provides very suitable host conditions for epiphytes (Sofiyanti, 2013).

Bryophytes, pteridophytes, lichens and angiosperms were all abundant epiphytes on the oil palms surveyed near Kuala Lumpur. Among the angiosperm epiphytes were *Ficus* species (Moraceae) that can exist as true epiphytes or hemiepiphytes. The epiphytic habit during juvenile stages is said to be an adaptation to avoid deep shade in the forest understory (Hao et al., 2013). Hemiepiphytic *Ficus* are strangler fig trees that later can give negative effects on the host plants by completion for light and even preventing growth by strangling. There are about 500 hemiepiphytic *Ficus* species that start life as epiphytes but become normal trees once their roots reach the ground (Hao et al., 2016). There are few truly epiphytic *Ficus* species that never have contact with the ground. Varieties of the evergreen shrub *F. deltoidea* can live as an epiphyte or as a tree that can reach up to seven meters tall (Zimisuhara et al., 2015). In this study, the three *F. deltoidea* complex epiphytes never had contact with the ground and can be said to be true epiphytes as described by (Corner, 1969). True epiphytes usually do not give any harm to the host plants (Zotz, 2013).

Ten *Ficus* species were identified in five different oil palm plantations in Peninsular Malaysia in this study. They included creepers, hemiepiphytes and epiphytes. Most of the species do not produce fruit until they reach considerable size, by which time they can kill the palm trees. Plantation managers are unlikely to ever let them grow sufficiently large. However, there are a few *Ficus* did produce fruit even when small. These figs (including figs of *F. deltoidea*) can provide food resources to frugivores that can disperse the seeds (Shanahan et al., 2001). As a result, the smaller *Ficus* species are more likely to contribute to biodiversity in oil palm plantations.

Ficus species in the different plantations were at different heights on the palm trunks, but different species did not occupy different heights on their hosts. The height of the trunks might be the factor that determines this. Different plantations have different ages and have different types of soils that can affect the height of the trunks. Older palm trees can cater for more epiphytes but management largely determines the abundance of the epiphytes and only some managers retain the epiphyte communities on the trunks without cutting them down. Many epiphytes including the fig trees appear to do no harm to palm oil production and management that encourages them should be encouraged (Prescott et al., 2015).

8.2 *Ficus deltoidea*

Ficus is a unique genus that has a closed inflorescence formed by an outer receptacle lined on the inside by tiny florets (a syconium). The closed inflorescence protects the floral structures from harsh environments and predators. Only specialised fig wasp pollinators (Agaonidae) can enter the figs. The pollinators make their way into the fig cavity through an ostiole and then gall, oviposit and pollinate the female flowers. *Ficus* and Agaonidae have been evolving together for a long time (van Noort, 2003). In this obligate mutualistic relationship the fig wasps provide pollen transfer and the fig wasps receive development sites for their offspring (Li et al., 2016). In dioecious fig trees such as *Ficus deltoidea* the nature of the relationship is modified. Female trees receive pollination services but they not only provide no reward, they prevent their pollinators from escaping to reproduce on other trees.

The group of fig trees comprising *Ficus deltoidea* and its relatives is one of the most interesting in the whole genus, displaying recent evolution, diversification and novel growth forms in and around the Sunda Shelf. The group can therefore be a model for patterns of speciation and co-diversification with fig wasp pollinators, as well as providing biogeographical insights from this hyper-diverse region. The chemistry of the group has also received considerable attention in recent years, due to their role in traditional medicine and economic potential.

Corner (1969) considered that the *F. deltoidea* group had originated in Borneo and then spread relatively recently across Sundaland. This is the region extending from Peninsular Malaysia to Borneo and Bali where shallow seas have meant that the changes in sea levels experienced during the Pleistocene resulted in the total land area varying greatly. Islands formed and fused during ice ages and become isolated or disappeared beneath the sea during warmer inter-glacials, such as that being experienced today. As a result of these sea level changes, plants and animals in the region have been periodically restricted to refugia and then allowed to expand their distributions when larger areas of land (roughly twice that present today) became exposed during the ice ages (Lohman et al., 2011). The recent spread of *F. deltoidea* has allowed them to diversify but whether they have diversified sufficiently to be considered as separate species is uncertain. Currently *F. deltoidea* is regarded as one species, with different varieties, but some of these occur in sympatry, which suggests they are distinct biological species.

It is likely that all the varieties of *F. deltoidea* are passively pollinated by their associated *Blastophaga* fig wasps. The absence of pollen pockets and coxal combs in their pollinators indicate that they passively pollinate the flowers. Passively pollinated *Ficus* produce many male flowers because pollen transfer is inefficient as their specific pollinators have a very limited pollen load in their pocket (Kjellberg et al., 2001). The ratio of anthers to ovules was high in all the varieties of *F. deltoidea* investigated, but was higher in some than others. *F. deltoidea* also possesses two traits of passively pollinated species as described by (Kjellberg et al., 2001). They have anthers that dehisce and project into the fig cavity so that as the female pollinators emerge from their galls they can be covered with pollen during their way out (Figure 8.1).

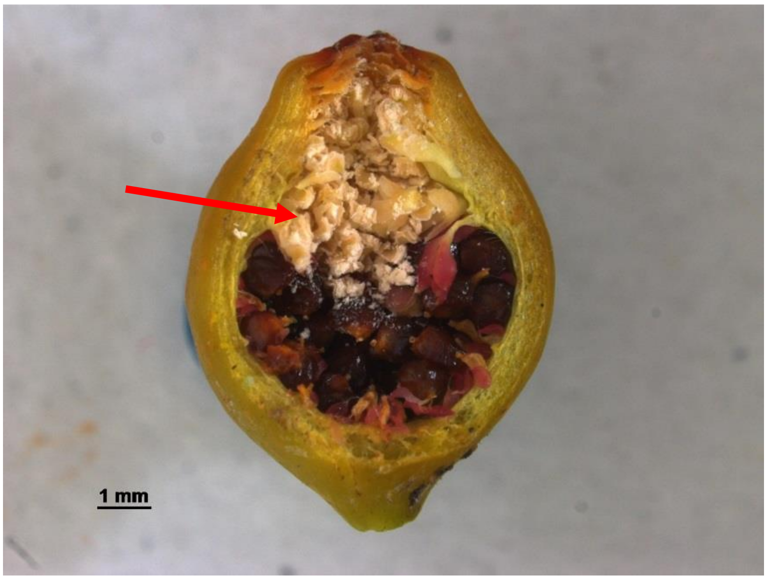


Figure 8.1 Dehiscing anthers projecting into the fig cavity in an early E phase male fig of *F. deltoidea* var. *angustifolia*. (Red arrow points to a dehiscing anther).

Ficus species are described as typically having from 30 to 1600 seeds per fig (Lansky et al., 2008). However, the varieties of *F. deltoidea* investigated had as low as 3 seeds in var. *angustifolia* and even one seed in another variety. Apart from having small numbers of seeds, seed size in *F. deltoidea* is also extremely large compared to other *Ficus*. The small numbers of flowers in each inflorescence allows for each seed to be large without increasing the overall size of the figs that contain them. The larger seeds than is normal for fig trees is a presumed adaptation for an epiphytic life style, as it means that young seedlings have more resources available initially (Kraft et al., 2015b). Larger seed size also can prolong the respiration process during carbon limited times by supplying more nutrient reserves (Westoby et al., 2002).

Seed sizes are also correlated with leaf toughness, and are suggested to form a syndrome associated with slow development in resource-limited environments (Kraft et al., 2015b). Having larger seeds can increase the chance of survival under extreme environments (Li et al., 2016). This trait might produce larger seedlings to reach deeper in soil pockets to absorb more water or higher into the air to have a better photosynthetic process, and avoid carbon deficit by having more metabolic resources (Westoby et al., 1996). Slow growth is a featured trait of *F. deltoidea* described by Berg and Corner (2005). An epiphyte usually undergoes slow growth to tolerate water deficits in the upper environment above the ground (Hao et al., 2016). A study by (Kraft et al., 2015a) showed that well-defended leaves are usually associated with a plant that has larger seeds and slow growth. *F. deltoidea* has all the traits typical of epiphytes which are small leaves, slow growth and large seeds (Hao et al., 2016).

Seeds and seedlings of the hundreds of species of hemiepiphytic fig trees face similar conditions to those of epiphytic *F. deltoidea*, but unusually large seeds have not developed in these lineages (Hao et al., 2016). The large seeds of *F. deltoidea* may therefore have evolved under different selection pressures (and within the *F. deltoidea* complex, some varieties are not epiphytic, or only facultatively epiphytic). The large seed of *F. deltoidea* was said to be the ancestral condition and the small seeds of other *Ficus* a derived condition (Corner, 1969). However, this may be unlikely because monoecious fig trees have probably the original breeding system in the genus (Harrison and Yamamura, 2003). There is no evidence that pollinators were ever much larger in the past (Compton et al., 2010), so seed and pollinator gall sizes would need to be much different in shared figs. Figs containing large seeds and many pollinators would have had to be larger than those seen today.

Large red tepals are a feature of subsection *Frutescentiae*, which includes the *F. deltoidea* group and species belonging to Series Podosyceae (Berg and Corner, 2005). Varieties of *F. deltoidea* all possess large tepals, with four tepals surrounding each ovule (sometimes 3-5 tepals). The size and colour varies between sexes. The female figs of all varieties tended to have larger tepals than the male figs. The larger size in females might reflect that there are fewer flowers, so each tepal needs to be bigger, but the function of the tepals is unknown. Their prominent appearance and colour is hidden inside enclosed, dark figs, so they are unlikely to have a role in photosynthesis or be visible to pollinators.

The tepals in *F. deltoidea* figs were largest during receptivity, before shrinking post pollination. This suggests that they might have a role in pollinator attraction. Other possibilities include an anti-microbial function to deal with microbes brought in to the figs by the pollinators or to help position female pollinators above the flowers to aid pollen transfer on to the stigmas (Hong et al., 1998). *Ficus awkeotsang* Makino is called the jelly fig because of the large amounts of gel that is produced inside the figs. This species also has red tepals and biochemical studies have shown that they contain pectinesterases involved in gel formation (Komae and Misaki, 1993). The seeds of *F. deltoidea* var. *angustifolia* have a thin layer of gel around them that seems to inhibit mould formation (S.G. Compton, personal communication), which supports the idea of an antimicrobial function for the large tepals in this species.

In nursery pollination mutualisms and especially the fig wasp-fig tree mutualism, timing is everything. The emergence of short-lived adult fig wasps should be at the same time as the receptivity of other figs. In seasonal environments, some male dioecious fig trees release most pollinators at times of the year when the most female figs are receptive (Patel, 1996). In contrast, Malaysia is a country close to the equator with little seasonal variation in climate. *F. deltoidea* produced new figs and new leaves throughout the year and individual plants often had figs at all stages of development. This asynchronous development can avoid the local extinction of the fig wasp (van Noort, 2003) and can provide food resource to frugivores all the time (Shanahan et al., 2001).

Flowering phenology may have a strong effect on the extent of intersexual chemical mimicry because good quality mimicry is believed to be more important in species where receptive male and female figs are available at the same time and the fig wasps can make a choice (Hossaert-McKey et al., 2016). However, this study showed that the fig wasps associated with var. *angustifolia* are more likely to enter figs on male plants. This suggests they were able to distinguish between the sexes of their host, and that inter-sexual mimicry is not very effective in this species.

Previously, it was believed that once a foundress fig wasp enters a fig she will always be trapped inside. More recent studies have showed many fig wasps are able to re-emerge after pollination and oviposition (Moore et al., 2003) and the foundresses can enter other figs after walking between them (Suleman et al., 2013b). The tightness of the ostiole can be seen as being beyond the control of fig trees, with pollinator release an accidental consequence (Gibernau et al., 1996) or as an adaptation by the plants to allow foundresses to pollinate several figs rather than just one. There are very few flowers in female figs of *F. deltoidea* and it would not be efficient use of pollinators if they were trapped in the first fig they entered and could only pollinate one fig. This study showed that *Blastophaga* sp. routinely re-emerge from male and female figs of var. *angustifolia* after pollination and oviposition. The life span of adult female *Blastophaga* sp. was not measured in this study but based on personal observations, their active behaviour lasts only for a few hours. This limited time requires each foundress to choose a fig to enter quickly and means that only adjacent figs can be entered after they re-emerge. The figs of var. *angustifolia* are produced quite close together which makes it easier for foundresses to walk between them.

Suleman et al. (2013b) said that re-emergence is likely to be due to a lack of oviposition sites inside male figs together with the added benefit of spreading the risk of offspring deaths by having offspring located in more than one fig. Inflorescence design in var. *angustifolia* helps to stimulate foundress re-emergence because many of the male figs have fewer female flowers than the number of eggs in the female fig wasps. Females that re-emerged also produced more offspring. Similar results have been found in other pollinators that can re-emerge (Gibernau et al., 1996; Moore et al., 2003). The sex ratio is female biased in *F. deltoidea* and this event benefited the plants as more pollen can be dispersed. However, this ratio changed in the first and later figs that were entered. The size of the clutches might have affected the sex ratio of the pollinator offspring (Kjellberg et al., 2005a). Because male eggs in *Blastophaga* may be laid first, as in some other species (Raja et al., 2008b), a bigger clutch will produce a smaller proportion of male offspring.

8.3 Is *Ficus deltoidea* a complex of different biological species?

Species boundaries are maintained by limiting gene flow between taxa. In flowering plants pollinator behaviour can strongly influence gene flow, especially if the pollinators are host specific (Sedeek et al., 2014). Floral scents emitted by receptive flowers act as cues to influence pollinator behaviour (Souto-Vilaros et al., 2018) and in the nursery pollination mutualism between fig trees and fig wasps, long distance detection of suitable host figs is mediated by the scent of receptive figs (Hossaert-McKey et al., 2010). Apart from the scent cues, pre-pollination filters that reduce the chance of entry by non-adapted pollinators include non-volatile cuticular cues, ostiole size and shape, and floral arrangement within the figs (Borges, 2016). For the fig wasps to be able to reproduce the style lengths in male figs must also not be longer than the ovipositors of the foundress (Weiblen 2004; Kjellberg et al., 2005b). The foundress must also be able to gall the ovule to allow their offspring to develop (Ghana et al., 2015a). Divergence in volatile signals between figs could represent an initial isolating mechanism between individuals which is later reinforced by pollinator behaviour and morphological co-adaptation with their hosts (Soutos-Vilaros et al., 2018).

One of the outcomes of pollinator sharing is the production of hybrid plants and increasing numbers of *Ficus* hybrids are being recorded (Wang et al., 2016). Introgession between *F. auriculata* Lour. and *F. oligodon* Miq. in China is facilitated by similar volatiles emitted by their receptive figs (Wang et al., 2016). The attractant volatiles released by *F. deltoidea* varieties have not been compared. The varieties vary in the size of their figs and the numbers of flowers they contain, but the lengths of the styles in male figs are rather similar and it seems likely that pollinators from one variety could oviposit in the ovules of other varieties, if they enter the figs. Our behavioural experiments showed that the fig wasp (*Blastophaga* sp.) only enters their natal fig species (var. *angustifolia*) if that species is present. In the absence of the natal fig trees, small numbers of this pollinator will enter figs of var. *deltoidea* and can induce the gall formation, but if eggs had been laid in these figs, then the larvae that emerged failed to develop until maturity. Limited reciprocal experiments with the pollinators reared from var. *deltoidea* suggest that these were also unwilling to enter receptive figs of var. *angustifolia*.

These results can explain the field observations at Batu Pahat, where epiphytic var. *angustifolia* and var. *deltoidea* grow closely together and sometimes on the same host trees. However, no plants with intermediate features are present and gene flow between them seems to be absent. Two other different varieties (var. *kunstleri* and var. *trengganuensis*) were also planted in Batu Pahat outside of their natural ranges. No sign of pollination was observed, suggesting that these varieties are also avoided by the pollinators of var. *deltoidea* and var. *angustifolia*, but the unwillingness of the pollinators to travel far could also explain their absence. The two varieties var. *deltoidea* and var. *angustifolia* have different leaves and figs so other features as well as attractant volatiles may provide cues to their pollinators.

The two varieties remain distinct even when in sympatry and they have pollinators with behaviour that prevents gene flow. This suggests that they represent two different biological species. Some at least of the many other varieties of *F. deltoidea* probably are also likely to be distinct biological species. Molecular evidence supports this conclusion (S. Segar personal communication) and different species of pollinator fig wasps have been reared from different varieties of *F. deltoidea* in Brunei (F. Kjellberg, personal communication to S.G. Compton, 2016).

Most studies fail to find a sexual preference, but the experiments showed that *Blastophaga* sp. preferred to enter male figs. It also seems unwilling to leave its natal host unless the host has no receptive figs present. This clearly benefits individual pollinators because they are more likely to survive and reproduce, but is not so good for the plant.

8.4 Conclusions

To our knowledge, this is the first biological study of *Ficus* in oil palm plantations. In Peninsular Malaysia *F. deltoidea* was the only true epiphyte. There were three varieties of *F. deltoidea* recorded as epiphytes in this study (var. *angustifolia*, var. *deltoidea* and var. *trengganuensis*). There are morphological differences between the figs of these and other varieties. In Batu Pahat plantation, two of the varieties live sympatrically. The absence of hybrids between var. *angustifolia* and var. *deltoidea* in Batu Pahat plantation suggests that there is a barrier to gene flow between them. The limited dispersal of the pollinators away from natal trees may make gene flow less likely and experiments showed that pollinator behaviour is normally preventing gene flow between these varieties. The fig trees are likely to produce volatiles that only attract their own specific fig wasps to pollinate them. The overall conclusion is that these two varieties of *F. deltoidea* are distinct species.

As a further conclusion, we might say that the specificity between fig trees and fig wasps in the many different *F. deltoidea* varieties is likely to be high. No evidence of gene flow or intermediate forms were found when two varieties were in sympatry and they remained distinct. The implication from this study is that all or most of the varieties might be different species. Previously all the varieties under *F. deltoidea* were defined based on morphological characters only. However, according to the biological species concept, they are said to be a same species if they interbreed with each other when living together. There are other examples of what were considered to be a single *Ficus* species being more than one. For example *Ficus insipida* Willd. in Mexico and *Ficus inspida* in Panama were described as the same species but they are now understood to be totally different species (Su et al., 2008). The major implication from this study is the taxonomists may have underestimated the number of *Ficus* species.

8.5 Future work

Future work should include study of the composition of the volatile organic compounds released by receptive figs of all varieties of *F. deltoidea* to find if they produce similar blends or not. Male and female plants need to be examined separately as the pollinators of var. *angustifolia* seem to prefer male figs, which suggests that their volatiles are not identical. Molecular population genetics can be used to see if gene flow is occurring between varieties and to establish a phylogeny. Detailed morphological and molecular examinations of the fig wasps should be conducted to determine whether they are different species or not. Previously, the classification of *F. deltoidea* varieties was based solely on plant morphology. These studies can give additional information to update the classification of this complex and explore their evolution.

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