Fig Trees and Fig Wasps: Their Interactions with Non-mutualists

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The candidate confirms that the work submitted is her own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriates credit has been given within the thesis where reference has been made to the work of others.

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“Praise be to Allah, the Lord of the worlds.”

Abstract

Non-mutualist species that interact with mutualists occur commonly in nature. Non-mutualists associated with the mutualism between fig trees and their pollinating fig wasps include mites and nematodes. This thesis focusses on the interaction of nematodes with fig trees and fig wasps in South Africa and Indonesia, with additional investigation on how mites, nematodes and fig pollination respond to highly seasonal environments. The reproduction of monoecious *Ficus burtt-davyi* in Grahamstown, South Africa slows down but does not stop in winter. There are fewer fig pollinating wasps *Elisabethiella baijnathi* flying in the air during winter, but most figs are probably pollinated by locally-produced fig wasps. *Elisabethiella g5baijnathi* females transport mites (*Tarsonemella* sp. nr. *africanus*) and nematodes (*Parasitodiplogaster* sp.) between figs. Contrasting dispersion patterns and relationships with fig wasp foundress numbers indicate that the mites, but not the nematodes, disperse between figs after being carried there by the pollinators. Three nematode species *(Caenorhabditis sp.*, *Schistonchus* centerae, and S. *guangzhouensis*) developed inside both male and female figs of dioecious *F. hispida* tree in Sumatra, Indonesia. *Caenorhabditis* sp. was transferred between figs as juveniles, whereas *Schistonchus* spp. were transferred mostly as juveniles, and occasionally as adults. The nematode community of eight species in Sumatran monoecious *F.racemosa* was the most diverse recorded anywhere. No mites were found in both species of fig trees. Peak nematode populations occurred in D-phase figs, when the fig wasp offspring are ready to emerge. The nematodes attach themselves to newly-emerged female pollinators, which then carry them away. Usually more nematodes attached on the first fig wasps to emerge. Entry into figs by early-emerging pollinators resulted in higher numbers of the next generations of nematodes within the figs. Details of the ecology of each nematode species may be different, but as a group they did not seem to significantly affect seed and wasp development in both *F. hispida* and *F. racemosa figs*.

Assistance provided

I am thankful for assistance that I had during the course of my PhD. Data in Chapter 2 and Chapter 3 in this thesis were collected by Dr Stephen Compton in Grahamstown, South Africa. There were 1967 young figs of *Ficus burtt-davyi* collected from 146 crops and 99 trees during October 1987 until February 1990. The data were presented to me as raw data in spread sheets in which I started working from. I thank Eka Putra for climbing up several trees when I needed to introduce pollinating fig wasps into receptive figs and collected them in later phase for data collection in chapter 6.

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Chapter 1 General Introduction

1.1 Interactions among species: mutualisms and exploitation of mutualisms

Positive mutual interactions between individuals belonging to different species, termed as mutualism, occur commonly in nature but vary widely in levels of specificity (Dufay and Anstett 2003; Foster and Wenseleers 2006; Ezoe 2012). Different species of diverse taxa of a wide range of ecological types may be involved in these associations that exchange resources and services (Leigh 2010). Mutualisms could include symbiotic interactions when two species are physically connected and non-symbiotic ones when two species are physically unconnected or contact may be brief (Thompson 1994). They are involved in a variety of ecosystems as indispensable elements, e.g. mutualisms between flowering plants and pollinators, plants and seed dispersers, plants and ectomycorrhizal fungi, legumes and rhizobia, reef-building corals and dinoflagellates, etc. These all are important components that maintain global biodiversity. Furthermore, mutualisms are supposed to have played key roles in some major evolutionary transitions in the history of life (Sachs and Simms 2006). Obligatory mutualistic relationships between plants and their pollinators such as yucca plants and their pollinator yucca moth, and fig trees and their pollinating wasps, include species that depend totally on each other for reproduction. These relationships often represent an example of coadaptation and coevolution between pairs of species (Lunau 2004).

Mutualisms may act as reciprocal exploitations, where each partner is selected to increase its own benefits and reduce the costs of the interaction (Bronstein 1994; Anstett, HossaertMcKey and Kjellberg 1997a). In other words, there may be conflict between mutualistic partners because the fitness of one partner often increases by reducing the fitness of the other (Dufay and Anstett 2003)

The associations between mutualist species often involve several other species that exploit this mutualism. These non-mutualist species (sometime referred to as exploiters or cheaters) utilize available sources but may not benefit either mutualist partner. The presence of such species of non-mutualists probably influences the net benefits within many mutualisms (Heithaus, Culver and Beattie 1980; Bronstein 2001b; Bronstein 2001a). Individuals which gain the benefits in a mutualistic system without paying the cost of providing service to the mutualists are expected to have higher fitness than the mutualist partners. To ensure the long-term stability of mutualism, it is likely that a mechanism that limits cheating or overexploiting exists in many mutualistic systems (Jander and Herre 2010).

The non-mutualist species may negatively affect one or both mutualism partners, even though their effects have not often been quantified (Bronstein 1994; Segar and Cook 2012). Depending on the circumstances, the presence of the non-mutualize species may also stabilize or destabilize the mutualisms (Dunn et al. 2008a; Segar and Cook 2012). Despite the clear exploitation by non-mutualist on the mutualisms, very few of mutualist-exploiter interactions have been studied in detail (Cardona, De Ulloa and Kattan 2007; Bronstein 1991; Segar and Cook 2012).

The interaction between fig trees (Moraceae, *Ficus*) and fig wasps (Hymenoptera, Agaonidae) is a well-known example of mutualism. This mutualism has evolved over 60 million years (Ronsted et al. 2005; Compton et al. 2010), and has been well suited for studying the evolution and stability of mutualisms (Jander and Herre 2010). Fig trees depend on certain fig wasps for pollination, thus seed production, and also transmission of their pollen. On the other hand, the wasps depend on figs for the development and reproduction of their offspring (Janzen 1979a; Compton 1993; Weiblen 2002; Herre, Jander and Machado 2008). Figs are a rich resource for pollinating and non-pollinating fig wasps, mites, nematodes, and others that live within the figs, also the communities of frugivorous vertebrates and invertebrates that eat ripe figs (Compton 1993; Shanahan et al. 2001; Harrison 2005; Krishnan et al. 2010; Jauharlina et al. 2012).

1.2 Biology of fig trees and their pollinating wasps

Ficus is one of the most diverse genera of flowering plants with over 800 species distributed in tropical and subtropical regions. They vary in size from small shrubs to huge rainforest trees. Many fig trees are free standing and develop from seeds left on rocks or the soil. Some other trees grow from seeds deposited on other trees, and ultimately destroy and replace their hosts. These fig trees are called ‘strangler figs’ (Compton, Wiebes and Berg 1996; Herre et al. 1996).

Despite the differences among fig tree species, all fig trees have a unique form of inflorescence, which is called a fig or syconium. The fig comprises hundreds or thousands of tiny flowers distributed on the inside of the outer wall (Berg 1989; Anstett, HossaertMcKey and Kjellberg 1997a). Ecologically, this structure is a fruit, and the cavity inside the fig is called a pseudolocule (Janzen 1979a). The size of figs varies greatly, depending on the tree species and they can range in diameter from a few millimetres up to 10 cm or more (Compton, Wiebes and Berg 1996). A fig tree can produce crops of between one and 1,000,000 figs that are found on the leaf axils on the stems or trunk (Janzen 1979a).

About half of described fig trees species are monoecious, while others are morphologically gynodioecious but functionally dioecious (Berg 1989; Verkerke 1989; Berg and Corner 2005). In monoecious fig trees, individual figs have both male and female flowers that can produce viable seeds as well as pollen and fig wasp offspring that transport the pollen. In gynodioecious fig trees, individual trees produce either viable seeds (seed figs or female figs) or pollen (gall figs or male figs) (Janzen 1979b; Verkerke 1989; Chen et al. 2013).

Pollination within the figs, both in monoecious and gynodioecious trees, totally depends on female fig wasps. These pollinating fig wasps belong to a single family, Agaonidae. They all have relatively similar life cycles (Kerdelhue, Rossi and Rasplus 2000; Segar and Cook 2012). The female wasps (foundresses) enter the figs through a narrow bract-lined opening called the ostiole, when the figs are ready to be pollinated (receptive). In monoecious figs, when inside the figs, the foundresses will lay eggs in some of the ovules of female flowers, and pollinate others. This is the only way that the pollen carried by the wasps can reach the female flowers for pollination. Each fig then produces wasps and seeds (Peng, Compton and Yang 2010). In some species, pollination by the fig wasps results in higher larval survivorship when developing in pollinated flowers. This is the benefit gained for the wasps by pollinating the figs, and this is why many fig wasps actively ensure pollination occurs in the flowers they oviposit. When figs are occupied by the wasps without carrying pollen (no pollination occurs), larval mortalities may increase and the figs can be more likely to abort from the tree (Tarachai, Compton and Trisonthi 2008).

The life cycle of the fig wasps precisely coincides with the developmental cycle of the figs. The figs are only suitable for the female pollinating wasps when the figs reach the female stage (receptive figs) (Compton 1993; Hu et al. 2009). The stage-specific changes in the volatile cues of the plants involve the production of additional plant volatile compounds that attract the wasps to visit at this time (van Noort, Ware and Compton 1989; Ware and Compton 1994). These semiochemicals attract only the specific fig wasp pollinators to the figs (Ware et al. 1993; Zhang et al. 2012). In all figs, the pistillate (female) flowers are receptive to pollination by female wasps several weeks before the staminate (male) flowers produce pollen (Giblin-Davis et al. 1995). The length of the receptive period for the figs to be attractive to the female pollinating wasps (foundresses) lasts for several days, and is quite short compared with the other stages during fig development (Bronstein and HossaertMcKey 1996; Zhang et al. 2012). This duration, however, could be prolonged in individual figs when they are still unpollinated (Zhang et al. 2012)

Female pollinating wasps are highly modified to enter the ostiole (van Noort and Compton 1996). The wasps also show sexual dimorphism that is related to the different activities of males and females. Female pollinators have functional wings and eyes, and are responsible for seeking and colonizing new figs. In contrast, males do not have wings, and have only vestigial eyes and short antennae. Their participation in the life cycle is mainly limited to the cavity of the fig fruits (Weiblen 2002).

There are over twenty recognized genera of pollinating fig wasps (Wiebes 1981; Cruaud et al. 2009). Each fig tree species was thought to be pollinated by a fig-specific wasp species; however it has been shown that many fig tree species are pollinated by two or more wasp species (Berg 1989; Wiebes 1979; Compton, Grehan and van Noort 2009). The female, pollen-carrying wasps enter the fig, pollinate, and lay eggs in the ovaries of female flowers in the figs. The foundress female wasps are generally assumed to die within the fig, however this is not always the case (Gibernau et al. 1996).

The larvae of the pollinator feed on some of the female flowers that have been pollinated. Each larva develops at the expense of a single galled ovary (Anstett, HossaertMcKey and Kjellberg 1997a), thus (in monoecious figs) damages some of the flowers that might otherwise have produced seeds (Chen et al. 2013) . When the fig seeds and anthers are mature, so are adult wasps. The male wasps emerge first and chew their way out of their galls. The males then chew a hole into the adult female's galls and proceed to fertilize the females. When the female wasps exit the galls, they either actively collect pollen, or they simply are coated with pollen as they pass through the male flowers (Kerdelhue, Rossi and Rasplus 2000). The females exit the fig through a hole dug by the males in the fig wall, and search for new receptive figs to enter and to start the life cycle all over again (Weiblen 2002). It has been reported that female pollinating wasps are able to disperse over large distances of more than 160 km (Ahmed et al. 2009), and some use fast-moving wind above the canopy of the forest (Compton et al. 2000; Compton et al. 2005). Seeds in the fig, after the adult wasps leave, are dispersed by vertebrates including birds and mammals (Shanahan et al. 2001).

In gynodioecious fig tree species, the female wasps that enter the figs of a female tree ensure pollination and seed production. However, due to the structure of the flowers, they cannot lay eggs and die without reproducing. On the other hand, when the wasps enter the figs of male trees, they will lay eggs and complete their life cycle within the female flowers. The flowers do not produce seeds even if they have no eggs laid in them. Thus, some gynodioecious fig trees are female and produce seeds, while some other trees are male and produce pollen and pollen-carrying wasps (Anstett, HossaertMcKey and Kjellberg 1997a; Berg and Corner 2005; Compton et al. 2010).

Fig trees and their pollinating wasps represent probably the most extremely co-specific pollination system known. The relationship between fig trees and fig wasps has been studied increasingly, particularly to help understanding of coevolution and mutualism (Janzen 1979a; Weiblen 2002; Ronsted et al. 2005; Compton et al. 2010). A wide range of tropical vertebrate frugivores, including many species of birds, bats, and monkeys, depend on ripe figs to eat. Therefore, the population size of these animals also depends on the pollination of the figs by the wasps (Shanahan et al. 2001; Weiblen 2002; Herre, Jander and Machado 2008). Fig trees are often considered as a 'keystone' group in tropical forests. Their often all-year-round production of fig fruits potentially maintains frugivore populations, particularly, at times of the year when other fruits are not easily available (Shanahan et al. 2001; Harrison 2005).

Fig trees are known to have figs on the trees throughout the year, however their phenology is affected by climatic conditions in the area where they are growing. In seasonal areas, the different climates in different seasons affect the production of figs, thus also affect the development of seeds and wasps offspring within the fig (Janzen 1979a; Wang, Yang and Yang 2005). Dry and cold seasons are known to reduce fig fruiting frequency (Janzen 1979a; Compton 1993). In Northen Australia, leaf production in *Ficus variegata* is related to seasonal rainfall patterns (Spencer, Weiblen and Flick 1996). It has been recorded that the development time of *F. racemosa* figs in the dry season (winter) is much longer than in the (warmer) rainy season in Yunnan, China (Wang and Sun 2009).

1.3 Non-pollinating fig wasps

In addition to pollinating wasps, a diverse assemblage of non-pollinating wasps also develops within the fig fruits. They provide no obvious benefit to the figs. Their presence within the figs is generally viewed as harmful to one or both mutualist partners (West and Herre 1994; Bronstein 1994; Segar and Cook 2012). These non-pollinating fig wasps (NPFW) include gallers, inquilines, cleptoparasites, parasitoids of pollinators, and parasitoids of other non-pollinating wasp species (Bronstein 1991; Kerdelhue, Rossi and Rasplus 2000; Chen et al. 2013).

The non-pollinating wasps tend to also be specific to a single fig species (Cook and Segar 2010), at least in the African figs (Jousselin et al. 2008). On the other hand, data collected in Asia (Weiblen and Bush 2002) and in Panama (Marussich and Machado 2007) show that NPFWs can be less specific to the host fig than pollinators. Regardless of these differences, generally there are more than one species of non-pollinators inhabiting a single fig tree species (West et al. 1996; Cook and Segar 2010; Segar and Cook 2012). These non-pollinating wasps belong to several Families (Eurytomidae, Ormyridae) and Sub-families, i.e. Sycophaginae, Otitesellinae, Sycoecinae, and Epichrysomallinae (Kerdelhue, Rossi and Rasplus 2000) from the super family of Chalcidoidea (Segar and Cook 2012). These subfamilies of NPFWs are mostly placed in the family Pteromalidae (Heraty et al. 2013).

The effect of different species of non-pollinating wasps on the reproductive success of their hosts depends on their larval diet. Species that are competitors or parasitoids of the pollinators have a direct effect on the reproductive success of the pollinating wasps and the host figs, by reducing the fig's ability to disperse pollen (West and Herre 1994). The non-pollinating species that only form galls on the fig wall or unoccupied ovaries may have a less apparent cost to their hosts (West et al. 1996). Recent studies have shown that *Philotrypesis taiwanensis* (Pteromalidae) a NPFW species associated with *F. microcarpa,* is a seed predatory species. The presence of this NPFW species within the figs significantly reduces numbers of seeds produced, but has no negative impact on pollinators offspring numbers (Rong et al. 2014 in press).

Each particular species of NPFW lays its eggs at a specific stage of fig maturation. However, all non-pollinating wasps typically emerge from the gall in the fig cavity at the same time as the pollinating wasps. The winged individual adult females of most species exit through a hole chewed by the male pollinating wasp to escape the fig (Kerdelhue, Rossi and Rasplus 2000), but some NPFW species have males that chew their own exit holes (Suleman, Raja and Compton 2012)

The NPFW species mostly oviposit from the surface of figs. These non-pollinators exhibit long to extra-long ovipositors compared to their head or bodies (Kerdelhue and Rasplus 1996). Some of the non-pollinating wasps have similar body size to the pollinators. These non-pollinators compete with the pollinators over the individual flowers that provide a resource for larval development. This competition usually reduces the number of pollinator offspring (West and Herre 1994; West et al. 1996; Segar and Cook 2012) and may influence the pollinator sex ratios (Pereira and do Prado 2005). Another group of non-pollinating wasps that are relatively larger in size induce the formation of large galls in which the larvae develop and seem to prevent unpollinated figs from being aborted. These wasps reduce the number of pollinator offspring as well as seed production, since they use up the resources from the fig (West et al. 1996) and may prevent pollinator entry. The true parasitoids of the pollinators or the other gall formers are the last group of non-pollinating to lay eggs, from the outside through the walls of the fig (West and Herre 1994; West et al. 1996). Although the majority of non-pollinating wasps lay eggs from outside of the fig, a few species manage to crawl through the ostiole and lay eggs inside the fig (Compton and van Noort 1992). *Diaziella yangi* (Sycoecinae) and *Lipothymus* sp. (Otitesellinae) are two NPFWs that enter the figs of *F. curtipes* through the ostiole to lay eggs (Chen et al. 2013).

1.4 Other insects and invertebrates occupying the figs

A diverse group of other insects and invertebrates are also found in the figs, such as weevils (*Omophorus*, Coleoptera: Curculionidae) and pyralid moths (Lepidoptera: Pyralidae). These two insects can remove all other occupants within the figs, leaving the figs empty inside. The larvae of Drosophilidae and Phoridae (Diptera) may also be present (Compton 1993).

The pollinating wasps sometimes act as vectors for phoretic mites, such as *Tarsonemella* sp (Acari: Tarsonemidae) on *F. burtt-davyi* (Compton 1993; Jauharlina et al. 2012). Other genera of mites associated with fig wasps are *Paratarsonemella* found in Ghana, *Ficotarsonemus* found in Taiwan (Ho 1994; Lindquist 1986), and *Paratarsonemella giblindavisi* which was found in the figs of *Ficus virens* in Australia (Walter 2000). A fig mite species was also recorded on the females of *Pegoscapus* sp, the pollinating-wasp of *Ficus eximia* Schott in Brazil. These mites were attached to the lower part of the thorax of female pollinators. When the pollinators enter the fig through the ostiole, the mites fall off in the bract of the ostiole and reproduce in the figs. When the newly emerged female pollinating wasps leave the fig through the exit hole, the mites attach themselves to the pollinators and are brought away to other figs when pollinators enter other receptive figs. All of the mites mentioned above belong to the tribe Tarsonemellini. The associations between these mites, fig wasps, and figs was not discovered until recently, however, considering that there are many fig tree species the diversity of fig mites is potentially high (Walter 2000).

1.5 Fig trees and nematodes

Associations of several nematode species with fig wasps have been reported in different places. The nematodes develop and reproduce within the figs, they are then transported internally by the pollinator offspring to other receptive figs (Martin, Owen and Way 1973; Giblin-Davis et al. 1995; Kanzaki et al. 2009; Kanzaki et al. 2012a;

Davies et al. 2013). High numbers of nematodes, up to 50,000, has been recorded from one fig (Martin, Owen and Way 1973). The nematode *Schistonchus caprifici* is able to complete its development and reproduces on caprifig (*Ficus carica sylvestris)* and also in the pollinating wasp *Blastophaga psenes.* A histological study indicated that *S. caprifici* is a parasite of the plant and may result in premature inflorescence decay. The flowers infested by the nematode show necrosis but there is no evidence of any adverse effects on the pollinating wasps (Vovlas, Inserra and Greco 1992). *Schistonchus caprifici* is transferred from infected to healthy caprifigs during oviposition of the pollinator wasp *B. psenes*. This nematode is carried in the form of eggs, juveniles, and adults in the haemocoel of female pollinating wasps. The nematodes colonize and start reproducing in the haemocoel of the pollinator larvae, and persist in pupae and female adult pollinators. There are no morphological differences between nematode populations that develop in the haemocoel of the pollinating wasps and in the caprifig flowers (Vovlas, Inserra and Greco 1992). The same species of nematode associated with *B. psenes* was also recorded later on in *F. carica* in Turkey (Gulcu et al. 2008). Another species of nematode, *S. racemosa* Reddy & Rao, is associated with the pollinating wasp *Ceratosolen* sp. which pollinates *Ficus racemosa* L. in India. The nematodes are carried as second stage juveniles, however the association between the nematode and the fig was not examined (Reddy and Rao 1986).

Female wasps *Elizabethiella baijnathi*, the pollinator of *F. burtt-davyi* has been recorded to carry the nematode *Parasitodiplogaster* sp (Diplogasteridae) internally. These nematodes feed on their decaying remains (Compton 1993; Jauharlina et al. 2012). *Schistonchus* spp. are commonly found associated with pollinating fig wasps and transferred into the figs of different species of fig trees in many different places. It is also known that more than ones species of *Schistonchus* nematode can occupy the same figs (Vovlas and Larizza 1996; Vovlas et al. 1998; Center et al. 1999; Zeng, Giblin-Davis and Ye 2007; Davies et al. 2010; Zeng et al. 2011a; Davies et al. 2013)

The above nematodes are associated with the unique habitat within the figs where they develop and feed. They have complicated life cycles and rely only on fig wasps for dispersal between figs. These biological facts make these nematodes an interesting example to study biodiversity considering that there are over 800 species fig trees worldwide (Berg 1989). The interactions among the fig tree, fig wasp, and nematodes is a suitable system to investigate the evolution and maintenance of host specificity, especially the phenomenon of phoresy by the nematodes (Krishnan et al. 2010).

1.6 Objectives and outline of thesis

The studies in this thesis were mainly aimed at investigating the biology and impacts of non-mutualist species on the interaction between two mutualist species and how non-mutualist species interact. The system studied was fig trees and their pollinating wasps, adding mites and nematodes as the non-mutualist species that interact with the mutualist partners. Not all fig trees are tropical, and mutualistic interactions are likely to vary over time in strongly seasonal environments. A study of fig tree mutualism in a seasonal environment is also therefore part of the thesis. The questions addressed include: how does seasonal variation in temperature affect pollination, how do mites and nematodes use fig wasps as vectors, what are the life cycles of fig nematodes, what is their impact on fig wasp and seed production in monoecious and dioecious fig trees and can nematodes develop in female figs?. The outline of the thesis is as follows:

Chapter 1. This chapter describes basic information and provides references related to mutualisms and how other species exploit the mutualism

Chapter 2. This chapter investigates how a fig tree population *(Ficus burtt-davyi*) responds to seasonal variation in temperature in terms of fruiting frequency, crop size, and crop duration. Also the consequences of this seasonal variation for the numbers of fig wasp offspring released and the numbers flying in the air, and whether an apparently isolated population of pollinating wasps was truly independent.

Chapter 3. This chapter focuses on how mites and nematodes use fig wasps as vectors to disperse between figs. The biology of a mite (*Tarsonemella* sp. indesc. nr. *T. africanus* Hirst., Tarsonemidae) and a nematode (*Parasitodiplogaster* sp. indesc., Diplogastridae) associated with *F. burtt-davyi* and its pollinator (*Elisabethiella baijnathi*) is described. Their prevalence within crops is also investigated, and whether their prevalence is influenced by the size of a fig crop, and how their life cycles are different from each other.

Chapter 4. It has been known for years that nematodes are transferred by fig wasps, and develop and reproduce with the figs of several fig tree species in many countries. In dioecious figs, the study of nematodes was only focussed on male figs. In this chapter, several questions are addressed in relation to the nematodes associated with a dioecious fig tree, *F. hispida*, in Indonesia, both in male and female figs. How many nematode species are present locally, and how does their abundance vary? What are their life cycles and can they develop inside female figs?

Chapter 5. This chapter investigates the nematode species associated with the monoecious figs of *F. racemosa* and their relationship with the fig tree and its pollinating wasp. This chapter addresses the following questions. How many nematode species are present in figs of *F. racemosa*, in Sumatra and how does their abundance vary? How does fig wasp foundress number affect the number of nematodes within a fig? What is the impact of nematodes on seed and pollinator production? What is the structure of the broader fig wasp community? Do the nematodes only seek out female pollinators for transmission between figs?

Chapter 6. In this chapter I test the hypothesis female fig wasps that emerge at different sequence from figs may carry different number of nematodes. Additional experimental work is done to investigate whether position of the emergence sequence of foundresses introduced into receptive figs affect the number of new generation of nematodes and the nematode community in the figs.

Chapter 7. This chapter summarizes the important findings from the whole study and discusses the broader significance of the results.

1.7 Study species and sites

The first two of result chapters of this thesis are based on a field study of the monoecious figs of *F. burrt-davyi* database which were collected earlier by Dr Stephen Compton in Grahamstown, South Africa. The data were presented to me as raw data in spreadsheets. The other three results chapters of the thesis consist of field studies on the dioecious figs of *F. hispida* and monocious figs of *F. racemosa*. The study was undertaken in the northern part of Sumatra Island in Aceh Province, Indonesia. The study was carried out for 19 months from July 2011 until February 2013. Details of each species studied are described in each chapter of the thesis.

Chapter 2 The dynamics of fig tree pollination in a highly seasonal environment

2.1 Abstract

Fig trees and their pollinating wasps rely on each other to survive. Reproductive success of both partners is determined by the wasps' ability to reach receptive flowers suitable for pollination or oviposition, which depends on successful dispersal of the pollinators between trees. Female pollinators are weak flyers and live less than 48 hours. I investigated the effect of seasonal changes on *Elisabethiella baijnathi* (pollinating fig wasps) production and numbers in flight from December 1987 to January 1990 in the Makana Botanical Gardens, Grahamstown, South Africa. I also examined the independence of its host fig tree fig (*Ficus burtt-davyi*) population, which was separated by more than 1 km from the nearest conspecifics, by comparing the presence of pollinating wasps flying in the air with whether wasps were being released locally. The abundance of flying wasps and to a lesser extent the number of wasp released, was correlated with temperature. No pollinators were released locally in certain weeks during winter, but wasps were still captured on the traps, indicating that the wasps had dispersed from somewhere else and that the population was not independent. These findings emphasise the dispersal capabilities of fig wasps and the strong effects of seasonal changes on the dynamics of fig tree pollination.

2.2 Introduction

Ficus (fig trees, Moraceae) is one of the most diverse genera of flowering plants, with over 800 species distributed in mainly tropical and subtropical regions. Fig trees vary in size from creepers and small shrubs to huge rainforest trees (Berg 1989; Compton, Wiebes and Berg 1996; Herre et al. 1996). The inflorescences of fig trees known as syconia or figs (hereafter called as figs) are urn-shaped, enclosed structure contain from tens to thousands of tiny flowers depending on the fig tree species (Kjellberg et al. 2005). Pollination of figs is dependent on female fig wasp belong to family Agaonidae. Fig trees have a reciprocal, obligate mutualism with these pollinating wasps that lay eggs and develop their offspring in galled female flowers within the figs. The female, pollen-carrying wasps enter the fig, pollinate, and lay eggs in the ovaries of some female flowers of the fig trees. These females enter a receptive figs through a narrow bract-lined tunnel called ostiole which is only loosen for a short period of time (Janzen 1979a; Weiblen 2002). The females are generally assumed to die within the fig, however this is not always the case, as some pollinating females can re-emerge from the pollinated figs (Gibernau et al. 1996). The larvae of the pollinator feed on some of the female flowers that have been pollinated (one pollinator larva in each early galled ovule) (Compton, Wiebes and Berg 1996). In monoecious fig trees, individual figs have both female and male flowers which produce seeds, pollen and fig wasp offspring that transport the pollen. In gynodioecious fig trees, individual trees produce either viable seeds (female trees/female figs) or pollen (male trees/male figs) (Janzen 1979b; Verkerke 1989; Chen et al. 2013). Most of fig trees species are pollinated by only one or few pollinating wasp species and most pollinating wasps are only associated with one species of fig tree (Cook and Rasplus 2003; Cook and Segar 2010).

Figs also provide food for other groups of insects. These include numerous species of non-pollinating fig wasps (NPFW) that are inquilines, gallers and parasitoids, together with the larvae of moths, flies and beetles (Compton and van Noort 1992; Bai and Li 2008; Chen et al. 2013; Wu et al. 2013). Female pollinating wasps may also transfer mites and nematodes into the syconium of fig trees, when the wasps enter to lay eggs and pollinate the female flowers (Compton 1993; Krishnan et al. 2010; Jauharlina et al. 2012).

Fig crop development is usually synchronised within each tree in most monoecious *Ficus* species (those species where all trees are similar, with figs that contribute to both the plant’s females and males reproductive success, via seeds and pollen-carrying fig wasps respectively). The time gap between one crop and another in any particular tree can last from several weeks up to several years (McKey 1989; Compton 1993; Compton et al. 2005; Suleman, Raja and Compton 2013). This condition forces the newly emerged female wasps to fly away from their natal trees to search for other trees with receptive figs for them to enter and lay eggs (Ware and Compton 1994a; Ware and Compton 1994b; Anstett, HossaertMcKey and Kjellberg 1997a; Anstett, HossaertMcKey and McKey 1997b; Harrison 2003; Compton et al. 2005; Harrison and Rasplus 2006).

The presence of fig trees with receptive flowers at any time of the year affects the survival of pollinator populations. Any gap in the fig tree's flowering cycle will result in local failure of pollinator reproduction and in a reduction of the fitness of the fig trees (Anstett, HossaertMcKey and McKey 1997b). At the population level, fruiting and development of fig crops is typically asynchronous, so that at any one time there are only small proportions of the trees that are either releasing the wasps or having figs ready to be pollinated. The nearest suitable figs can be kilometres away from the tree in which the wasps are released, which means they have to increase the distance that they need to travel (Compton et al. 2005; Ahmed et al. 2009)

It has been reported that some female pollinating wasps are able to disperse over large distances of more than 160 km (Ahmed et al. 2009), by using the wind, above the canopy of the forest (Compton et al. 2000; Compton et al. 2005). The pollinating fig wasps are small insects, usually less than 2 mm in body length which only live for a short time (less than 2 days), and do not feed (Compton 1993; Ware and Compton 1994a; Ware and Compton 1994b; Dunn et al. 2008b). Reproductive success of these female wasps is strongly dependent upon their ability to disperse and reach receptive flowers inside the fig (Harrison and Rasplus 2006). Compared with pollinating fig wasps of dioecious fig tree species, the female wasps of monoecious fig trees able to fly higher and are passively dispersed over a longer distances before being attracted to receptive figs (Ahmed et al. 2009; Jeevanandam and Corlett 2013).

Fruit abundance in many species of fig trees varies greatly between the rainy and dry seasons, particularly in tropical rain forests. Some monoecious fig trees species only have very small crops during the dry seasons or winter. Most fig trees fruit more often and have larger crops during the warmer months and just have sufficient figs over the winter to allow insect reproduction (Janzen 1979a; Bronstein 1989; Wang and Sun 2009).

This chapter is aimed at investigating how a population of the veld fig (*Ficus burtt-davyi),* one of the most southerly-distributed fig trees in South Africa, responds to seasonal variation in temperature in terms of fruiting frequency, crop size, and crop duration. I also examined what the consequences of this seasonal variation were for the numbers of wasps released and the numbers flying in the air in relation to seasonal variation of temperature, and whether an apparently isolated population of pollinating wasps truly independent.

2.3 Study species

Ficus burtt-davyi (the veld fig) is a monoecious fig tree belonging to Subgenus Urostigma, Section Galoglychia, with a distribution that extends from the Eastern Cape of South Africa to Mozambique (Berg 1989). Growth form is variable: in sand dunes it grows as a creeping shrub whereas in temperate forest it is a strangler fig found on other trees. Elsewhere, as in the botanical gardens, it is a small free-standing tree or shrub, growing mainly among rocks. The tree is deciduous, and despite some variation of leaf condition within individual trees there are clear seasonal patterns of leaf condition. The leaves senesce and fall mostly during autumn and early winter (February to July) (Compton 1993; Ross 1994). More variation is found in the periods when young leaves are present, and the production of young leaves may be influenced to local rainfall patterns (Compton 1993). New leaves appear mainly during spring (between August and November), with mature leaves present in the summer (November to January).

The figs of *F. burtt-davyi* are small, reaching about 15 mm in diameter, and are produced in the leaf axils. Fruiting of *F. burtt-davyi* within trees is synchronized, resulting in discrete crops, but different trees fruit at different times, so figs at different stages are present on trees throughout the year. Crop sizes are highly variable, from a single fig up to more than twenty thousand (Compton 1993). Birds are the most prominent seed dispersal agents (Liu et al. 2013).

Ficus burtt-davyi is pollinated by *Elisabethiella baijnathi* and is also host to several additional species of non-pollinating fig wasps (NPFW) (Compton and Nefdt 1990; Compton 1993). *Elisabethiella baijnathi* flies during the day, emerging from the figs earlier in the morning during the summer (Ware and Compton 1994a). The female pollinators are attracted to receptive (B-phase) figs by species-specific volatiles released when the female flowers are mature and ready to be pollinated (van Noort, Ware and Compton 1989). The short-lived foundresses enter into the centre of the fig through the ostiole, which temporarily loosens to allow entry of the wasps. *Elisabethiella baijnathi* females have not been observed to re-emerge, and die inside the figs within 24 hours of entry. During the following inter-floral (C- phase), pollinator larvae and seeds develop. Maturation of the male flowers (D-phase), coincides with the emergence of flightless male pollinator fig wasps from their galls. They mate with the females, which are still in their galls, and then make a communal exit hole through the fig wall to allow the females to depart. The females emerge after they have filled their pollen baskets and then fly to receptive figs on other trees. The figs then become ripe and mature (E-phase). As other monoecious figs, individual figs of *F. burtt-davyi* also produce seeds, pollen and fig wasps.

A total of 105 *F. burtt-davyi* individuals, of varying sizes were recorded in the Makana Botanical Gardens. All the plants were regularly monitored, but some of the smaller individuals failed to produce figs during the survey period. A small number of plants were subsequently found to not be genetically distinct, despite being separated by several metres (S. Ross & J. Nason, unpublished), showing that the plants can also spread vegetatively. The separate parts of the plants nonetheless tended to produce fig crops at different times, and were treated as distinct individuals. The nearest known population of *F. burtt-davyi* was about two kilometres away from the Botanical Gardens, separated from them by the Rhodes University campus.

2.4 Methods

2.4.1 The study site

The studies were undertaken in the Makana (previously known as 1820 Settlers) Botanical Gardens in Grahamstown South Africa, from September 1987 to January 1990. Grahamstown (33°22'S, 26°29'E) is located in the Eastern Cape, 60 km inland from the south-east coast of South Africa at an altitude of about 550 m. The climate is semi-arid. Rainfall patterns are unpredictable, but typically more rain falls during the winter than summer months.

2.4.2 Meteorological information

Minimum and maximum temperatures were obtained from records made daily between September 1987 and February 1990 at a meteorological station adjacent to the gardens, located on the Rhodes University campus. Wind speed data for the sampling period is not available, but an indication of annual and diurnal variation in wind speed in Grahamstown was obtained using the daily measures available in the Rhodes University archive, based on the years 2005-2007 (http://oldwww2.ru.ac.za/weather/ARCHIVE/OLDSTATION/). Limited wind speed measurements taken during the study period showed similar diurnal patterns (Ware and Compton 1994a; Ware and Compton 1994b).

2.4.3 *Ficus burtt-davyi* fruiting phenology, pollination and fig wasp production

The *F. burtt-davyi* population of 105 trees was monitored at fortnightly intervals for 27 months in the period between September 1987 and February 1990, but with a gap in September 1988. The presence or absence of figs on each tree, and their developmental stages, were recorded on each occasion. Any fallen or clearly about-to-abort figs were also collected and damage or other likely causes of failure were recorded. A total of 205 such figs were examined. Initial crop sizes were estimated shortly after B-phase, when the figs had recently been pollinated. Samples of these figs were also removed in order to record the number of pollinator foundresses that had entered each fig. A total of 1967 early C phase figs were collected, from 149 crops.

Crop sizes on the 105 trees were estimated a second time when the figs were at late C or early D phases (releasing or about to release the next generation of figs wasps). A total of 93 crops reached maturity, from which 1014 figs were dissected to count the number of pollinators and NPFW present. The presence of damage caused by larger chewing insects such as moth caterpillars and beetle larvae was also recorded. The duration of each crop was calculated as the length of time elapsed from the dates of initiation and the dates when mature figs were observed. The estimated total number of female pollinator wasps released from each crop was calculated by multiplying mean numbers per fig by the final crop size. Approximate weekly pollinator release rates were estimated by dividing total female pollinator production by the number of weeks that D and E phase figs remained on the trees (the figs abort quickly or are eaten after the wasps emerge).

2.4.4 Pollinators in flight

The number of pollinators flying in the gardens was estimated using passive sticky traps. Five vertical plastic poles, 20 cm in diameter and painted black, were positioned within the *F. burtt-davyi* population, but in open areas at least 20 m away from any *F. burtt-davyi* and at least 50 m from each other. Each trap consisted of two clear cellulose acetate sheets (width 10 cm, length 42 cm) sprayed with pruning sealant (produced by Frank Fehr, Durban). The lower edges of the sheets were 1.5 m above the ground. The traps were replaced weekly and any trapped fig wasps were identified and counted. Catch numbers depend on the number of wasps in the air and the speed that the air is travelling, so they do not directly measure aerial density, but provide an indication of how many of the insects were passing a specific location.

2.4.5 Data analysis

Continuous response variables (crop duration and mean number of foundresses per crop) were analyzed by linear mixed effect models using the *nlme* package. Estimated numbers of pollinators released and crop sizes were also treated as continuous variables since their means were high. Assumptions were checked by looking at the normality of residuals. Log transformations were applied on crop sizes (young and mature), and number of pollinators released to meet the assumption of normality. For the response variables of count data (numbers of crops, number of pollinators trapped and number of figs aborted), analyses were done using generalized linear mixed models in the *lme4* package. Poisson distribution was used as error in these latter models. The models were accepted when dispersion number ranged between 0.50 and 2.0. Mixed effects models were applied because the crops were nested within trees, generating both fixed and random effects. Analyses were done separately on (1) effect of temperature and initial crop size on crop duration, (2) effect of temperature on crop size (initial and mature sizes), (3) effect of season and temperature on estimated pollinators released, (4) effect of season and temperature on number of pollinators trapped, (5) effect of initial crop size and temperature on number of foundresses inside a fig, (6) effect of temperature on number of crops (young and mature crops), and (7) effect of initial crop size on aborted figs. Trees were included in the models as random effects. The effects are considered to be significant at P-values <0.05. They are reported with P-value, degrees of freedom (df) and t-value when analyzed using *nlme* package or P-value together with z-values when analyzed using lme4 package. Significance values for the analysis that included season as an explanatory variable was assessed using likelihood ratio tests (LRT). The effect was considered to be significant at P-values <0.05, and reported together with degree of freedom (df) and LRT χ^2 value. All statistical analysis throughout the thesis were carried out using R programme (Ihaka and Gentleman 1996) version 2.11.2 or 2.12.2 on the first two result chapters and version 2.14.12 in the last three result chapters.

2.5 Results

2.5.1 Seasonality

The seasons in Grahamstown can be divided into summer (November- January), autumn (February-April), winter (May-July), and spring (August-October). Temperatures in Grahamstown are strongly seasonal with average daily temperatures noticeably higher during the summer months and lower during the winter months (Fig. 2.1.). The highest daily temperatures each year were recorded in January (24.5, 29.4, and 27.3 ^{o}C in 1988, 1989, and 1990 respectively). The lowest daily minimum temperatures during the winter months were 2.0 ^{o}C, recorded in July 1988, and 2.9 ^{o}C in June 1989.

Throughout the year, wind speeds in Grahamstown display a strong diurnal pattern, with the strongest winds typically recorded during the middle of the day. Seasonal variation is not pronounced, but the fastest mean day-time winds occurred during the winter months in all three years (Fig.2.2.) illustrates example wind speeds from four months of 2007). The fastest mean day-time wind speed during summer occurred at 13.00 hours and was 9.4 km/hour (Fig. 2.2.).

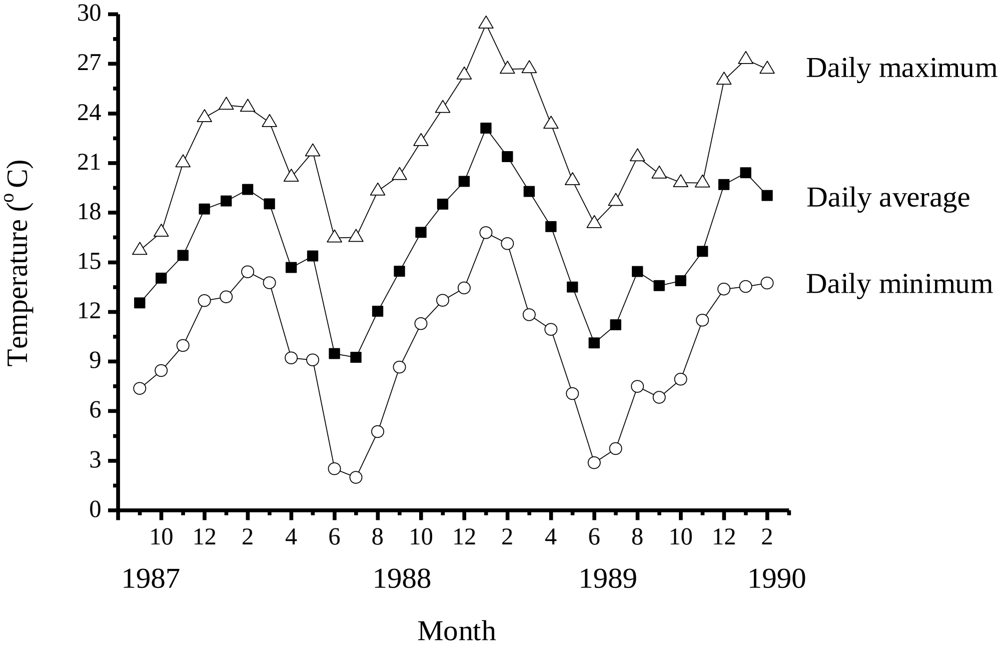


Fig. 2.1. Monthly variation in maximum, minimum, and mean temperatures in Grahamstown, South Africa between September 1987 and January 1990.

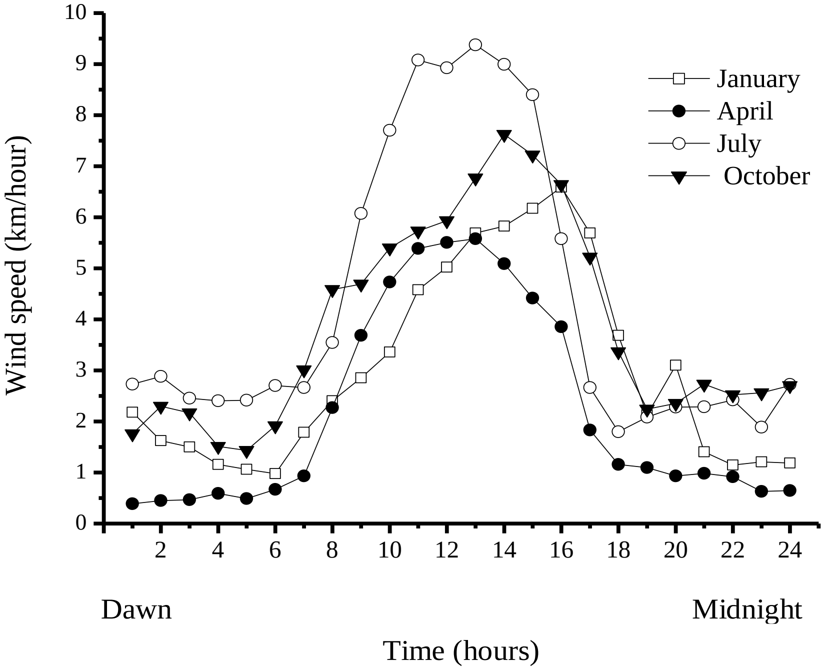


Fig. 2.2. Diurnal variation of mean wind speeds in Grahamstown during four months of 2007.

2.5.2 Fruiting phenology

Sixty four of the 105 *F. burtt-davyi* trees produced at least one crop of figs during the 29 month study period. Trees that failed to produce any crops were typically small, and were assumed to have failed to reach maturity. Seven crops was the maximum recorded from any one tree. Some of the smallest crops failed to have any figs that successfully completed their development. Figs were present on the trees throughout the year, although the number of trees bearing figs varied greatly (Fig. 2.3.). There were no consistent seasonal trends, but initiations of new crops were lowest during winter (Fig. 2.4.) and fewer crops reached maturity in winter and spring (Fig. 2.5.). These differences were reflected in significant positive relationships between daily seasonal temperatures and the number of crops initiated and maturing (lmer, $z = 8.95$, $P<0.0001$, and lmer, $z = 11.30$, $P <0.0001$ respectively). Average daily temperatures for each season were calculated based on average daily maximum and minimum temperatures over each period of three months (season).

The duration of crop development from B-phase to D-phase ranged from 30 to 113 days (but note that sampling intervals were every 14 days, so these values are approximate). Crops initiated in warmer months needed a shorter time to complete their development (Fig. 2.6.), with the duration of crop development negatively related to mean temperature during the period when a crop was developing (lme, df = 59, t-value = -9.255, $P <0.0001$). Development times were independent of initial crop sizes (lme, df = 59, t-value = 1.085, P = 0 .282,).

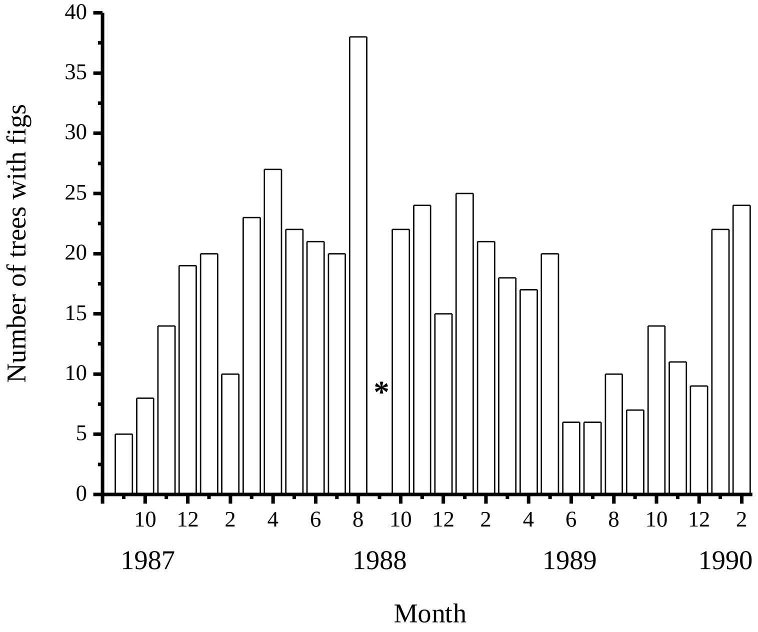


Fig. 2.3. Monthly variation in the numbers of *Ficus burtt-davyi* trees bearing figs (* no data available for September 1988).

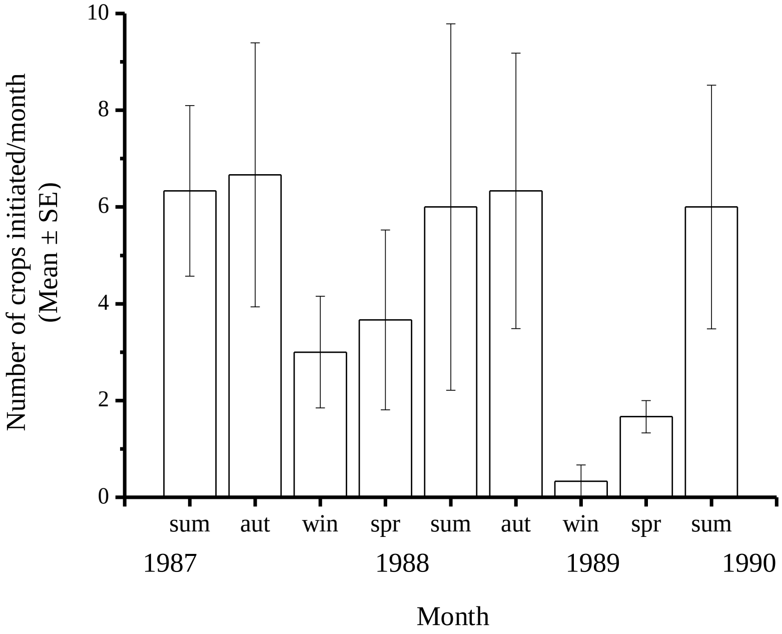


Fig. 2.4. Seasonal variation in the numbers of new crops of *Ficus burtt-davyi* initiated each month between October 1987 and January 1990).

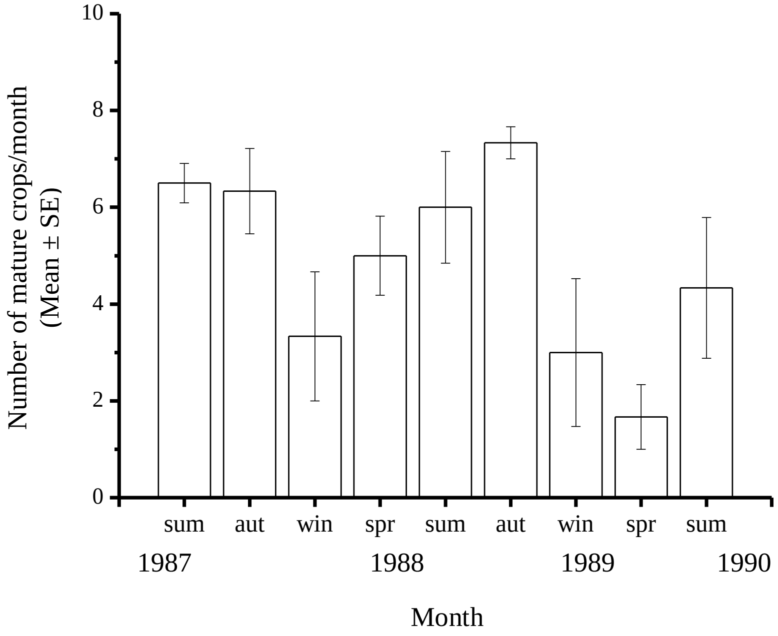


Fig. 2.5. Seasonal variation in the numbers of mature crops of *Ficus burtt-davyi* each month between October 1987 and January 1990.

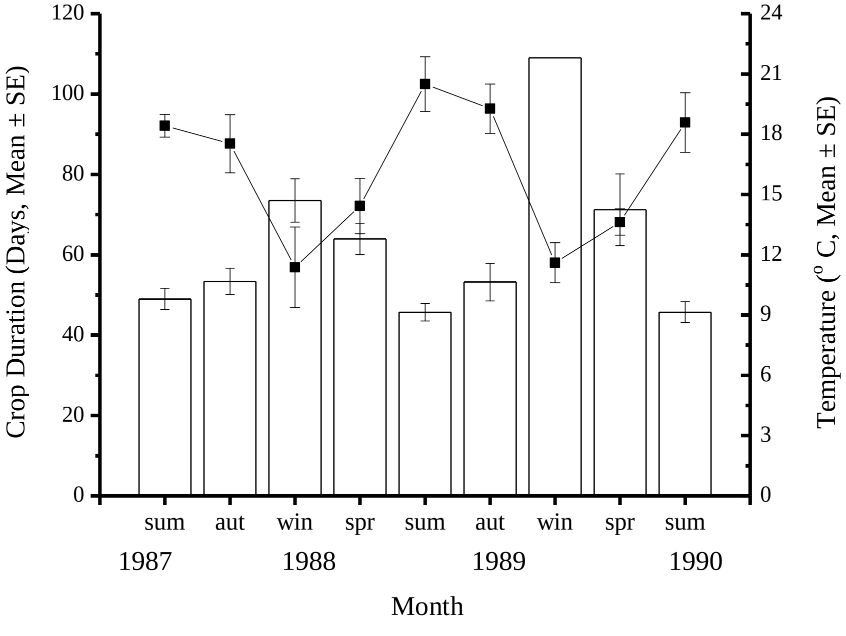


Fig. 2.6. The time taken to reach maturity by crops of *Ficus burtt-davyi* in relation to mean monthly temperatures. The durations were recorded from the time when any figs were first pollinated to when the first figs were recorded to be releasing fig wasps (November 1987-January 1990).

Crops initiated or maturing in the same months varied greatly in size. The largest crop initiated was estimated to contain 45800 figs and was counted during February 1988, when the smallest crop initiated in the same month was 2200. The largest crop of mature figs, estimated at 30324, was in October 1988 while the smallest crop size maturing that same month was 521. There were no obvious seasonal patterns in initial or mature fig crop sizes (Figs. 2.7. and 2.8.) and neither estimate of crop size (initial or final) was significantly related to mean temperatures during the months when the counts were made (lme, df = 60 , t-value = -0.193, P = 0.848, and lme, df = 60, t-value = 1.504, P = 0.138, respectively).

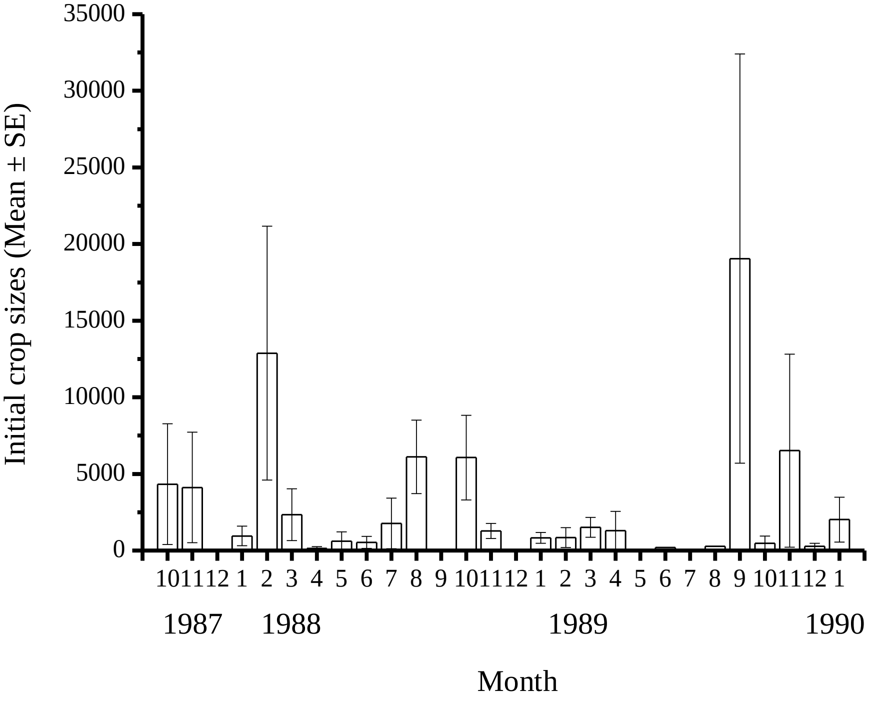


Fig. 2.7. Initial sizes of *Ficus burtt-davyi* crops.

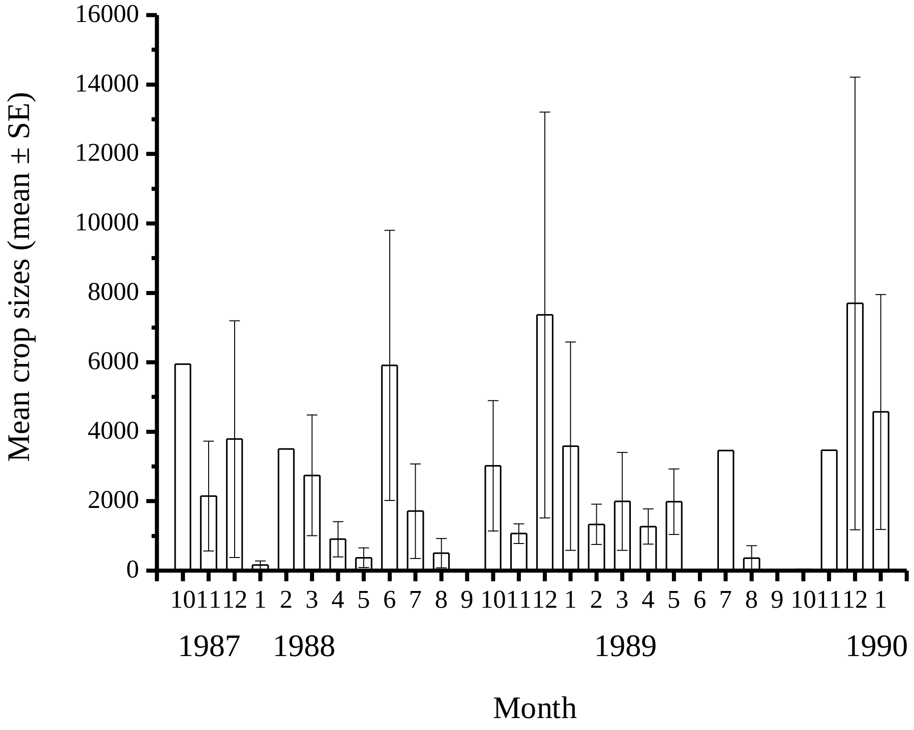


Fig. 2.8. Sizes at maturity of *Ficus burtt-davyi* crops

2.5.3 Pollinators release and numbers in flight

Fig wasps were being released from the trees almost throughout the study, with D and E phase figs (when fig wasps were being released or had recently done so) present on the trees for 56 of the 62 sample periods. Estimated numbers of female pollinators released weekly varied significantly with season (lme, df = 102, t-value = -2.150, P = 0.034), as well as with mean temperature (lme, df = 102, t-value = 0.009, P = 0.04) (Fig. 2.9.). The most female pollinators to have emerged from a single crop was estimated to be 1,642,260, with pollinator release from this large crop spread over eight weeks. The least number of pollinators estimated to emerge from a single crop that produced any mature figs was 192, with release spread over two weeks.

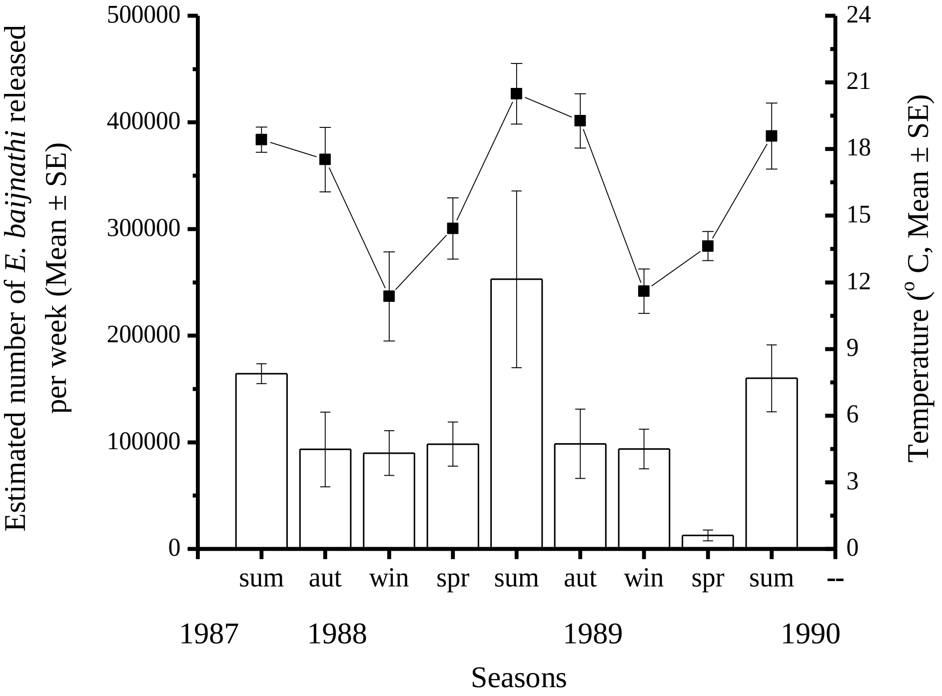


Fig. 2.9. Estimated numbers of *E. baijnathi* females released by a population of *Ficus burtt-davyi* and mean temperatures during the same period.

The number of pollinators flying between trees and captured on the sticky traps was related significantly with temperature (LRT, $\chi 2$ = 16.801, df = 1, P <0.0001) (Fig. 2.10.), but not significantly related to season (LRT, $\chi 2$ =, df = 3, P = 0.218). Wasp production and wasps in the air peaked in the summer months (Figs. 2.9. and 2.10.). The number of pollinators emerging each week varied between season, averaging above 150,000 during the summer months and 100,000 during colder months of the year. The numbers of pollinators on the traps was recorded during 101 weeks of the study. Variation in the numbers trapped largely corresponded with the numbers of pollinators estimated to have been released locally (Fig. 2.11.). The maximum number of pollinators trapped was 95, recorded during December 1988.

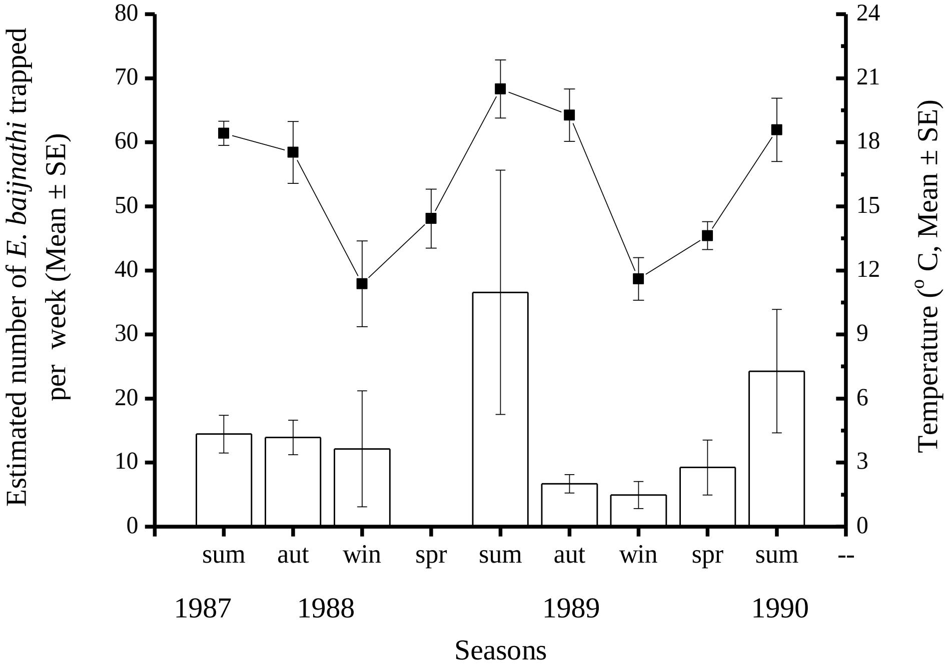


Fig.2.10. The numbers of *E. baijnathi* females trapped each week in relation to temperature (No trap data was available during Spring 1988).

During the sampling period, there were nine weeks in total when no pollinators were recorded in the traps. They occurred during winter and early spring 1988 and 1989. Two of these weeks corresponded with periods when no pollinators were being released from the trees locally (winter 1989). This indicates that even though pollinators were being released locally, they were not always releasing sufficient numbers to be detected on the traps. The number of *E.baijnathi* trapped each week was significantly correlated with the estimated number of wasps being released during the same week (P <0.0001, Pearson's R= 0.52) (Fig. 2.11.). There were ten weeks (during spring and early summer 1989) when no pollinators were being released locally, but they were still being captured on the traps (Fig. 2.12.). The wasps had clearly dispersed from somewhere else, confirming that pollination was also being carried out by *E. bajnathi* that have emerged from trees outside the local population.

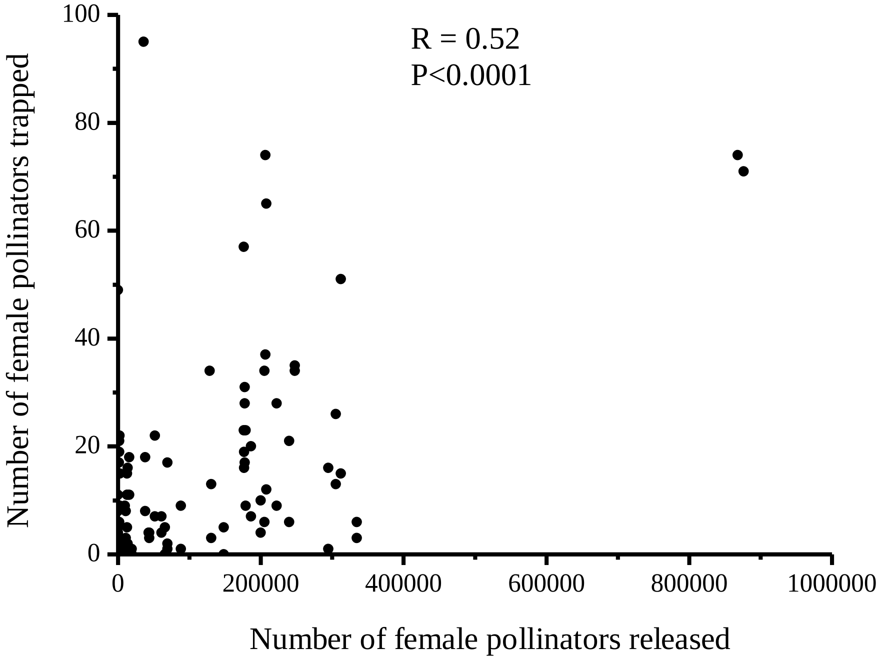


Fig. 2.11. Numbers of female *E. baijnathi* caught in the air in relation to the number of the wasps being released locally.

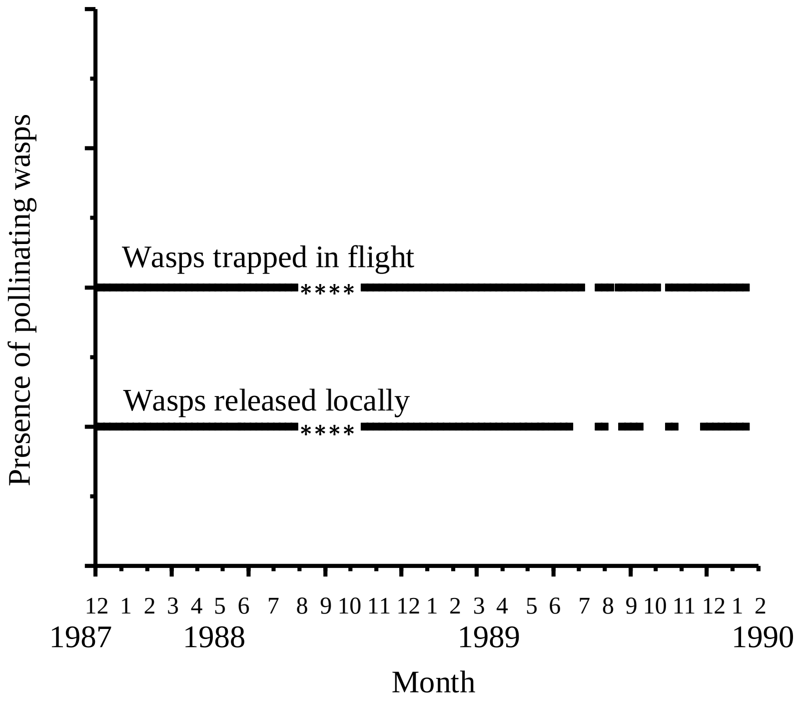


Fig. 2.12. The periods when *E. bajnathi* females were trapped in flight, compared with local releases of the fig wasps from their natal figs (Solid lines indicate wasps presence, line breaks indicate when wasps were not trapped or were not being released, * represents a period when no data is available).

2.5.4 Number of foundresses

Initial crop sizes varied from only one fig up to 45,800 figs, averaging 4257 ± 869 (mean ± SE) figs per crop. Almost all crops had almost all of their figs entered by pollinators, irrespective of crop size (Fig. 2.13.). The numbers of foundresses that entered a fig ranged from 0 up to 13 wasps, with a grand mean of 1.5 wasps per fig, although 1269 figs out of 1967 (61.46 %) had one pollinator. The number of foundresses entering a fig was independent of crop size (lme, df = 55, t-value = -0.938, P = 0.352), and temperature (lme, df = 55, t-value = -1.093, P = 0.279). Almost no figs showed signs of being pollinated, but did not contain any pollinator, showing that re-emergence from the figs is very rare.

Receptive figs that do not receive pollinators eventually fall off without any further development. The number of figs that received no pollinators was small, usually ranging one to 20 figs in each crop, although one large crop (with 20500 figs) had about 100 figs with no pollinators. These figs were un-pollinated and no seed or pollinators young developed inside. Initial crop size affected marginally the number of figs that aborted as a result of not being pollinated (lmer, z = 1.910, P = 0.0561) (Fig. 2.14.).

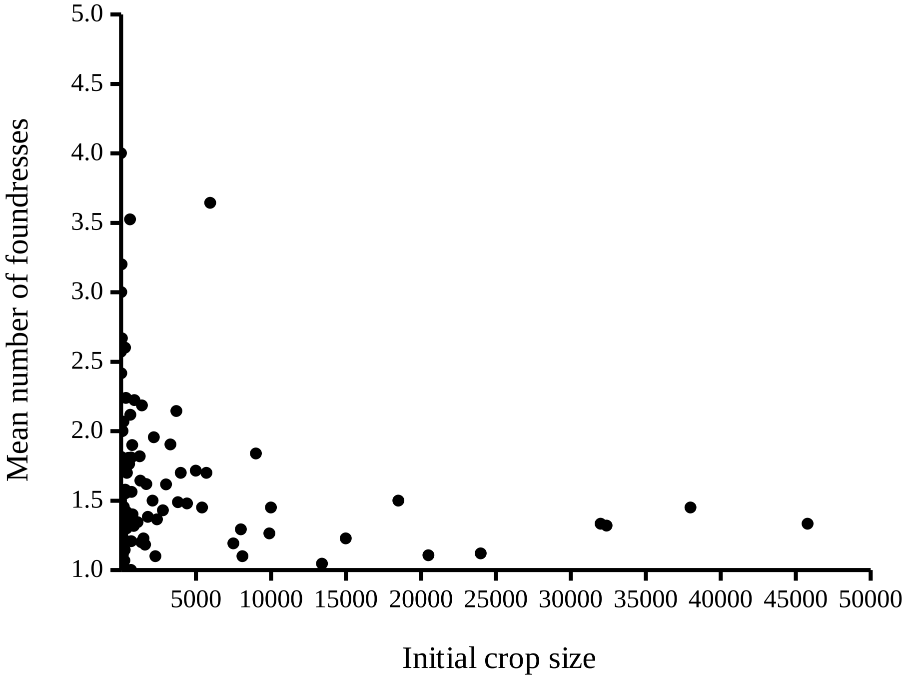


Fig. 2.13. Mean numbers of *E. baijnathi* foundresses per crop entering individual figs in relation to crop size. Only figs that had been entered by at least one pollinator are included.

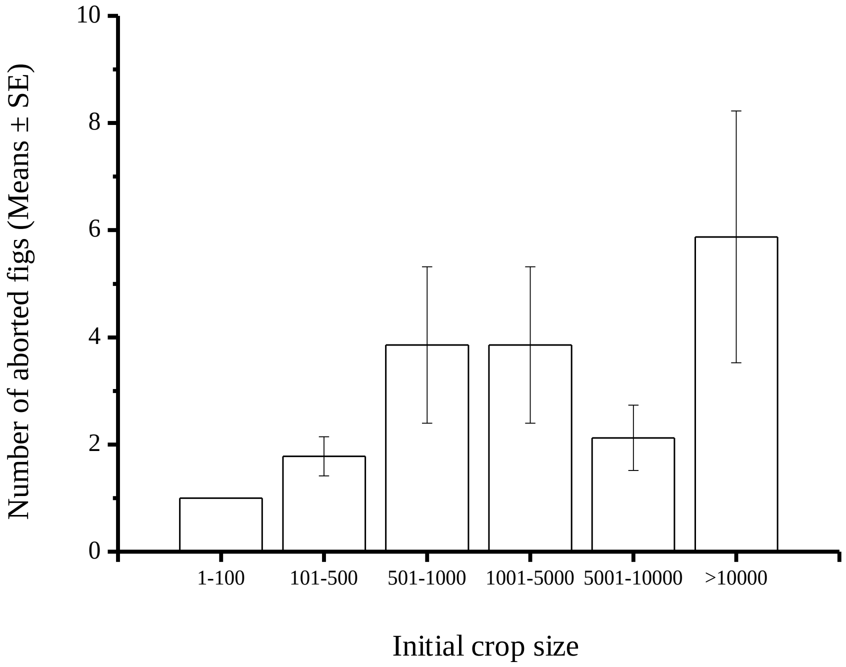


Fig. 2.14. The number of aborted figs that resulted from being un-pollinated in relation to crop size of *Ficus burtt-davyi.*

2.6 Discussion

2.6.1 Seasonal fruiting phenology and pollinating wasps

The fruiting phenologies of many monoecious fig trees have been described previously, but the relationships between phenology and pollinator population dynamics have not been inferred. Here I have shown how pollinator populations are maintained in a fig tree population growing close to the southern limit of the genus in Africa. The abundance of fig fruits varies greatly in many species of fig trees according to season. Sometimes they only have small crops during winter (Janzen 1979a; Bronstein 1989). In this study, however, I found that the fig fruits were present throughout the year with no consistent seasonal trends. The effect of temperature was nonetheless apparent with the initiation of new crops and the number of crops that mature, both of which were lower during the winter season in Grahamstown. Most mature figs were present during the warmer months, but there were still have enough mature figs in the winter to allow pollination and propagation of the pollinator population. During winter, fewer trees in the population were in reproductive phase, but the pollination population can persist. Adult pollinating wasps are short-lived, they have to find receptive figs soon after they emerge from a natal tree. They cannot wait until the next crop on their natal tree has receptive figs, because crops are highly synchronised and usually discontinuous, with long periods when no figs are present on each tree. Therefore, the pollinator females have to look for another tree having receptive figs at that time, which ensures that the plants are cross-pollinated. If no receptive figs are being produced within the local fig tree population, all emerging pollinators during that period will not be able to lay eggs, and will die without leaving any offspring if they cannot find receptive trees in other populations. Truly isolated local pollinator population can only survive if crops of their host plants are produced throughout the year (Bronstein 1989; Kjellberg and Maurice 1989; Harrison and Rasplus 2006). Fig trees can always produce another crop after previously having crops that failed to be pollinated, but pollinating wasps do not have another chance to reproduce if receptive figs are unavailable during their short life-spans (Smith and Bronstein 1996). When seasonal variation occurs in fruiting intensity, more trees are needed to ensure wasp populations survive the winter, to increase the chances that at least some trees will be supporting the wasps. The development time of the wasps may also be an important factor, as a longer development time may enable the wasps to bridge periods of very low fruiting intensity (Kjellberg and Maurice 1989).

Environmental variation between seasons is highly likely to affect the reproductive success of both fig trees and their pollinating wasps and possibly also the nature of the interaction (Bronstein 1989). In seasonal areas, the different climatic conditions in different seasons have an obvious impact on the production of viable seeds and wasp offspring in the fig tree/fig wasp system (Janzen 1979a; Yang et al. 2000; Wang and Sun 2009). Figs of *F. burrt-davyi* and its associated fig wasps develop more quickly during summer than winter (Baijnath and Ramcharun 1988; Ware and Compton 1994a) and this is likely to be true of other species. The development time of figs of *F. racemosa* is similarly longer in winter than during warmer seasons in China (Wang et al. 2005; Wang and Sun 2009). The results of the present study confirm this trend, with a significant effect of temperature on crop development. In warmer months when daily temperatures were up to 20^{o}C on average, the crops could reach maturity in as little as 45 days. On the other hand the crops that were initiated when the mean monthly temperature fell down to 10^{o}C, had their times to reach maturity extended up to 90 days or more. When crop durations and intervals between crops are longer, crop failures may be likely more to occur, thus seed production and ultimately population growth will be lower, too (Bronstein 1989). I found that the duration of crop development from the start of B-phase to the start of D-phase was independent of initial crop size. Trees that matured larger crops did have longer total periods when figs were present however, because of slight asynchrony in fig development.

Despite the significant effect of daily temperature on the number of young and mature crops, surprisingly there was no evidence of seasonal or monthly temperature effects on both initial and mature crop size of *F. burtt-davyi.* There are very few studies looking at what factors affecting the crop size of fig tree species. A larger crop will release more volatiles which in turn may attract more pollinators to visit (Harrison and Rasplus 2006). Fig crop size is also known to influence the diversity of visiting frugivorous animals (Shanahan et al. 2001; Sanitjan and Chen 2009). Because foraging behaviour of the attendant animals is also influenced by the crop size, it will also affect the reproductive success of the trees (Thompson et al. 2013).

2.6.2 Pollinators release and numbers in flight

The offspring production of pollinating wasp *Ceratosolen fusciceps*, which is associated with *F. racemosa* in China, varies greatly during different months of the year. Gall production (with the wasp developing inside) is higher in the cooler months than in warmer months. The seed production is however quite stable throughout the year (Wang, Yang and Yang 2005). I have shown that temperature clearly affects the number of adult female *E. baijnathi* released by the local population of *F. burtt-davyi* and also the numbers of wasps trapped from the air as they disperse between trees. There is a trend that the higher the weekly temperature in particular months the higher the numbers of both wasps released and those captured in flight. Temperature plays an important role in the flight activity of many insects, as well as other environmental factors such as humidity, wind speed and atmospheric pressure. All these factors may stimulate or inhibit insect flight activity (Wang et al. 2013b). The timing of emergence and dispersal flights by *E. baijnathi* are highly influenced by ambient temperature. Laboratory observation shows that minimum temperature for *E. baijnathi* to take off is between 15 and 16 ^{0}C, however in the field the ambient temperature when the wasps were observed flying can be much lower (Ware and Compton 1994a).

The fig wasps that pollinate *F.burrt-davyi* are known as day flying insects, and they usually emerge from their natal figs in the morning between 0600 h and 1200 h. In mid-summer some wasps may emerge earlier due to the earlier day break and warmer morning temperatures, but no wasps emerged before 0600 h during the winter months (Ware and Compton 1994a). Many fig wasps are able to fly above the canopy and use wind to disperse (Harrison 2003; Compton et al. 2005). The vegetation around Grahamstown would naturally have been a mixture of vegetation types, consisting of fynbos and thicket, with few trees. Along with ornamental trees, the vegetation at the time of this study comprised mainly woody shrubs (S.G. Compton, personal communication). In Grahamstown, wind speeds start to increase from 0600 h and reach highest speeds between 1200 h and 1300 in four different seasons observed. This pattern corresponds intimately with the time of *E.baijnathi* emergence from the natal trees. Soon after emerging, *E. baijnathi* will disperse to find another tree bearing receptive figs. Despite their small size, the ability of the pollinators to find the receptive figs is impressive. It is reported that the pollinator *Ceratosolen arabicus* Mayr could transfer pollen between the monocious African fig trees *F. sycomorus* L. separated more than 160 km away from each other along the Ugab River in the Namib Desert, Namibia (Ahmed et al. 2009). Small numbers of pollinating wasps are able to reach their host fig trees even if they are grown away from their conspecifics or outside their natural distribution range (Compton and Nefdt 1990; Ware and Compton 1992), but it is likely that most pollination events are achieved by wasps that have flown shorter distances.

The pollinating wasps locate suitable figs to enter by using volatile attractant released from figs in female phase when they are ready to be pollinated (Ware et al. 1993; Cardona, Kattan and de Ulloa 2013). The small size of *E. bajnathi* adults do not allow them to fly quickly in search of suitable figs, therefore wind speed plays an important role during this search. Their ability to fly high, where wind speeds are greater, may however be limited by their ability to detect and respond to the volatile attractants released by receptive trees (Harrison and Rasplus 2006). *Elisabethiella baijnathi* wasps leave their natal tree by flying upwards and then are brought down-wind by the prevailing air currents. This initial vertical flight ensures the wasps rapidly enter the air column and are blown passively downwind. When the wasps perceive the fig tree volatile attractants, they may lose height, or the wasps may decide to descend when they have been flying a certain length of time. The wasps then use controlled upwind flight to search for the receptive figs releasing the volatiles (Ware and Compton 1994b).

The weekly observations of trapped *E. baijnathi* in this study indicated that not all pollinators flying in the air came from local population of *F. burtt-davyii*, since the pollinators were still captured even when there were no pollinators being released locally. This finding confirms previous studies that pollinating wasps are effective colonisers of isolated host trees despite their small size (Thornton, Compton and Wilson 1996; Compton 2002; Compton et al. 2005). Their willingness to fly passively downwind, combined with the effectiveness of the volatiles released by the figs to attract the pollinators over short distances (Ware and Compton 1994b) allows the mutualism to be maintained (Compton 2002).

2.6.3 Numbers of foundresses and crop size

The amount of pollinator volatile attractant is estimated to be proportional to crop size (Smith and Bronstein 1996). A larger crop size will produce more of a volatile trail which in turn may attract more pollinators to visit and secure higher pollination success (Harrison and Rasplus 2006; Yeo and Tan 2009). Alternatively, larger crops may swamp the local supply of pollinators, as seen with a monoecious fig tree species *F. superba* in Singapore, pollinated by *Platyscapa corner* Wiebes, which is shown to have fewer pollinators per fig in larger crops (Yeo and Tan 2009). In this study I have shown that the number of foundresses entering a fig of *F. burtt-davyi* was independent of crop size, with most figs receiving between one to two foundresses each (Fig.2.13). Only one unusually large crop had many figs that failed to be pollinated, suggesting that at my study site, fig production and pollinators being released were well matched.

Chapter 3 Fig wasps as vectors of mites and nematodes

3.1 Abstract

Females of the pollinator fig wasp *Elisabethiella baijnathi* Wiebes carry mites (*Tarsonemella* sp. nr. *africanus*) and nematodes (*Parasitodiplogaster* sp.) between figs of *Ficus burtt-davyi* in Grahamstown, South Africa. The mites are phoretic on the outside of the wasps and phytophagous, feeding on galled flowers. The nematodes are transported inside the wasps and eventually eat them. Both mites and nematodes were present throughout the year. The prevalence (fig occupancy rates) of mites and nematodes in different crops ranged between zero and 100%. Crop size did not influence the prevalence of either mites or nematodes. Contrasting dispersion patterns and relationships with fig wasp foundress numbers indicate that the mites, but not the nematodes, disperse between figs after being carried there by the pollinators, and they may also utilise non-pollinating fig wasps as vectors.

3.2 Introduction

Figs (*Ficus* spp., Moraceae) are keystone resources in many tropical areas, producing fruits that are eaten by more species of vertebrates than any other plants (Shanahan et al. 2001). The trees are entirely dependent on fig wasps (Hymenoptera, Agaonidae) for pollination and the fig wasps can only reproduce inside the figs (syconia) produced by their hosts. Most fig trees rely on a single species of pollinator, but an increasing number are known to support two or more species. Figs and their pollinators have co-evolved in a general sense (Jansen-Gonzalez, Teixeira and Santinelo Pereira 2012). Figs also provide food for other groups of insects. These include numerous species of non-pollinating fig wasps (NPFW) that are inquilines, gallers and parasitoids, together with the larvae of moths, flies and beetles (Compton and van Noort 1992; Bai, Yang and Compton 2008; Wu et al. 2013). Mites and nematodes also colonise figs, but because they are flightless they depend on insects, usually pollinator fig wasps, to transport them between trees (Bronstein 1991; Pereira, Semir and Menezes Junior 2000; Walter 2000; Cardona, De Ulloa and Kattan 2007).

Phoretic tarsonemid mites (Tarsonemidae, Tarsonemellini) are associated with fig wasps throughout the world (*Paratarsonemella* R. Ochoa in West Africa and Australia, *Ficotarsonemus* Ho in Taiwan, *Tarsonemella* Hirst across Africa, plus undescribed species in Brazil (Pereira, Semir and Menezes Junior 2000) and China (Y-Q Peng, pers. comm. 2009). In southern Africa, *Platyscapa soraria* Wiebes (from *F. ingens* (Miq.) Miq.), *Elisabethiella glumosae* Wiebes (from *F. glumosa* Delile) and *E. baiijnathi* Wiebes (from *F. burtt-davyi* Hutch.) each transport different undescribed species of *Tarsonemella* (E.E. Lindquist, unpublished), which suggests that host plant specificity is high and that there may be hundreds of species of fig mites (Walter 2000).

No mites have so far been described from fig trees with a dioecious breeding system. There are few dioecious fig tree species in Africa, but they outnumber monoecious fig trees in Asia. Dioecious *Ficus* have separate sexes, with figs on male trees producing only fig wasps and female trees producing only seeds. Any mites transported to female trees would have no chance of reproduction and this potential loss of half the mites each generation appears to have been sufficient to stop them from colonizing dioecious hosts. Nematodes are frequently present in dioecious figs, and it is unclear why a dioecious breeding system has only prevented colonization by mites.

Female pollinator fig wasps act as hosts to many species of nematodes by transporting them within their bodies between figs of both monoecious and dioecious fig trees. The nematodes then vacate their hosts and the next generation develops in time to colonise the next generation of fig wasps (Martin, Owen and Way 1973). Giblin-Davis et al. (1995) described how juvenile *Parasitodiplogaster* sp. nematodes enter adult fig wasp females, then increase in size and moult while still in the fig wasp hemoecoel, before leaving the cadaver. Moulting into the adult stage, mating and laying of eggs then occurs in the fig cavity (lumen). As many as 50,000 nematodes have been recorded from a single fig (Martin, Owen and Way 1973).

Some fig nematodes are phytophagous while others are parasitic or saprophytic, killing the fig wasps that transported them or feeding on their bodies after they die. *Schistonchus caprifici* Gasperrini (Aphelenchoididae) is an example of a phytophagous species, and reproduces in male figs of the edible fig, *Ficus carica* L.. Flowers in figs infested by the nematode show necrosis, but there is no evidence of any adverse effects on the pollinating wasps (*Blastophaga psenes* L.) that transport them (Vovlas, Inserra and Greco 1992). *Schistonchus caprifici* nematodes will enter both pollinator and NPFW females, but there is no evidence that those transported by NPFW ever manage to enter figs and reproduce (Vovlas and Larizza 1996). Host specific *Parasitodiplogaster* Poinar nematodes (Diplogastridae) feed on the female wasps that transport them, with varying degrees of impact on the wasps' reproductive success, depending on how quickly they are consumed (Herre 1993). Both feeding strategies can be present in the same figs, as the fig wasp *Elizabethiella stuckenbergi* Grandi that pollinates *F. burkei* (Miq.) Miq. (= *F. thonningii* Blume) transports both *Parasitodiplogaster sycophilon* Poinar (Poinar 1979) and *Schistonchus africanus* Vovlas (Vovlas et al. 1998) in southern Africa.

Previous studies of fig mites and fig nematodes have generally focused on taxonomic descriptions and establishing their host relationships (Giblin-Davis et al. 1995; Pereira, Semir and Menezes Junior 2000; Walter 2000; Giblin-Davis et al. 2006) and their general biology is poorly known. How mites and nematodes use fig wasps as vectors has not been compared previously. Here I describe the biology of a mite (*Tarsonemella* sp. indesc. nr. *T. africanus* Hirst., Tarsonemidae) and a nematode (*Parasitodiplogaster* sp. indesc., Diplogastridae) associated with *F. burtt-davyi* and its pollinator (*Elisabethiella baijnathi* Wiebes) in South Africa (Compton 1993). After describing their life cycles and whether they are only found in association with pollinator fig wasps, I examine whether their prevalence within crops varies seasonally and is influenced by the size of a fig crop. By relating their presence or absence to the numbers of fig wasp foundresses entering a fig, I also examine whether there is secondary dispersal between figs, after their initial transport between trees by the wasps.

3.3 Study species

Ficus burtt-davyi (the veld fig) is a monoecious fig tree belonging to Subgenus Urostigma, Section Galoglychia, with a distribution that extends from the Eastern Cape of South Africa to Mozambique (Berg 1989). In the area around Grahamstown (33°22'S, 26°29'E) in the Eastern Cape, it grows as a shrub or small tree, mainly on rocks (Compton and Nefdt 1990; Burrows and Burrows 2003). Grahamstown is located 60 km inland from the south-east coast of South Africa at an altitude of about 550 m. The climate is semi-arid with unpredictable rainfall patterns.

The figs of *F. burtt-davyi* are small, reaching about 15 mm in diameter, and are produced in the leaf axils. Fruiting within trees is synchronized, resulting in discrete crops, but different trees fruit at different times, so figs at different stages are present on trees throughout the year. Crop sizes are highly variable. *F. burtt-davyi* is pollinated by *E. baijnathi* and is also host to additional species of NPFW (Compton and Nefdt 1990; Compton 1993). Most of the NPFW oviposit from the outside of the figs, but *Philocaenus liodontu*s (Wiebes) enters the figs to oviposit, like the pollinator (van Noort 1994). *Elisabethiella baijnathi* females are attracted to receptive (B) phase figs by species-specific volatiles released when the female flowers are mature and ready to be pollinated (van Noort, Ware and Compton 1989). The short-lived foundresses enter into the centre of the fig through the ostiole, which temporarily loosens to allow entry of the wasps. *Elisabethiella baijnathi* females have not been observed to re-emerge, but die inside the figs within 24 hours of entry. During the following inter-floral (C) phase, which lasts at least six weeks, pollinator larvae and seeds develop. Maturation of the male flowers (D phase) coincides with the flightless male fig wasps emerging from their galls. They mate with the females, which are still in their galls, and then make a communal exit hole through the fig wall to allow the females to depart. The females emerge after they have filled their pollen baskets and then fly to receptive figs on other trees, as described for other active pollinators (Galil and Eisikowich 1968).

3.4 Methods

3.4.1 Study site

In the area around Grahamstown (33°22'S, 26°29'E) in the Eastern Cape, *F. burtt-davyi* grows as a shrub or small tree, mainly on rocks (Compton and Nefdt 1990; Burrows and Burrows 2003). Grahamstown is located 60 km inland from the south-east coast of South Africa at an altitude of about 550 m. The climate is semi-arid with unpredictable rainfall patterns.

3.4.2 Sequential samplings

A population of 105 *F. burtt-davyi* trees growing in the 1820 Settlers Botanical Garden in Grahamstown was monitored between September 1987 and February 1990, with samples taken from each crop shortly after the figs had been pollinated. The numbers of pollinating wasps (foundresses), and the presence of nematodes and mites inside a total of 1 967 figs were recorded from 149 crops, together with crop sizes at that time. All the figs present at a tree were counted if crops were small. The sizes of larger crops were estimated using counts of randomly selected sections of the trees in combination with visual estimations of the proportion of the total canopy volume that had been counted. Additional samples from a single crop were taken at regular intervals in 1986 to establish the life histories of the mite and nematode.

3.4.3 Data analysis

The prevalence of mites and nematodes was analysed by generalized linear mixed effect models with binomial errors using the lme4 package. Linear mixed effects models were applied, because the crops were nested within trees, generating both fixed and random effects. The number of foundresses, crop size, and sampling time were treated as fixed effects. Binomial error was added into the models. Crops and trees were added in the models as random effects. The sampling dates were grouped into three-month periods (October - December, January - March, April - June, July - September) to examine seasonal effects in different years. Significance values were assessed using likelihood ratio tests (LRT). The effects are considered to be significant at P-values <0.05. They are reported as P-value, together with degrees of freedom (df) and LRT χ^2 value.

3.5 Results

3.5.1 Mite and nematode life cycle

Sequential samples from a single crop during winter (Table 3.1.) showed that adult female mites *Tarsonemella* sp. are the dispersive stage. After entering a B phase fig they feed on the developing ovules, causing enlargement, browning and a concavity on the ovule surface. They have unusually elongated, recurved and deeply retractable mouthparts, reflecting their phytophagous habits. The mites are strongly aggregated within a fig, so that only a small proportion of the ovules show signs of feeding damage. Males are more orange in colour than the females and never formed more than about 5% of the adult population. The apparent presence of two distinct cohorts of males before the figs had matured suggests that two generations of mites were produced during the course of the crop. Generation times of about this length (four weeks) have been reported for other tarsonemid mites when developing at relatively low temperature (Jander, Herre and Simms 2012; Al-Beidh et al. 2012). More generations may be possible in summer, though figs also complete their development more rapidly during warmer months. The aggregation of the mites breaks down once the next generation of fig wasps start to emerge from their galls and the mites disperse throughout the interior of the figs.

Male pollinators emerge first and sometimes have large numbers of mites crawling on them as they seek out female wasps. The males chew holes in galls containing females to mate and some mites make use of the mating holes to enter the galls and attach themselves to the females before they emerge. The mites become firmly attached between the segmental plates of the gaster and occasionally the thorax (Fig. 3.1.) and in extreme cases several dozen mites will attach to a single female, covering the entire surface of the gaster. Female pollinators can also pick up additional mites when they emerge from their galls into the lumen of the fig, before they eventually leave the figs. The host preferences of the mites are illustrated by a sample of figs from which wasps were allowed to emerge naturally through the exit holes produced by the males (Table 3.2.). Mobile mites were present on the bodies of both males and females of several fig wasp species. Whether the mites can be transported successfully without becoming firmly attached is unknown. Firm attachment was much more selective, with pollinator females preferred over *Philotrypesis* sp. (P < 0001, Fisher exact test), *Otitesella* spp. (P = 0.023, Fisher exact test), and *Sycoryctes* sp. (P = 0.001, Fisher exact test). No males of any species were found with mites firmly attached to them (Table 3.2). One of the two *P. liodontus* females present had mites firmly attached and additional *ad hoc* samples also recorded rare instances of the mites being firmly attached to *P. liodontus* females. This species enters the figs to oviposit, and is potentially as valuable a vector for the mites as the pollinator. In the main survey, however, this NPFW was only recorded from 12 figs (out of a total of 1967 figs), none of which contained mites, so its role as a vector remains unconfirmed.

Adult mites were also observed on the surface of mature figs that did not yet have fig wasp exit holes, sometimes clustered around the ostioles. These may have included female mites that had managed to emerge independently from those figs, via the ostioles, but mites which failed to attach to fig wasps in their natal figs were also seen to disperse to other figs. This gives the mites a second chance to encounter suitable vectors. A crop with many un-pollinated, aborting early C-phase figs (sampled 25 September 1989) showed that the mites can enter B-phase figs without being carried in by female wasps, but that they are also poor at controlling subsequent fig development and preventing such figs from aborting. Among 52 aborting figs that showed no signs of having been eaten by caterpillars or otherwise damaged, 30 had pollinator females that had become trapped while attempting entry through the ostiole, but had no females that had succeeded in entering. Sixteen of these had flowers damaged by mites while the remaining 14 did not. A further 20 aborting figs had not been visited by pollinators, but 16 nonetheless contained mites and mite-galled flowers. The remaining two figs were aborting despite having been entered by pollinators.

The biology of the nematode *Parasitodiplogaster* sp. in *F. burtt-davyi* figs is similar to that described for congeneric nematodes elsewhere. The nematodes were carried by the pollinator *E. baijnathi* as juveniles. Before emerging through the exit hole prepared by the males, the young adult female pollinators walk around the interior of the fig, collecting pollen. During this period, tiny nematodes were very active on the surface of the female flowers inside the figs, ‘standing on their tails’ and waving their bodies. On contact with female pollinators they entered their bodies, through inter-segmental membranes, and possibly also the gut. Adult *E. baijnathi* longevity is short, with 48 hours probably a maximum, and most successful females are likely to have found a suitable fig within a few hours (Compton 1993), giving little time for the nematodes to damage them. After laying her eggs and pollinating the flowers the foundress dies and the nematodes consumed the cadaver, growing rapidly and leaving the wasps within a few days. Whether this rapid feeding starts before or after the wasps have died is unclear and had not been established for other *Parasitodiplogaster* species feeding on different pollinators

(Poinar 1979). The nematodes did not appear to feed on the plant tissues, but eventually produced larvae in time for the next generation of fig wasps to emerge.

Table 3.1. Changes in composition of a *Tarsonemella* sp. population from a single crop of *F. burtt-davyi* figs in Grahamstown during winter 1986. The development of the figs in this crop was not perfectly synchronized. Day 1 was 27th May. At least 20 figs were examined on each date.

Day	Activity
Day 1	Figs recently entered by foundresses. Adult female mites aggregating and feeding on developing ovules, causing enlargement, browning and a concavity on the ovule surface. Eggs present on the flowers galled by mites.
Day 7	Adult females and eggs, some hatching.
Day 16	Adult females, eggs and juveniles.
Day 24	Adult females, males, eggs and juveniles.
Day 30	Adult females, eggs and juveniles.
Day 45	Eggs and juveniles, few adults.
Day 54	Adult females, males and juveniles. Few eggs.
Day 60	Adult females, males and juveniles. Few eggs.
Day 68	Some figs with wasp exit holes. Aggregation around feeding sites ended. Frenzied mite activity. Male pollinators sometimes covered with mites.

Fig. 3.1. Four female *Tarsonemella* sp. mites attached to the gaster of *Elisabethiella baijnathi*.

Table 3.2. *Tarsonemella* sp. mites on fig wasps that had recently emerged from 11 figs of *F. burtt-davyi*. Some fig wasps had both mobile and attached mites present.

Fig wasp species	Sex	Individual wasps					
		Total	With mobile mites	Proportion with mobile mites	Range per wasp (Total mites)	With mites attached	Percentage with mites attached (%)
Elisabethiella baijnathi	F	16	11	0.69	1-15	6	38
	M	2	0	0.00	-	0	0
Philotrypesis sp.	F	88	16	0.18	1-2	0	0
	M	78	13	0.17	1	0	0
Otitesella spp.	F	12	6	0.50	1-2	0	0
	M	9	0	0.00	-	0	0
Sycoryctes sp.	F	37	11	0.30	1-2	1	3
	M	29	2	0.07	1-2	0	0
Philocaenus liodontus	F	2	1	0.50	3	1	50
	M	8	0	0.00	-	0	0

3.5.2 Variation in prevalence between crops

Within individual crops, the prevalence (proportion of figs occupied) of mites and nematodes in recently-pollinated figs ranged from zero to 100 %, with nematodes generally more abundant than mites (Fig. 3.2.). Both mites and nematodes were present throughout the year, with the prevalence of mites ranging between about 6% and 35% and nematodes ranging between about 20% and 45% in different seasons (Figs. 3.3. and 3.4.). There was no significant effect of sampling period on mite prevalence (LRT χ^2 = 14.03, df = 9, P =0.12), nor on nematodes prevalence (LRT χ^2 = 8.51, df = 9, P = 0.48). The prevalence of mites varied between different periods, but the peak periods for mites varied in different years. Nematode prevalence was more stable and displayed no evidence of seasonal patterns. Estimated crop sizes at the time of pollination ranged between one and 45,800. Crop size did not influence the prevalence of either mites (LRT χ^2 = 1.42, df = 1, P = 0.23) or nematodes (LRT χ^2 = 1.01, df = 1, P = 0.32) (Figs. 3.5. and 3.6.).

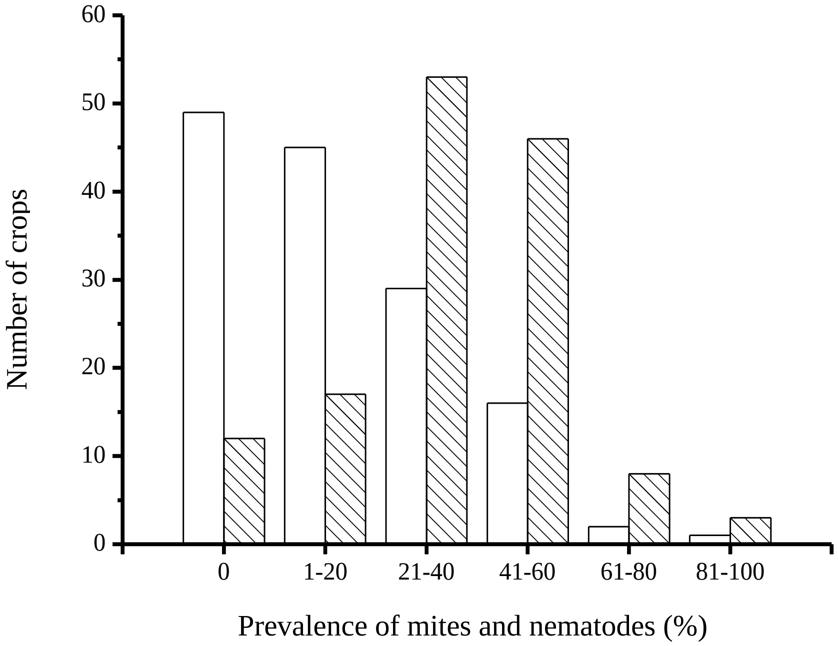


Fig. 3.2. Prevalence of *Tarsonemella* mites (open bars) and *Parasitodiplogaster* nematodes (hatched bars) across all crops of *F. burtt-davyi*.

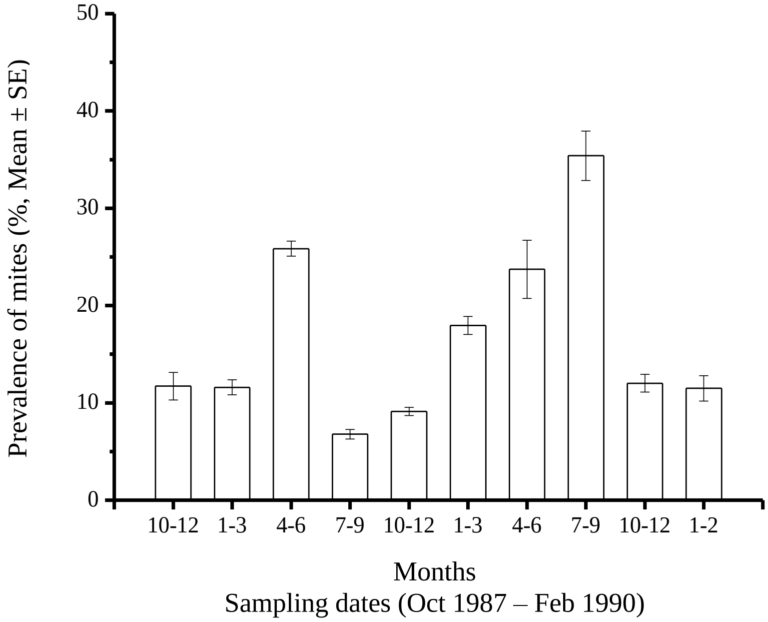


Fig. 3.3. Seasonal changes in the presence of *Tarsonemella* mites inside the figs of *F. burtt-davyi.*

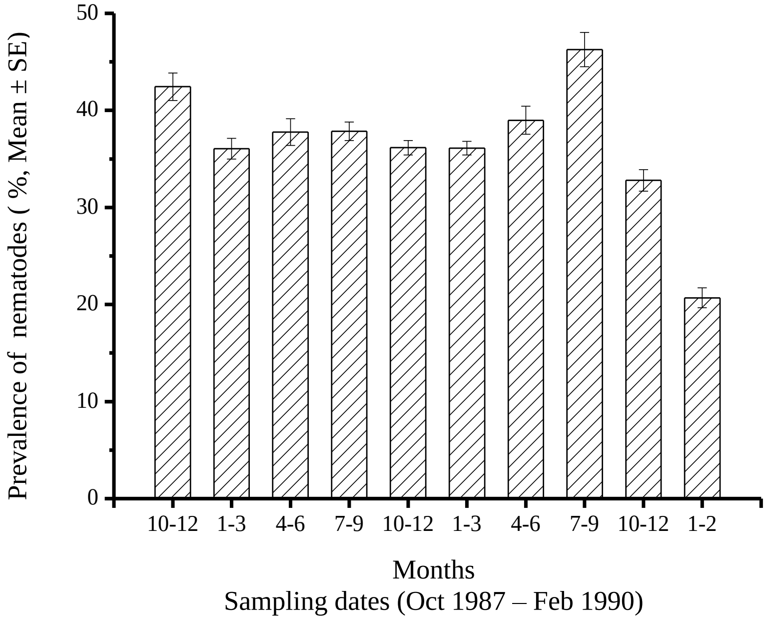


Fig. 3.4. Seasonal change in the presence of *Parasitodiplogaster* nematodes inside the figs of *F. burtt-davyi.*

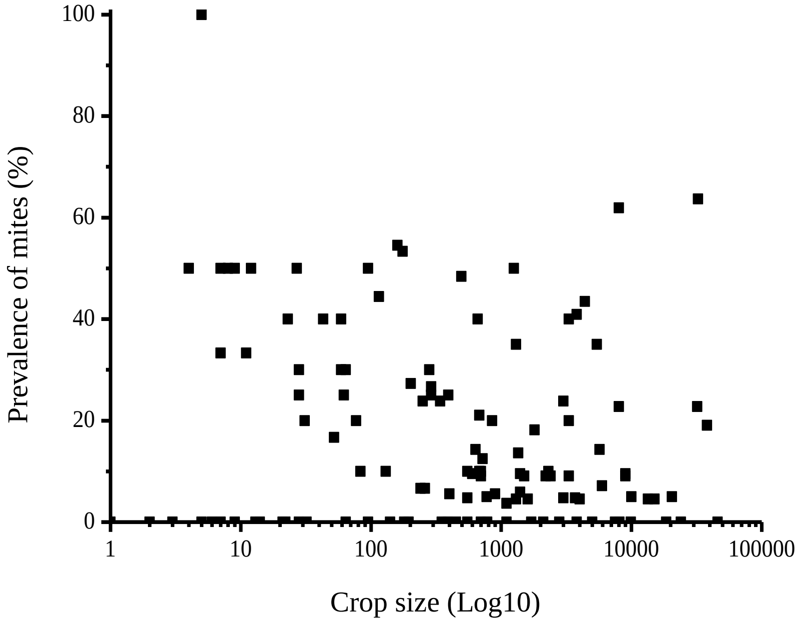


Fig. 3.5. Prevalence of *Tarsonemella* mites in relation to crop size.

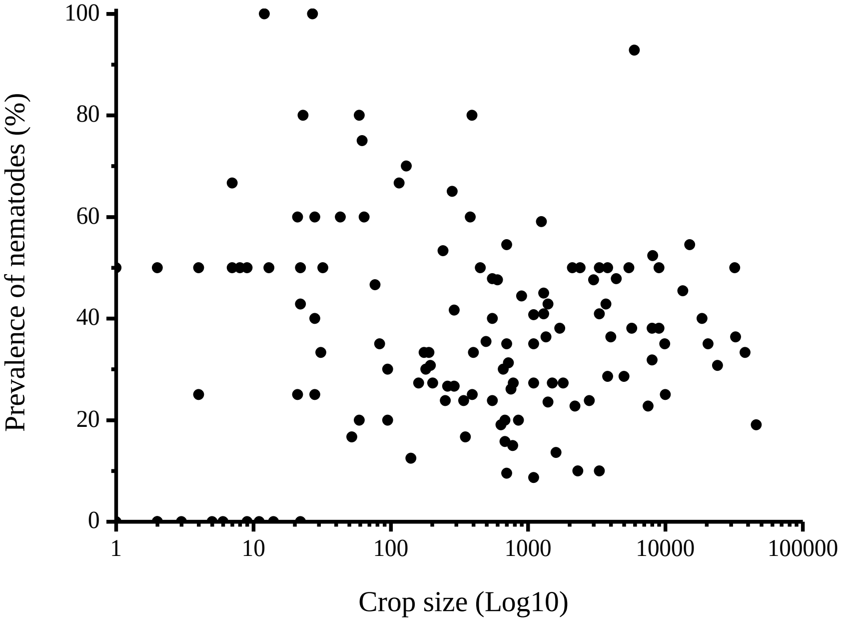


Fig. 3.6. Prevalence of *Parasitodiplogaster* nematodes in relation to crop size.

3.5.3 Foundress number, mite and nematode prevalence.

As many as 13 *E. baijnathi* foundresses were recorded from a fig, but most were only entered by a single foundress (Fig. 3.7.). The ability of mites to enter figs independently was suggested by the presence of mites in 11 of 94 early C phase figs where no fig wasp foundresses were present. I failed to observe any re-emergence of foundresses from figs, but this possibility cannot be entirely discounted. Furthermore, if mites and nematodes are only transported by pollinator foundresses and then do not re-emerge from the figs the wasps have entered, there should be a positive relationship between foundress number and the likelihood of mites and nematodes being present in a fig. This was not the case with mites, where the number of foundresses in a fig did not influence the likelihood of mites being present (LRT, $\chi^2 = 2.13$, df = 1, P = 0.14; Fig. 3.8.), but was evident with the nematodes (LRT, $\chi^2 = 66.15$, df = 1, $P < 0.001$; Fig. 3.9.), where prevalence increased in line with foundress number (the apparent decline in prevalence among the figs with more than four foundresses is probably an artefact of the small number of figs entered by so many fig wasps). Only two figs with no pollinators present inside (out of 94) contained nematodes. These may have emerged from foundresses that had become trapped in the ostioles of the figs, and so had failed to enter.

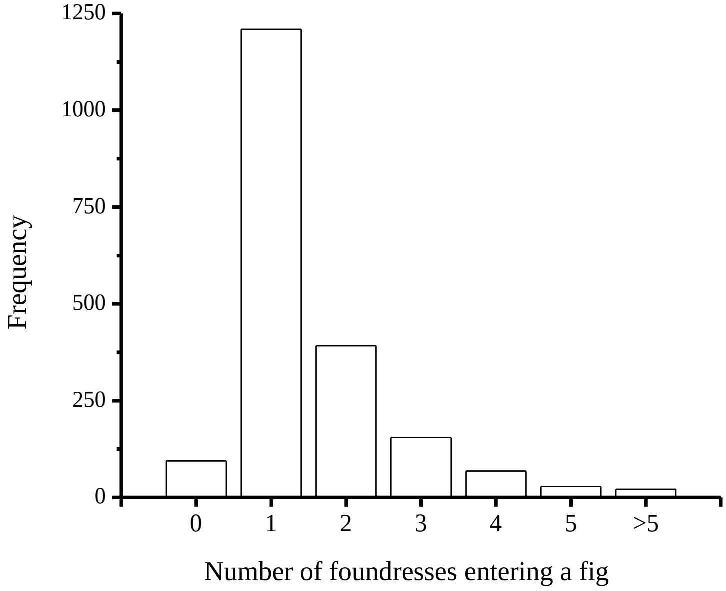


Fig. 3.7. Number of foundresses *Elisabethiella baijnathi* entering figs of *F. burtt-davyi.*

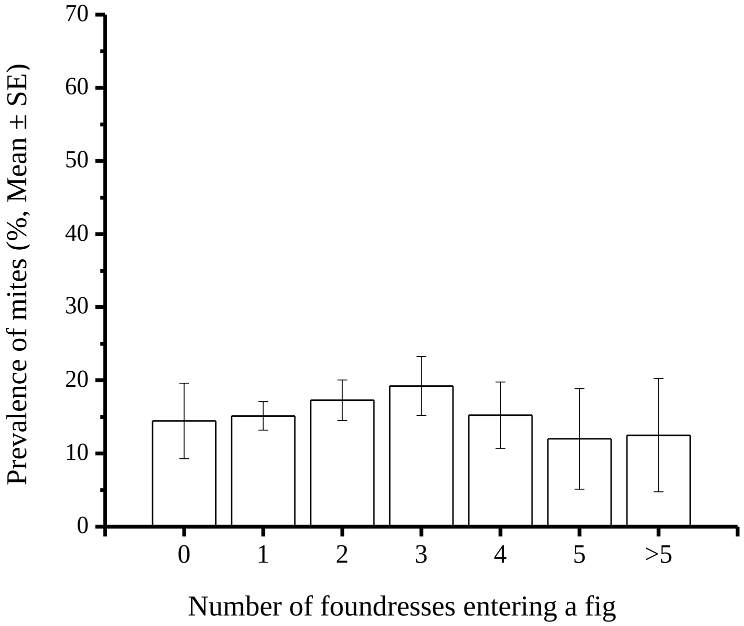


Fig. 3.8. Prevalence of *Tarsonemella* mites inside figs of *F. burtt-davyi* with different numbers of foundresses (Note that sample size varies between foundress categories).

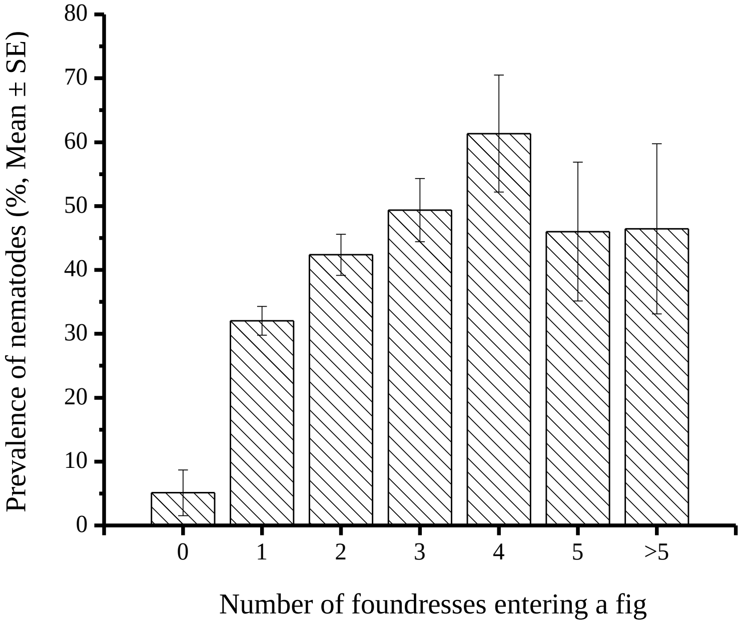


Fig. 3.9. Prevalence of *Parasitodiplogaster* nematodes inside figs of *F. burtt-davyi* with different number of foundresses (Note that sample size varies between foundress categories).

3.6 Discussion

Monoecious fig trees typically produce synchronized crops of figs, with inter-crop periods of several months. This fruiting pattern forces each generation of fig wasps to migrate between crops when seeking breeding sites. It also means that short-lived flightless animals such as mites and nematodes that can only reproduce inside figs need to utilize winged insects to transport them from crop to crop. The representatives of these two groups that breed in the figs of *F. burtt-davyi* differ in biology in that the mite is a phoretic phytophage whereas the nematode feeds on the body of its host and may be harmful to it (Herre 1993).

Both the mites and nematodes associated with *F. burtt-davyi* mainly use pollinator females for transportation between trees, as has been described for similar species elsewhere (Bronstein 1991; Pereira, Semir and Menezes Junior 2000; Walter 2000; Cardona, De Ulloa and Kattan 2007; Krishnan et al. 2010), but a small proportion of *Tarsonemella* sp. females were also found to attach themselves to NPFW and may be transported by them. The ability of this mite to walk between figs and independently enter them means that any NPFW females can potentially carry the mites to suitable host trees, as it is not necessary for vectors to carry the mites into the figs. Because species such as *Sycoryctes* sp. however are attracted to trees with older figs, that already contain pollinator larvae (Compton 1993), they will still represent poorer quality vectors.

The ability of the mite to move between figs is probably more significant in terms of allowing secondary spread of individuals between figs on the same tree. This ability is absent in the nematode *Parasitodiplogaster* sp., yet the nematode was present in more figs than the mite, which suggests it achieves higher vector colonization rates. Most of the *F. burtt-davyi* figs were pollinated by a single fig wasp foundress, a situation that selects for reduced nematode virulence, because if the nematodes kill their host too quickly then the fig wasp will fail to reproduce and there will be no offspring to transport the next generation of nematodes (Herre 1993). *Parasitodiplogaster* sp. prevalence was greater in figs with several foundress fig wasps and if the nematodes have any negative impact on their hosts then it should increase selection on foundresses to avoid figs that have already been entered by other females, because this will reduce the likelihood of their offspring being infected by nematodes.

Chapter 4 Interaction between nematodes, figs, and fig wasps inside the figs of *Ficus hispida*

4.1 Abstract

Female pollinating fig wasps (Agaonidae) transport nematodes into receptive figs when they enter them to lay their eggs. Despite numerous taxonomic studies of nematodes associated with figs, their ecology is poorly understood. This study examined the nematodes carried by *Ceratosolen solmsi marchali* into figs of *Ficus hispida* in Sumatra, Indonesia. *Ficus hispida* is dioecious, with male trees where pollinator offspring develop and female trees with figs where only seeds are produced. Three nematodes routinely developed inside figs of both sexes: C*aenorhabditis* sp., *Schistonchus centerae*, and *S. guangzhouensis*. *Schistonchus* spp. are plant parasitic nematodes that feed on flowers within the figs. No *Caenorhabditis* species have previously been reported from figs. Congeneric species feed on bacteria. *Caenorhabditis* sp. was transferred between figs as juveniles, whereas *Schistonchus* spp. were transferred mostly as juveniles, and occasionally as adults. Peak populations of nematodes occurred in D-phase figs, when the next generation of fig wasps were ready to emerge. The nematodes in male figs attached themselves mainly to female pollinators. All three species of nematodes developed successfully in female figs, but had no means of dispersal. They had no discernible negative impact on seed and pollinator offspring numbers.

4.2 Introduction

Fig trees (*Ficus* spp.) and their pollinating fig wasps (Agaonidae) have evolved together as partners in an obligate mutualism. Female fig wasps are the exclusive pollinators of the trees, while the trees provide the wasps with the ovules inside their figs where their young to develop (Galil 1977). The trees depend on these small wasps for pollination and pollen dispersal and the fig wasps depend on the trees for larval development. When the female wasps' offspring emerge from their galls and have mated they disperse in search of other figs, carrying pollen from their natal figs (Nason, Herre and Hamrick 1998; Kerdelhue, Rossi and Rasplus 2000; Harrison 2005). The ecology and evolution of the mutualism between fig trees and their pollinating wasps has been studied worldwide and is also discussed in general reviews of co-evolution (Anstett, HossaertMcKey and Kjellberg 1997a; Jousselin et al. 2003; Weiblen 2004; Compton, Grehan and van Noort 2009; Compton et al. 2010; Cook and Segar 2010; Cornille et al. 2012).

Ripe figs serve as a source of food for many frugivorous vertebrates, especially in tropical forests (Compton, Wiebes and Berg 1996; Bai, Yang and Compton 2008; Krishnan et al. 2010). Many fig trees produce figs all year around, and together with their large crop sizes, this means that they are consumed by more species of animal than the fruits of any other plants (Shanahan et al. 2001). Figs also provide an appropriate environment for many different organisms to live in. These include non-pollinating fig wasps (NPFW), including gallers and parasitoids (Chen et al. 2012). Ecologically and taxonomically the NPFW are similar to the pollinating wasps, as they also belong to the Chalcidoidea, and their life cycles also coincide with those of the pollinator's (Compton and Hawkins 1992; Kerdelhue, Rossi and Rasplus 2000), but only rarely do they have a mutualistic interaction with their host plants. Mites and nematodes are also found in some figs (Compton 1993; Pereira, Semir and Menezes Junior 2000; Jauharlina et al. 2012) as well as microrganisms such as fungi (Lachaise 1977) and also protistans (J. Jauharlina and S.G. Compton, unpublished).

Pollination within the figs, both in monoecious and gynodioecious (functionally dioecious) species, almost always totally depends on female fig wasps belonging to a single family, Agaonidae (order Hymenoptera) (Wiebes 1979; West et al. 1996; Kerdelhue, Rossi and Rasplus 2000). Female pollinator fig wasps enter young figs through a narrow ostiole, where they pollinate and simultaneously lay eggs in some of the female flowers. Their entry into the figs means that they are potential vectors for any other organisms that are on or in the female fig wasps at that time (Compton 1993; Krishnan et al. 2010; Jauharlina et al. 2012). Nematodes are typically transferred by female pollinating wasps into figs when the wasps enter to lay their eggs and pollinate the flowers. They develop and reproduce inside the figs and their offspring are ready to attach themselves again to female pollinators when the new generation of pollinators are ready to leave the fig (Compton 1993; Krishnan et al. 2010). The female pollinators are often chosen specifically by nematodes to transfer them between figs because male fig wasps and most female NPFW do not enter figs to oviposit (Kerdelhue, Rossi and Rasplus 2000; Proffit et al. 2007). Female pollinators are appropriate and reliable ‘vehicles’ for the nematodes to use to disperse, since males are usually wingless and die within the figs. When the pollinator females are about to emerge from the galls, some nematodes are found to aggregate in male flowers (Vovlas, Inserra and Greco 1992). This condition may enhance the possibility for the nematodes to be attached to the female wasps as some wasps actively collect pollen on their way out of the figs.

Some fig trees are monoecious, while others are morphologically gynodioecious but functionally dioecious (Berg 1989; Verkerke 1989). In monoecious fig trees, individual figs have both female and male flowers that produce viable seeds as well as pollen and fig wasps. In dioecious fig trees, individual trees produce either viable seeds (female trees) or pollen (male trees) (Janzen 1979b; Verkerke 1989). In dioecious fig trees, the female wasps that enter the figs of a female tree ensure pollination and seed production. However, due largely to the structure of the flowers, they cannot lay eggs and die without reproducing. On the other hand, when the wasps enter the figs on male trees, they can lay eggs and complete their life cycle within the female flowers. The flowers do not produce seeds. Thus, in dioecious fig trees, some trees are female and produce seeds, while some other trees are male and produce pollen and pollen-carrying wasps (Anstett, HossaertMcKey and Kjellberg 1997a). Despite the difference in the tree’s function, nematodes are expected to be transferred into figs on female trees. Any nematodes that are transported by pollinating wasps into female trees will however, be facing a ‘dead end’, since the wasps will not reproduce in these trees, thus there will be no way out for the offspring of any nematodes. Unlike nematodes, no phoretic mites have been described so far from dioecious fig tree species. It is unclear why dioecious fig tree species have prevented colonization by mites and not by nematodes (Jauharlina et al. 2012).

Female NPFW are unlikely to be chosen by nematodes to transfer them between figs, because they usually lay their eggs from the outside of the figs (Proffit et al. 2007). Nematodes are not known to be transferred into figs via the ovipositors of NPFW (Vovlas and Larizza 1996; Krishnan et al. 2010; Jauharlina et al. 2012). *Shistonchus caprifici* Gasperrini is a phytophagous nematode that feeds and reproduces in male figs of the edible fig, *Ficus carica* L. This nematode is known to enter both pollinator and NPFW females, but there is no evidence that those transported inside NPFW ever manage to enter figs and reproduce (Vovlas and Larizza 1996).

The presence of nematodes has been recorded in the figs of many species of fig trees in different countries (Compton 1993; Giblin-Davis et al. 1995; Martin, Owen and Way 1973; Herre 1995; Vovlas and Larizza 1996; Kanzaki et al. 2009). Different species of nematodes were found to be associated with several species of fig trees and fig wasps in Africa (Martin, Owen and Way 1973). Nematodes belong to several genera including *Parasitodiplogaster* (Diplogasteridae) and *Schistonchus* (Aphelenchoidae). They have two different feeding styles. *Parasitodiplogaster* species are fig wasp predatory nematodes that develop at the expense of female pollinators either shortly before or after they have died. The nematodes mate and produce infective juveniles inside the figs that infect the next generation of female wasps as they leave to find new figs (Jauharlina et al. 2012; Poinar and Herre 1991; Giblin-Davis et al. 2006). The nematodes feed on the corpse of the female after she has laid her eggs, with varying levels of negative impact of pollinator fecundity (Herre 1993). In contrast, *Schistonchus* species are plant-parasitic nematodes that feed on the epidermis of flowers inside the figs, but also typically attach themselves onto the offspring of pollinator females to transport them from natal figs (Vovlas, Inserra and Greco 1992; Vovlas and Larizza 1996; Giblin-Davis et al. 1995; Gulcu et al. 2008).

Other nematodes associated with figs include the diplogastrid *Myctolaimus racemosa* (Anand 2005*)* and *Ceratosolenus hyderabadensis* (Cylindrocorporidae) that are associated with the pollinating fig wasp *Ceratosolen* sp. inside the figs of *Ficus racemosa* in India (Anand 2006). Another species belongs to the genus *Teratodiplogaster* (Diplogastridae) which is phylogenetically close to *Parasitodiplogaster*. *Teratodiplogaster fignewmani* is found in *Ficus racemosa* figs in Australia (Kanzaki et al. 2009). Another species of *Teratodiplogaster* has been described as *T. martini* From a *Ficus* sp. collected from Africa (Martin, Owen and Way 1973; Kanzaki et al. 2012a)

There are over 800 species of fig trees in the world, mainly distributed in tropical and subtropical regions, and only a small fraction of them have been checked for nematodes (Davies et al. 2013). The presence of stylets in nematodes is the common way to identify the nematodes as plant feeding species. Despite the variation in its length and shape, the stylet is consistently used as a specialized spear by nematodes to feed on many different parts of plants, including roots, stems, leaves, flowers and seeds. Nematodes use the stylet to puncture plant cells, to withdraw food and also to secrete proteins and metabolites that aid the nematode in utilising the plant (Lambert and Bekal 2002). Species of *Schistonchus* are widespread and apparently host tree specific, being found associated with many different fig tree species, including *S. caprifici* on *F. carica* in Italy (Vovlas, Inserra and Greco 1992), *S. aureus* on *F. aurea*, *S. laevigatus* on *F. laevigata* in Florida, USA (DeCrappeo and Giblin-Davis 2001), *S. africanus* on *F. thonningi* in South Africa (Vovlas et al. 1998), *S. aculeata* on *F. aculeate, S. virens* on *F. virens, S. benjamina* on *F. benjamina* in Australia (Bartholomaeus et al. 2009; Davies et al. 2010), *S. guangzhouensis* and *S. centerae* on *F. hispida, plus S. hirtus* on *F. hirta* in China (Zeng, Giblin-Davis and Ye 2007; Zeng et al. 2010; Bartholomaeus et al. 2012). These studies show that multiple species of the same genus can be associated with figs of a single fig tree species, as well as nematodes belonging to different genera.

The fig–fig wasp–nematode system is a triangular and potentially complex relationship. Despite numerous studies of nematode taxonomy, little is known about the ecology of nematodes associated with figs and fig wasps, nor the effects of nematodes in relation to the pollination mutualism. In this chapter, I address the following questions in relation to the nematodes associated with a dioecious fig tree, *F. hispida*, in Indonesia:

1. How many nematode species are present locally, and how does their abundance vary?
2. What are their life cycles and can they develop inside female figs?
3. How does pollinator foundress number affect numbers of nematodes within a fig?
4. What is the impact of nematodes on seed and pollinator production?
5. Which non-pollinating fig wasps are present, and are they also used as nematode vectors or do the nematodes only seek out female pollinators for transmission?
6. How frequently do newly emerged female pollinators carry nematodes with them? And how many do they carry?

4.3 Study species

Ficus hispida L., sometimes known as the hairy fig, belongs to subgenus Sycomorus, and section Sycomorus (Fig. 4.1.). Anatomically it is gynodioecious, and functionally a dioecious fig tree with distinct male and female trees. It is distributed throughout India, Nepal, Laos, Thailand, Malaysia, southern China (Yang et al. 2002), Sri Lanka, Myanmar, New Guinea, Australia, Andaman island (Ali and Chaudhary 2011), and Indonesia. It is a shrub or moderate-sized free standing tree up to 13 metres tall, with spreading branches. A common road-side species, they grow in evergreen forest and in moist localities such as banks of streams in deciduous forests, to an elevation of 1800 m above sea level. Almost all parts of this tree are commonly used as a folklore remedy for the treatment of various illnesses. Sometimes *F. hispida* is also cultivated for shade and its edible fruits (Ali and Chaudhary 2011; Murti, Lambole and Panchal 2011). This tree also plays an important role in restoration and renewal of tropical rainforests after they have been devastated naturally or artificially (Yang et al. 2002; Kuaraksa and Elliott 2013). *Ficus hispida* is a pioneer species that prefers high light conditions and is drought-tolerant. It appears early during land restoration towards secondary forest. The growth of *F. hispida* forms a shady micro-environment that allows shade-enduring and warm-preferring plants to grow vigorously. When other trees have grown up, they would replace *F. hispida* gradually. The succession continues to eventually develop into tropical rainforest (Yang et al. 2002; Corner 1967).

Fig. 4.1. *Ficus hispida* male tree (H1) with A-phase figs. This tree grew within dry area with several medium size rocks surrounding it.

Fig. 4.2. Different phases of figs of *Ficus hispida* on a male tree (H23).

Like many other dioecious fig trees, *F. hispida* sometimes shows asynchronous fruiting within a plant, with different phases of figs found at the same time on the same tree (Fig. 4.2). Being dioecious, *F. hispida* has individual trees that are functionally female, bearing figs with only female flowers which only produce seed. Other trees bear figs with male and female flowers, produce pollen and wasps, and are functionally male. In female trees, female pollinators enter figs and pollinate the flowers, but fail to lay eggs because the figs contain only long-styled female flowers and the wasps' ovipositors cannot reach the ovules. Hence, the female trees only produce figs with seeds inside. For the female trees, seed production represents the only component of reproductive success. The pollinators that enter figs of male trees, which contain male and only "gall" flowers (short-styled female flowers), can oviposit in any of the flowers. No seeds develop even if the flowers are pollinated. The figs produce fig wasp offspring and pollen that the female offspring can transport to other trees (Galil 1977; Weiblen, Yu and

West 2001; Harrison and Yamamura 2003a). The newly emerged female pollinators disperse to find receptive figs, and the life cycle is started again. If the wasps enter a fig on a male tree, they will be able to reproduce, but their natal tree receives no benefit in terms of ovules pollinated with the pollen the wasp was carrying. However, if the wasps enter a fig on a female tree, the wasps do not reproduce, but the natal tree benefits via the production of seeds fertilized with its pollen (Kjellberg, Doumesche and Bronstein 1988; Patel and Hossaert-McKey 2000); (Machado et al. 2001). Thus, the number of female pollinator offspring emerging from male trees represents the potential fitness for both the parent wasps and the male fig tree (Herre 1989; Patel and McKey 1998; Machado et al. 2001).

Figs of *F. hispida* are often borne in long clusters lying on the ground at the base of the tree, as well as higher up on the trees. They have a green colour during earlier phases and become bright yellow at later phases (Fig. 4.3.). Figs in earlier phases release more sticky white latex when they are dissected, which becomes less in later phases. The fig shows a single layered epidermis covered with thick cuticle having a few unicellular trichomes (Fig. 4.4.). The epidermis is underlain by four to six layers of hexagonal to polygonal collenchymatous cells (Ali and Chaudhary 2011).

Fig development is divided into distinct stages (Galil and Eisikowich 1968) with a slight modification among dioecious figs (Valdeyron and Lloyd 1979) (Fig. 4.5.). The youngest phase of the figs is Phase A which is a pre-receptive phase when the ostiolar bracts are still closed and the female wasps cannot enter the fig. The next phase is Phase B which is the receptive phase when the ostiolar bracts are loose so that the female pollinator can enter into the fig. Female pollinators are attracted to B-phase figs by species-specific volatiles release when the female flowers inside the fig are mature and ready to be pollinated (van Noort, Ware and Compton 1989; Chen and Song 2008). The following inter-floral (C) phase is the longest phase, and is when the progeny of wasps (male trees) and seeds (female trees) complete their development. In D-phase figs, having mated within their natal galls (one fig wasp develops in each galled ovule), female pollinator offspring of the next generation actively load pollen into their pollen baskets, and are then ready to leave the fig to fly away and find other receptive figs. Figs from female trees do not have this phase, as the wasps do not develop inside the figs. Figs of female trees then ripen and are attractive to seed dispersers (E-Phase). Male figs do not ripen in the same way and eventually fall from the tree. Like many other fig trees belonging to subgenus Sycomorus, C-phase figs of *F. hispida* often contain noticeable amounts of liquid (Berg and Corner 2005). The function of the liquid is unclear (Compton and McLaren 1989).

The pollinating wasps of *F. hispida* are *Ceratosolen* species. It is likely that two or more pollinator species are associated with this fig tree. They are variously identified as *Ceratosolen marchali* Mayr in India (Abdurahiman and Joseph 1976), and *Ceratosolen somlsi marchali* Mayr in Xishuangbanna, China (Yang et al. 2002; Chen and Song 2008). However, in Hainan, China, the pollinator of *F. hispida* was named as *Ceratosolen solmsi* (Wang et al. 2013a). Here, the pollinator of *F. hispida* is referred to throughout as *C. solmsi marchali* as this is the only subspecies/species recorded from Indonesia (Davies et al. 2013).

Fig. 4.3. Figs of *F. hispida* borne in clusters on a male tree (H3).

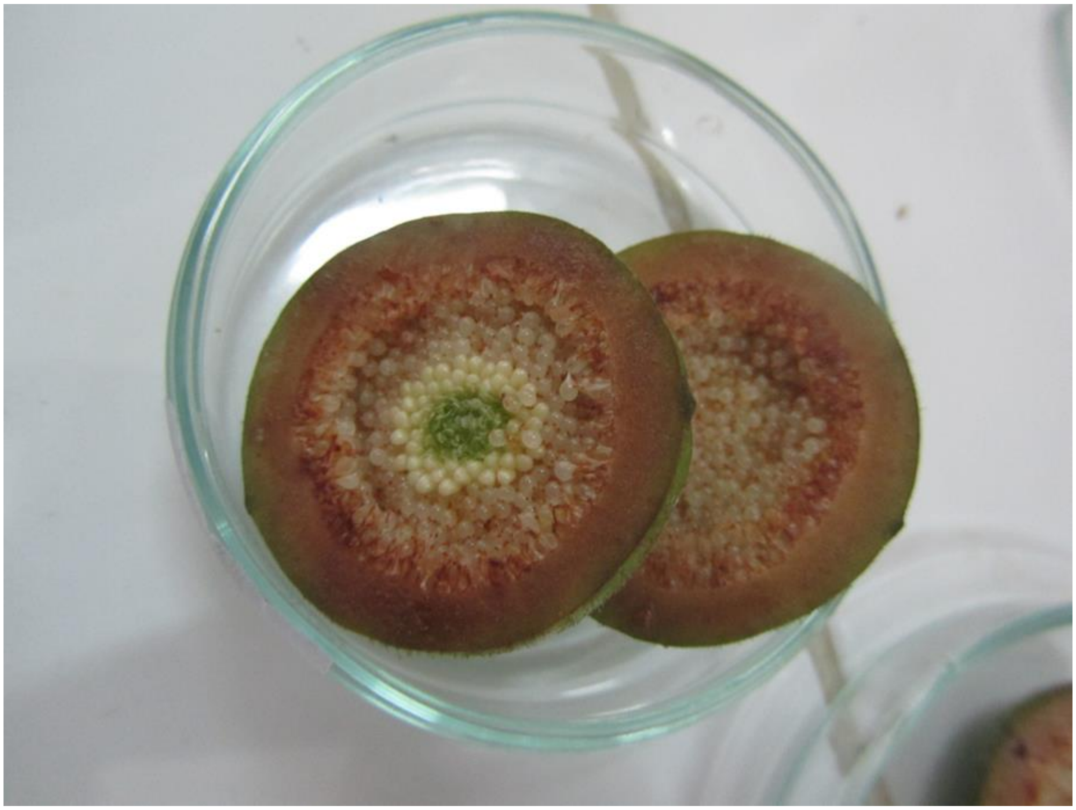


Fig. 4.4. A male (C-phase) fig of *F. hispida* cut into two halves, with the ostiole in the centre of the upper fig and a ring of male flowers around the ostiole.

Fig. 4.5. Male (top) and female (bottom) figs of *F. hispida*.

4.4 Methods

4.4.1 Study sites

The study sites for routine sequential sampling of the fig trees were located in the northern part of Sumatra Island in Aceh Province, Indonesia (Fig. 4.6.). The study was undertaken for 19 months from July 2011 until February 2013. The trees grew along the road in mountainous areas of Leupung and Lhoong Districts, about 25 km to 40 km from the provincial capital, Banda Aceh (Fig. 4.7). *Ficus hispida* trees are common in this area, growing along the road, where villagers grow vegetables such as chilies, tomatoes and maize, within villages and in the mountains. The trees chosen for sampling were mature trees with figs that were easy to access. They were between 4 and 12 meters in height, and 10-27 cm in diameter at one metre above the ground (Table 4.1.). The trees usually grew in clumps of several individuals, with both female and male trees present (Fig. 4.8.).

Meteorological information for the area was obtained from Blang Bintang Station, the closest Meteorological Station under the Indonesian Meteorological and Geophysical Agency, located about 35 km from the study area. The region has a tropical climate that supports rainforest vegetation, with fairly constant average temperatures throughout the year and little diurnal variation. The annual average temperature is 27 ^{o} Celsius. Monthly minimum and maximum temperatures are quite stable, with only slight variation between months. The average minimum temperature ranged between 22.1 and 23.9^{o}C, while maximum temperatures were between 30.2^{o}C and 34.0^{o}C (Fig. 4.9.). The year is divided into wetter and drier seasons. In the last ten years, July and August have been the driest months with monthly precipitation of less than 60 mm, while the wettest months have been from November to January, with more than 150 mm of precipitation each month. The number of days with rain each month ranged between nine days in August and 18 days in December (Fig. 4.10).

Fig. 4.6. Map of Indonesia, with Aceh Province highlighted. The arrow indicates where sampling took place.

Fig. 4.7. Sampling locations of *F. hispida.*

Table 4.1. Sampled trees and location coordinates of *Ficus hispida*.

No	Tree code	Tree sex	Circumference (cm)	Diameter (cm)	Height (m)	Location coordinates (East)	Location coordinates (North)
1	H1	male	54	17.2	5	95^{o} 15’ 34.92”	05^{o} 22’ 55.68”
2	H2	male	64	20.4	6	95^{o}15’ 31.56”	05^{o} 22’ 45.54”
3	H3	male	78	24.8	10	95^{o}14’ 30.21”	05^{0} 20’ 39.33”
4	H4	male	67	21.3	8	95^{o}14’ 37.02”	05^{o} 20’ 39.48”
5	H5	male	47	15.0	10	95^{o}14’ 33.42”	05^{o}20’ 38.53”
6	H6	female	56	17.8	10	95^{o}14’ 29.48”	05^{o} 20’ 49.94”
7	H7	male	62	19.7	10	95^{o}14’ 32.43”	05^{o} 20’ 42.93”
8	H8	female	57	18.1	4	95^{o}15’ 13.20”	05^{o} 18’ 57.00”
9	H9	female	42	13.4	4	95^{o}15’ 16.81”	05^{o} 19’ 52.10”
10	H10	male	36	11.4	7	95^{o}15’ 11.74”	05^{o} 19’ 49.55”
11	H11	male	35	11.1	7	95^{o}15’ 14.40”	05^{o} 19’ 58.58”
12	H12	female	40	12.7	4	95^{o}15’ 12.68”	05^{o} 19’ 53.09”
13	H13	male	50	15.9	6	95^{o}15’ 13.50”	05^{o} 19’ 55.16”
14	H14	female	40	12.7	12	95^{o}15’ 03.94”	05^{o} 19’ 23.00”
15	H15	male	84	26.7	10	95^{o}15’ 10.64”	05^{o} 19’ 20.13”
16	H16	female	42	13.4	10	95^{o}15’ 06.34”	05^{o} 19’ 18.10”
17	H17	male	45	14.3	10	95^{o}15’ 0 7.68”	05^{o} 19’ 23.29”
18	H18	male	30	9.5	4	95^{o}15’ 07.31”	05^{o} 19’ 26.27”
19	H19	female	32	10.2	8	95^{o}15’ 10.49”	05^{o} 19’ 24.78”
20	H20	female	40	12.7	10	95^{o}15’ 20.61”	05^{o} 19’ 16.83
21	H21	male	58	18.5	7	95^{o}15’ 23.74”	05^{o} 19’ 16.33”
22	H22	male	52	16.6	8	95^{o}15’ 21.52”	05^{o} 19’ 11.28”
23	H23	male	50	15.9	8	95^{o}15’ 19.45”	05^{o} 19’ 14.44”
24	H24	female	52	16.6	9	95^{o}15’ 18.91”	05^{o} 19’ 11.59”
25	H25	female	42	13.4	4	95^{o}14’ 32.10”	05^{o} 21’ 15.10”
26	H26	female	48	15.3	5	95^{o}15’ 05.88”	05^{o} 21’ 58.86”
27	H27	male	30	9.5	6	95^{o} 15’ 13.00”	05^{o} 21’ 56.80”
28	H28	male	35	11.1	8	95^{o} 14’ 47.90”	05^{o} 21’ 17.40”
29	H29	male	40	12.7	8	95^{o} 14’ 37.50”	05^{o} 21’ 15.50”

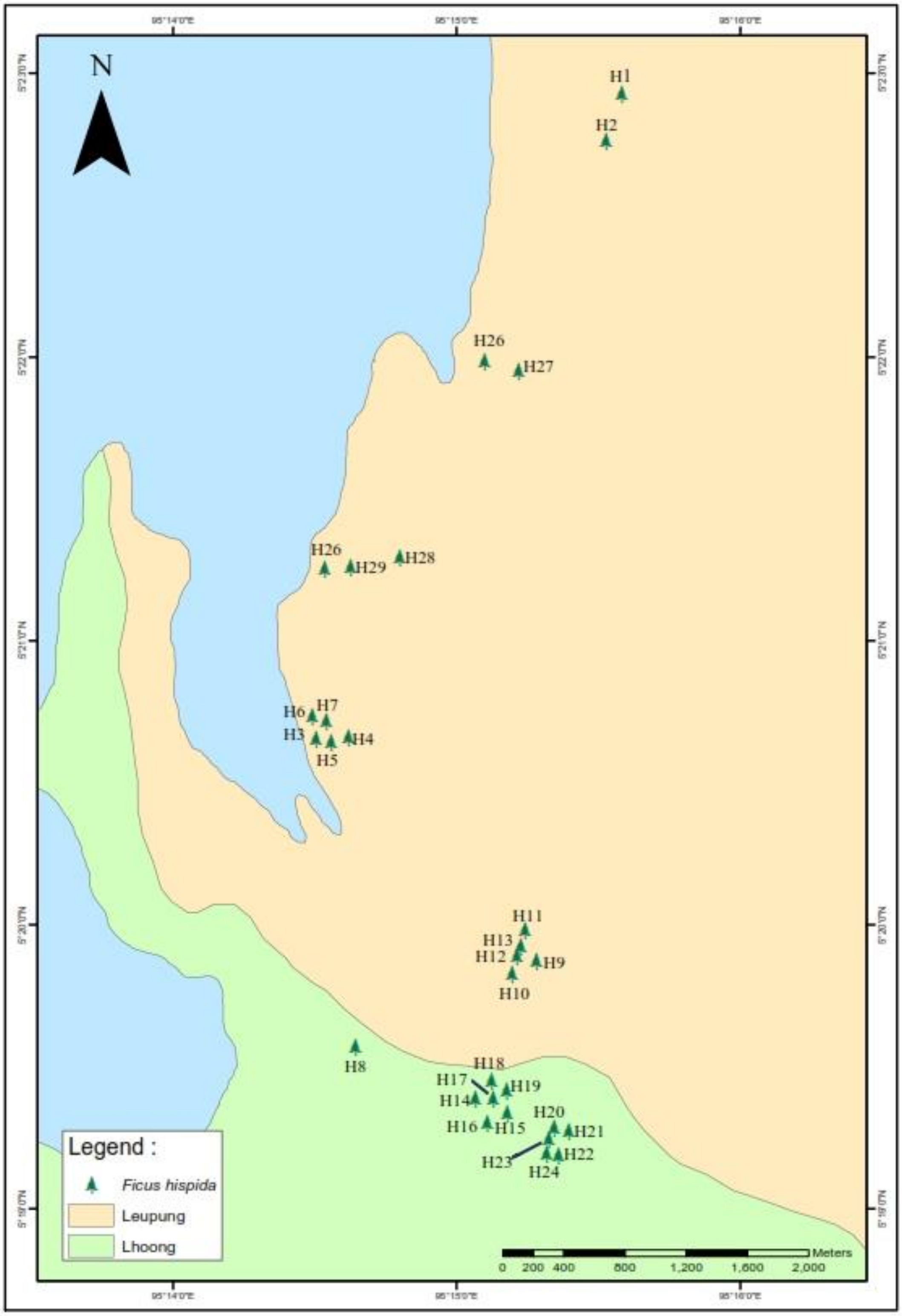


Fig. 4.8. Sampled trees of *F. hispida.*

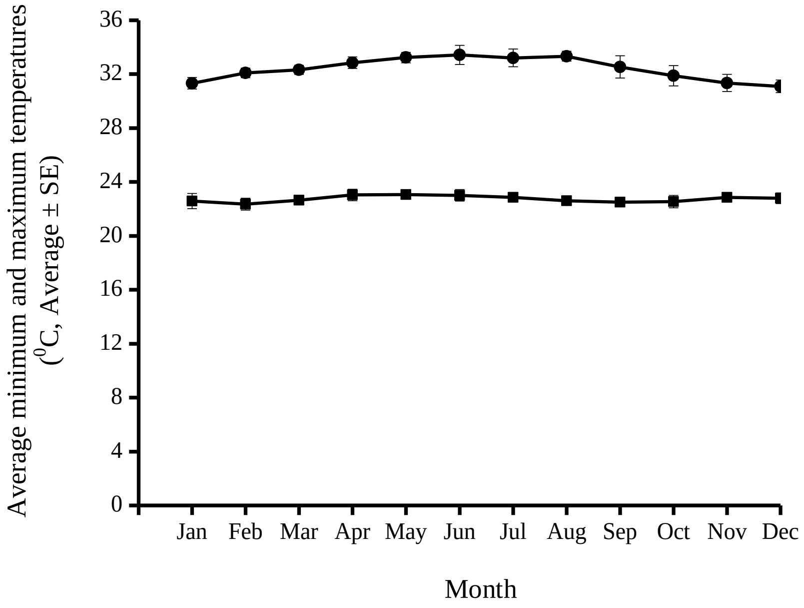


Fig. 4.9. Average monthly minimum (lower line) and maximum (upper line) temperatures for the years 2002-2012 at the study site: Leupung, Aceh Province, Sumatra, Indonesia.

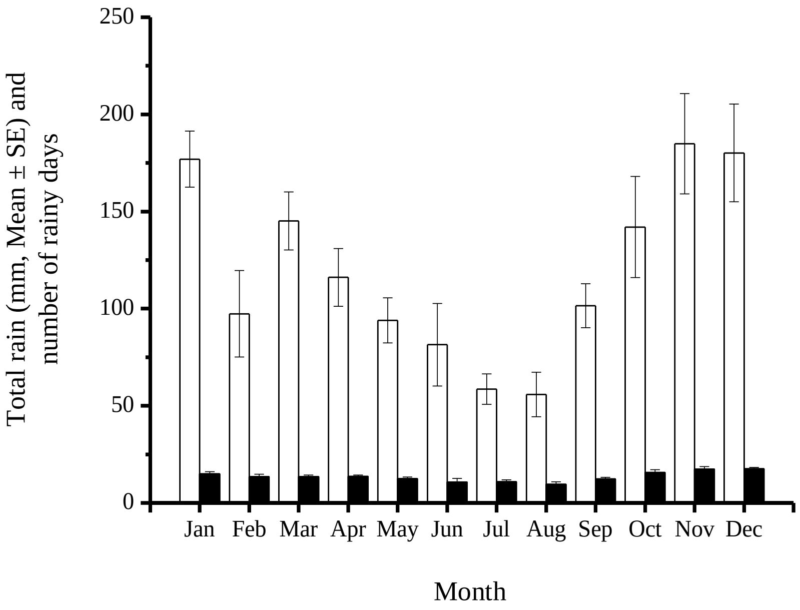


Fig. 4.10. Total monthly rainfall (open bars, Mean ± SE) and number of days with rain (filled bars) in the years 2002-2012 at the study site: Leupung, Aceh Province, Sumatra, Indonesia.

4.4.2 Routine sequential sampling procedures

Each tree was marked with black paint at the base and its location was recorded using GPS (Garmin GPS 60). During the course of the survey some of the trees were destroyed, due to road renovation or being cut by the villagers. Replacement trees were located and routine sampling was started, as necessary. All the fig samples were analysed in the Plant Disease Laboratory, Agrotechnology Department, Faculty of Agriculture, Syiah Kuala University, Banda Aceh, Indonesia.

A total of 29 *F. hispida* trees were marked (Table 4.1.), eight of which were used for weekly routine sampling and the others were used for ad hoc data collection. Five trees were not sampled, due to the absence of figs at particular times. For routine sampling, ten figs were sampled from each crop, from A-phase (when young figs first appear) through to E-phase (when wasp offspring had left the male figs, and female figs were soft). Some figs fell from the trees during sampling. If they were E-phase male figs, to prevent contamination from the soil, these figs were washed under running tap water or cleaned using a damp cloth.

For each fig, the developmental stage, the colour, and the diameter were recorded. On opening of the fig, the presence/or absence of liquid in the lumen was also observed. During B and C phases, the number of foundresses inside the figs was counted and the foundresses were then placed on glass slides in 40% sugar water. In younger figs where the foundresses were still intact (in B and early C-phases), they were placed individually on a glass slide. When the figs were in mid and late C-phases, the foundresses were still visible, but they were broken into pieces, so the fragments of all the foundresses were placed altogether on a slide. Any nematodes that emerged from the foundresess were observed and counted. The nematodes were counted within one hour of the fig wasps being placed on the slides and re-counted after 24 hours.

4.4.3 Fig extraction

The contents of each fig were extracted individually (Fig. 4.11.). The figs (including liquid if any) were placed in extraction funnels. The Baermann funnel method was used to extract the nematodes from the figs, adapted from Sriwati, Takemoto and Futai (2006) after preliminary trials. In preparation, the elastic pipes connected to the lower parts of the funnels were tightened to prevent the liquid coming out through the funnel. 60 ml of distilled water was poured into each funnel (this volume of water was used because it filled the funnel).

The figs were each cut into six to eight pieces. Each fig went into one extraction funnel. These pieces were placed onto a layer of fabric screen (60 mesh in size) which covered the funnel mouth and soaked in water inside the funnel. All the fig pieces were submerged.

In preliminary trials, the figs were left in the funnel for 24, 48, and 72 hours, and the liquid extracts were removed. Any nematodes that had not gone down and were left in the upper part of the tunnel were counted for each period of time. The number of nematodes that remained on the upper part of the funnel was less than nine for each period of time. Therefore I decided to adopt a 24 hour period to leave the cut figs on the funnels. By this time all the nematodes had gone down through the fabric and were clumped together in the base of the funnel. Leaving the system for only 24 hours had the additional advantage that fewer nematodes had died.

After 24 hours, the liquid was removed by opening the base of the funnels and was put into 20 ml reaction tubes (around 75 % of the tube capacity). After several trials, it was decided to leave the liquid in the tube for 3 hours. It was found that 2 hours was not long enough for all the nematodes to settle at the bottom, whereas after 3 hours there were no more nematodes swimming up to the liquid surface.

The upper part of the liquid was then removed using a small pipette so that only 5 ml liquid was left (each tube was marked with a line that indicated 5 ml). The process of taking the upper liquid was done carefully with the tube kept still, so that the nematodes stayed at the bottom. The 5 ml liquid left at the bottom of the tube (the fig extract) was observed under a microscope. The upper liquid from different tubes removed was checked several times to confirm that there were no nematodes present.

For nematode observations, one ml extracts were put on a one ml capacity nemacytometer glass slide (counting slide) and observed under a microscope. The nematodes found were counted and identified. Unfortunately due to the structure of the *nematocytometer*, I was only able to magnify the specimens up to 100 times, which limited observation on small juvenile of nematodes. Observations were repeated on the rest of the extract, giving five observations from each fig. The number of nematodes that were counted in each observation was added together as the number of nematodes in one fig. To ensure that no nematodes were left in the funnel, the remaining liquid was checked occasionally using the same process.

Fig. 4.11. The steps employed for figs extraction.

4.4.4 Measurements of nematodes

Measurements of nematodes were done on adults of each nematode species found within the figs. The measurements were done at the end of the study when each species of nematode was recognized and identified. Additional samples of late C-phase figs were taken for this purpose. Late C-phase figs were chosen due to the large numbers of adult nematodes at this phase. Figs were wiped clean and cut into several pieces, soaked in water inside a Petri dish. After two hours, adult nematodes were hand- picked individually from the dishes and placed onto slides. Each slide was then run above a container containing hot water (60-70 0C) to make the nematode relax but not damaged, so that measurements could be made under microscope. Simple measurements of nematodes were made (Goodey 1963). The measurements were the length of the nematodes, from head to the tail tip (L), the width, the widest part of nematodes (a), the length of the stylet (if present) from head to the knob, the length of the oesophagous (b), the length of the tail from anus to the tail tip (c), Spicule/Vulva: the distance from the head to the genitalia of male and female nematodes respectively. Measurements were taken using a measuring eye piece attached to a compound microscope having 100 times magnification.

4.4.5 Identification of nematodes

Preliminary identification of nematodes was done by observing the presence of a stylet and/or bulb and the shape of the mouth and tail. The stages of nematodes found were also recorded. Identification of nematodes was done morphologically by comparing with the previous investigation of nematodes found on *F. hispida* in China (Zeng, Giblin-Davis and Ye 2007). Further identification was helped by another fig nematodes researcher from Australia (K.A. Davies, personal communication). One species of nematode was also identified using molecular methods by N. Kanzaki and R. Tanaka (Forestry and Forest Products Research Institute, Tsukuba, Japan).

4.4.6 Nematode development and their transportation between figs

Ten early D phase figs from each crop of five male trees were brought to the laboratory for observation. Upon returning to the laboratory, each fig was cut into two halves, and a number of galls were taken away to check their contents. Between 30 and 50 galls were opened up from each fig to collect at least 10 female pollinators, one from each gall. The contents of other galls that did not contain female pollinators were also recorded (whether non-pollinator fig wasps or male pollinators). The wasp was taken out from each gall and placed on a microscope slide with a small amount of water added, then covered with a fine cover glass on top. When the wasp was placed into the water and covered with a cover glass with a little pressure, the nematodes came out from the wasps. Nematodes that emerged from pollinators were counted and the nematode stages were recorded. These observations were conducted from five crops of early D-phase figs from different trees.

Searches for developing nematodes inside the larvae/pupae of the wasps inside the galls were done by sampling mid C-phase figs and dissecting them as above. Ten galls containing larvae were opened up separately and observed under microscope. This work was done on 5-6 figs from each crop, with five crops in total from five different trees.

4.4.7 Impact of nematodes on female pollinator offspring and seeds

I investigated whether the number of nematodes in each fig has a detectable impact on the numbers of female pollinators or seeds produced in the same fig. Five to six early D-phase figs from male trees were brought to the laboratory. Each fig then was washed with distilled water to remove any contamination from its outer surface. Each fig was cut carefully into eight pieces, and then placed into an extraction funnel to undergo the extraction process. After 24 hours, the number of nematodes in each fig was counted. The fig pieces were removed and each gall was taken away from the fig to observe its contents. The numbers of galls containing female pollinators were recorded for each fig. These data were collected from five crops from five different trees. The same procedures were employed on late C-phase female figs to investigate whether the number of nematodes produced in each fig was correlated with the number of seeds present.

4.4.8 Fig liquid and nematode numbers

Fig liquid is mostly found within mid or late C-phase figs of *F. hispida.* Six mid or late C-phase figs from male and female trees were sampled and washed to prevent any contamination. Each fig was cut into two halves; the liquid was taken using micropipette and placed into a square glass of 3x3 cm in dimensions with a shallow hole in the middle. Nematodes within the liquid was recorded and counted under a microscope. This was done using figs from five crops from five different male and female trees.

4.4.9 Community structure of emerging fig wasps

Five to six early D-phase figs from male trees were sampled. Each fig was placed separately inside a net bag 12 cm x 9 cm in size and tightened with a string to prevent the emerging wasps from flying out. The figs were left for 24 hours in the laboratory under room temperatures. The next day when all the wasps had emerged, the bags containing figs and the wasps were place inside a freezer for 15 minutes to kill them. To make sure that most of the wasps had emerged from the figs, I waited for one hour before placing the bags inside the freezer. All the wasps from each fig were separately placed into a small plastic bottle containing 70% alcohol. All the wasps from each fig were identified and counted. Five crops from five different trees were recorded.

4.4.10 Newly emerged wasps with nematodes

The presence of nematodes on wasps emerging from ten D-phase figs from a male tree was recorded. Each fig was placed separately inside a net bag of 9x5 cm in dimensions and tightened with a string to prevent the emerging wasp escaping. Upon returning to the laboratory, I waited until all the wasps had emerged from a fig. Usually, within one hour after the first emergence of a wasp, all the wasps have come out. Then I sampled randomly ten female pollinators, ten male pollinators, and ten of each species of non-pollinators from each fig. When fewer than 10 male pollinators or non-pollinators were present, all those available were examined. Each wasp was placed individually onto a glass slide with a drop of water. Nematodes emerging from the wasps were counted within one hour, and counted again after 24 hours to check if anymore nematodes emerged from the wasps. I did these observations on five crops from five different trees.

4.4.11 Nematodes on female pollinators arriving at B phase figs

Open net bags with a dimension of 20 cm x 20 cm were secured to a 1.5 m long wooden stick. The net was swung three times near clusters of figs on male trees that were mostly at B-phase. Five net bags were used to catch the wasps from each crop. The net bags with wasps trapped inside were then brought to the laboratory for further observations. From each bag I sampled 10 female pollinators, and placed them separately onto glass slides with 40% sugar water. Emerging nematodes from the wasps were counted within one hour, and counted again after 24 hours. Fig wasps were collected from five crops which came from five different trees.

4.4.12 Data analysis

Crop duration was analysed by linear model since only one crop was observed in each tree. Number of seeds was analysed as a continuous variable with normal errors because the mean was high, and analysed by linear mixed effect models. The analysis was using *nlme* package. Assumptions were checked by looking at the normality of residuals. For count data (number of foundresses, number of nematodes on different number of foundresses, number of nematodes in D-phase, number of galls containing wasps, number of nematodes in emerging and arriving wasps), analysis were done using *lme4* package. Poisson distribution was used as error. Each of this analysis was over dispersed. This problem was solved by including fig identity as individual level random effect. The *lme4* packaged was also used to analyse proportion data including proportion of total adult nematodes, proportion of adult of each nematode species, proportion of female pollinator having nematodes (in emerging or arriving pollinators). The models were analysed using binomial error structures. Linear mixed effect models were applied (except for crop duration), because the crops were nested within the trees, generating both fixed and random factors. Temperatures and rainfall were treated as fixed effects for crop duration analysis. Numbers of foundresses present in B-phase figs was treated as a fixed effect for analysing the number of nematodes in B-phase figs. The tree-type (male or female) was treated as a fixed effect for analysing the number of total nematodes and number of nematodes of each genus in D-phase. Crops were included in the models as random effects. The terms were considered to have significant effects at P-values <0.05 and are reported together with t-values, and degrees of freedom (df) when analysed using *nlme* or z-values when analysed using *lme4.* Crop and fig phase were treated as categorical explanatory variables for analysing proportion of adults in different crops and fig phases, both in male or female trees. Significance values were assessed using likelihood ratio tests (LRT). Crops or fig phases were considered to be significant at P-values <0.05. They were reported with P-value, degrees of freedom (df), and LRT χ^2 value.

4.5 Results

4.5.1 Seasonality

There was no clear pattern of seasonal variation of daily temperatures during the 19 months of study. The average daily minimum and maximum temperatures were relatively stable (Fig. 4.12.). The average minimum temperatures recorded daily ranged from 22.0 to 23.2 ^{o}C, while the average daily maximum temperatures were between 30.6 and 34.2 ^{o}C. Rainfall tended to be lower during June, July and August and highest in November 2011 and 2012. The lowest minimum monthly rainfall was recorded in July 2012 (16 cm), and the highest was found in November 2012 (161 cm) (Fig. 4.13.)

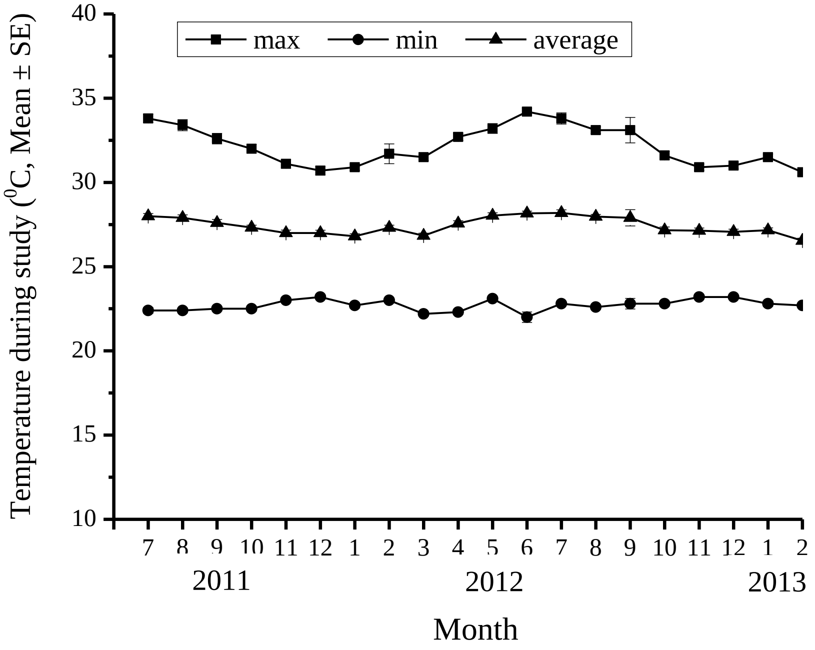


Fig. 4.12. Daily temperatures in the study area during the 19 months of study.

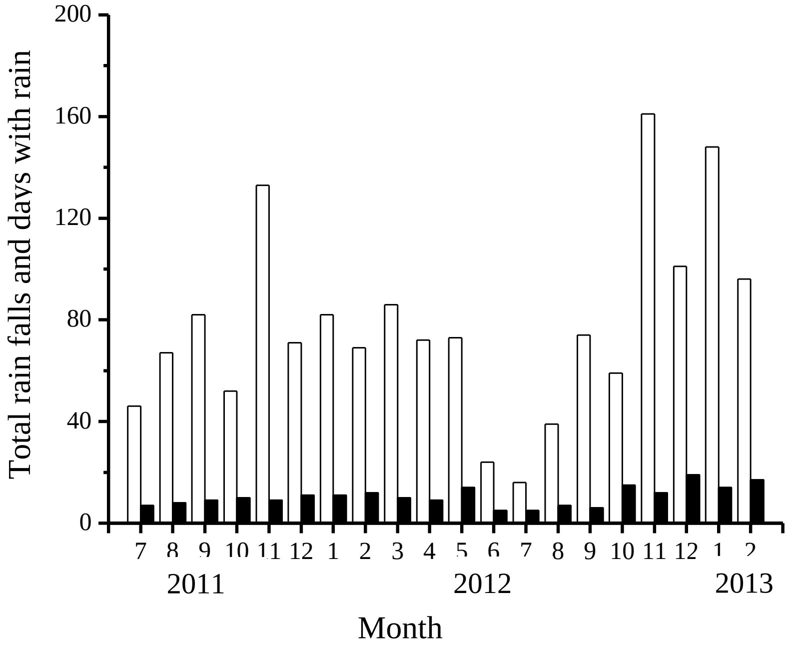


Fig. 4.13. Rainfall (open bars) and days with rain (closed bars) at the study area during the 19 months of study.

4.5.2 Fruiting phenology

During the period of study, I sampled figs sequentially at weekly intervals from four crops of figs from male and female trees. In total there were 183 male figs, and 186 female figs with nematodes extracted during this sequential sampling. The weekly sampling followed the full development of each crop. *Ficus hispida* shows asynchronous fruiting. The development of crops on each tree was almost continuous with overlap between consecutive crops. In many cases, D and E-phase figs were still on the trees when the new A-phase figs appeared. Additional samples for specific purposes involved 190 additional figs which came from 41 discrete crops were collected from both male and female trees.

The duration of crop development from A-phase until E-phase figs ranged from 49 up to 56 days for male trees, and 56-63 days for female trees. The average duration of crop development for male trees was 50.75 ±1.75 days (mean ± SE, N = 4), while female trees needed 57.75 ± 1.75 days (mean ± SE, N = 4) to complete the development of the crops. There was no significant difference between male and female trees in the time needed to complete the development of their crops (lm, $F_{(1,4)} = 1.276$, $P = 0.322$) (Fig. 4.14.). Crop duration was not affected by the relatively small differences in daily temperature during development (lm, $F_{(1,4)} = 0.252$, $P = 0.642$), or by monthly rainfall totals (lm, $F_{(1,4)} = 0.002$, $P = 0.968$) (Fig. 4.15. and 4.16.). B-phase was very brief in figs from male and female trees, with longer periods for A and C-phases (Fig. 4.17.). The figs on female trees had no D-phase, since there were no wasps that developed inside. Their figs matured (E phase) straight after a few weeks of C-phase. Male and female figs were similar in size. The size of male figs ranged between 1.3 and 1.8 cm in diameter when they were in A-phases, and between 3.2 and 4.1 cm in E-phases. Similarly female figs had a diameter ranged between 1.3 and 1.9 in A-phases, and between 3.1 and 4.0 cm in E-phases. They both had green colour during A-C phases, became green-yellowish in D-phase for the male figs (female figs do not have D-phase), and turned into bright yellow in E-phase.

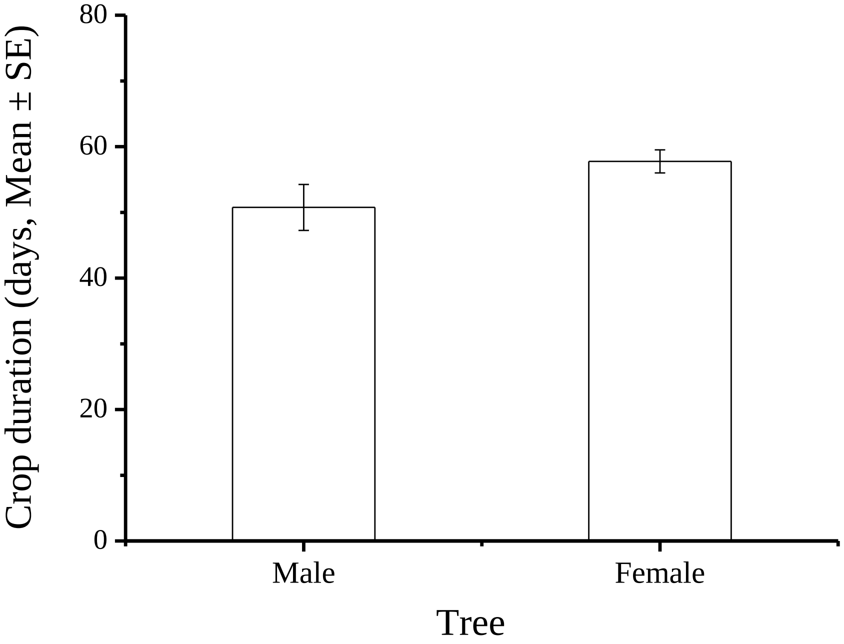
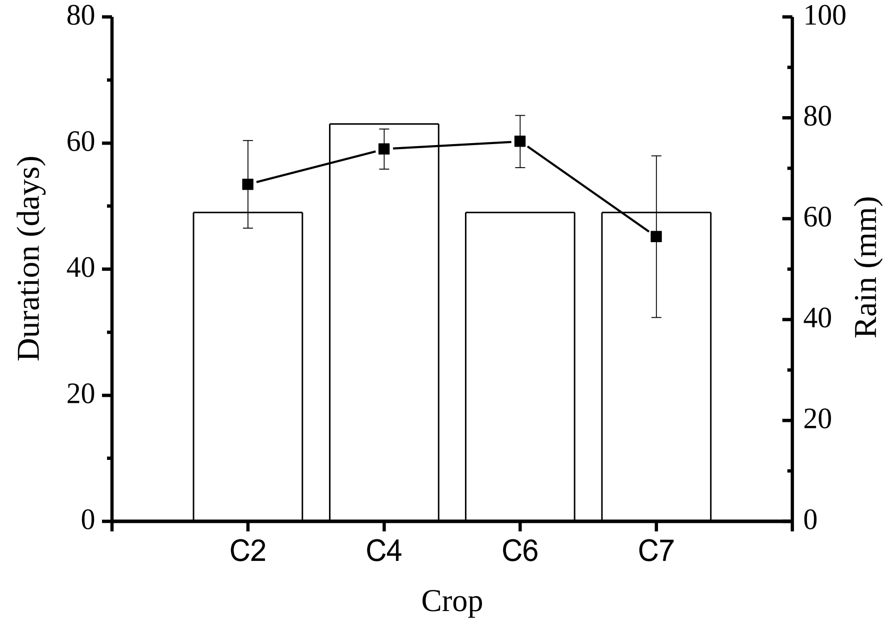


Fig. 4.14. Duration of crop development in *F. hispida* (from A to E phases) (N = 4 crops for each sex).



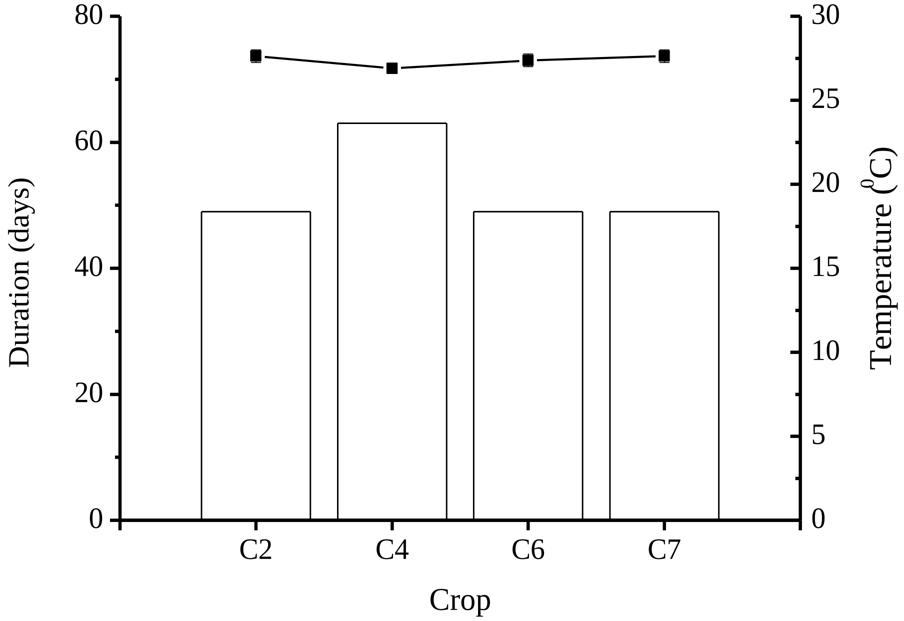


Fig. 4.15. Development times of each crop from male trees of *F. hispida* related to temperature (top) and rainfall (down). Open bars: crop duration; lines with error bars: temperature (top), rainfall (bottom). Standard error bars on mean temperature are not visible due to the very low variation of daily mean temperatures.

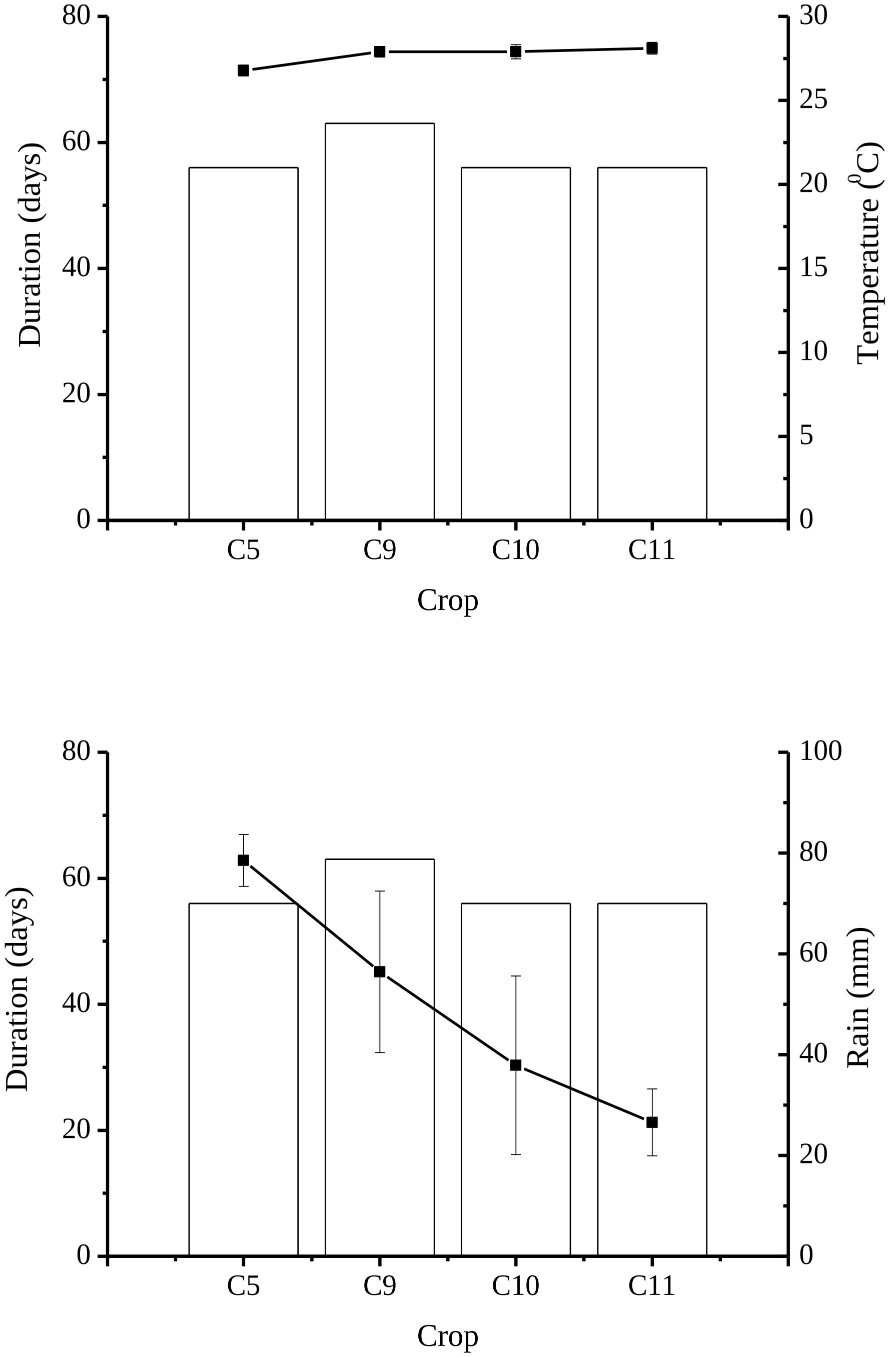


Fig. 4.16. Development times of each crop from female trees of *F. hispida* related to temperature (top) and rainfall (bottom). Open bars: crop duration; lines with error bars: temperature (top), rainfall (bottom). Standard error bars on mean temperature are not visible due to the very low variation of daily mean temperatures.

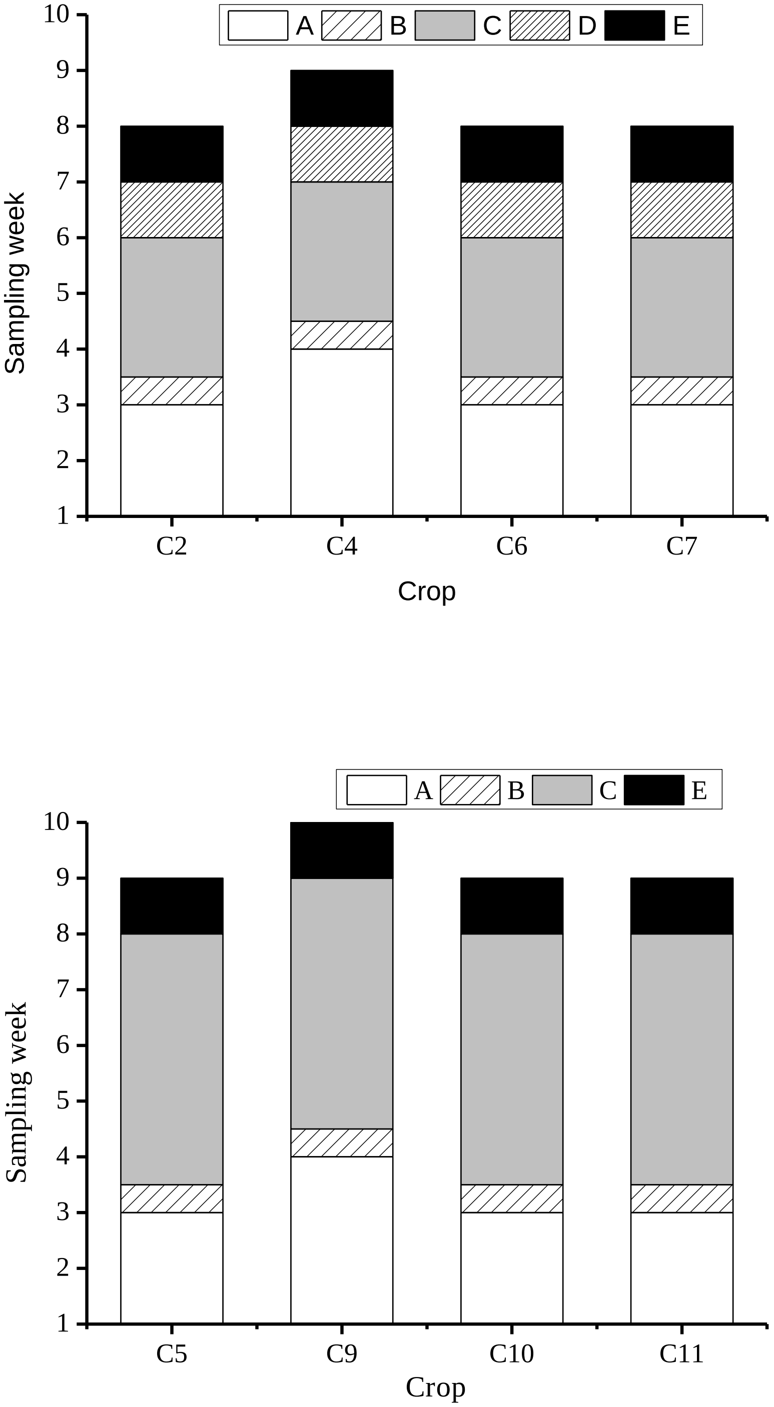


Fig. 4.17. Duration of each fig developmental phase on four male (top) and four female (bottom) trees of *F. hispida* during routine sequential sampling.

4.5.3 Species of nematodes found and their life cycles

There were two groups of nematodes being transferred by pollinating wasps which were regularly found in *F. hispida* figs both from male and female trees. The first group was *Caenorhabditis* sp. (Rhabditidae) (Appendix 1) which was molecularly identified at the Forestry and Forest Products Research Institute (FFPRI) Japan (Kanzaki and Tanaka Pers. Comm). The other group was *Schistonchus* spp. (Aphelenchoididae). During the study I failed to recognize that two different species of *Schistonchus* nematodes were present, so all quantitative data for these nematodes were grouped as *Schistonchus* spp. Later on after the field work has finished, the *Schistonchus* species in this study were identified on morphological grounds with the help of K.A. Davies, from the University of Adelaide, Australia. The two species of *Schistonchus* found in male figs of *F hispida* were *Schistonchus ?centerae* and another unknown *Schistonchus* sp. (Appendix 2 and 3). Further molecular identification done recently at Kyoto University, Japan, has confirmed the presence of *S. centerae* and identified the second species as *S. guangzhouensis* (R. Sriwati and Y. Takeuchiuko, Pers Comm). The two *Schistonchus* species and *Caenorhabditis* sp. were also transported and developed successfully in female figs. *S. guangzhouensis* was generally found to be more common than *S. centerae* both in male and female figs, based on non-quantified observations (R.Sriwati, Pers. Comm).

Caenorhabditis species have never been reported to be transported by fig pollinators before and its ecological role in this interaction remains undefined. *Schistonchus* spp. are well known plant parasitic nematodes. They have a stylet, a stomatal structure used to feed on plant tissues. This feature was absent in *Caenorhabditis* sp. Both species of *Schistonchus* were also much smaller in size compare to *Caenorhabditis* (Table 4.2.).

C*aenorhabditis* was transferred into the figs as juveniles, while *Schistonchus* spp. were transferred mostly as juveniles, and occasionally as adults. All nematodes were transported in the haemocoel of the female pollinators. They developed and produced new generations inside the figs, both in male and female trees. In male trees, peak populations of nematodes were found in D-phase figs, when the new generation of wasps was ready to leave (Fig. 4.18.). In female trees, where wasps could not reproduce, all nematodes that developed inside the figs would fail to be dispersed. The peak populations were found in late C-phase as there were no D-phase figs in female trees. The results showed that even though nematodes had no way out from female trees, new generations were still produced there (Fig. 4.19.).

The life cycles of the nematodes found in *F. hispida* were quite similar to those reported earlier in other figs. All nematodes developed and reproduced within the fig alongside the development of the fig itself. New generation(s) nematodes (mostly early juveniles) were ready to attach themselves to the pollinator offspring when the wasps were ready to leave the figs to find another suitable fig. This is how the nematodes disperse from one fig to another. Judging from the presence of adults in many different phases of figs (Figs. 4.20. and 4.21.), it is likely that there were more than one generation of both *Caenorhabditis* sp. and *Schistonchus* spp. during the development of the figs both in male and female trees. Adult nematodes from both genera could be found separately or together in each fig during each phase, both in male and female figs. The frequency of adult nematodes present in each phase varied among crops of both sexes (Figs. 4.20. and 4.21.). The proportion of adult nematodes from all genus in all fig phases and in crops did not vary significantly between male and female figs (lmer, z = 1.022, P = 0.307). When analysis was done separately, however, the proportion of each genus varied significantly among crops (LRT χ^2 = 26.153, df = 3, P <0.0001 for male figs, and LRT χ^2 = 321.37, df = 3, P <0.0001 for female figs) and among phases of fig development (LRT χ^2 = 67.147, df = 3, P <0.0001 for male figs, and LRT χ^2 = 169.55, df = 3, P <0.0001 for female figs). The development of the nematodes inside the figs occurred within the central lumen. Investigation of early D-phase of male figs when the wasps were still in galls showed that no nematodes were found on or attached to them, except for those female pollinators inside galls that had mating holes chewed by the males. In this case, nematodes could enter the galls through the holes and attach themselves to the female pollinators. There were no nematodes found on male flowers (Table 4.3.). Further observations of C- phase figs, when the wasps were still in the larval stage inside the galls, showed that from 320 larvae observed (from 30 figs, and 5 crops), none had nematodes attached. This result confirmed that all three species of nematodes did not develop within the galled female flowers or the wasps. *Schistonchus* spp. fed on the female and male flowers externally from the lumen of the fig. Whether the *Schistonchus* prefer male or female flowers to feed on was not investigated, but female flowers are much more numerous.

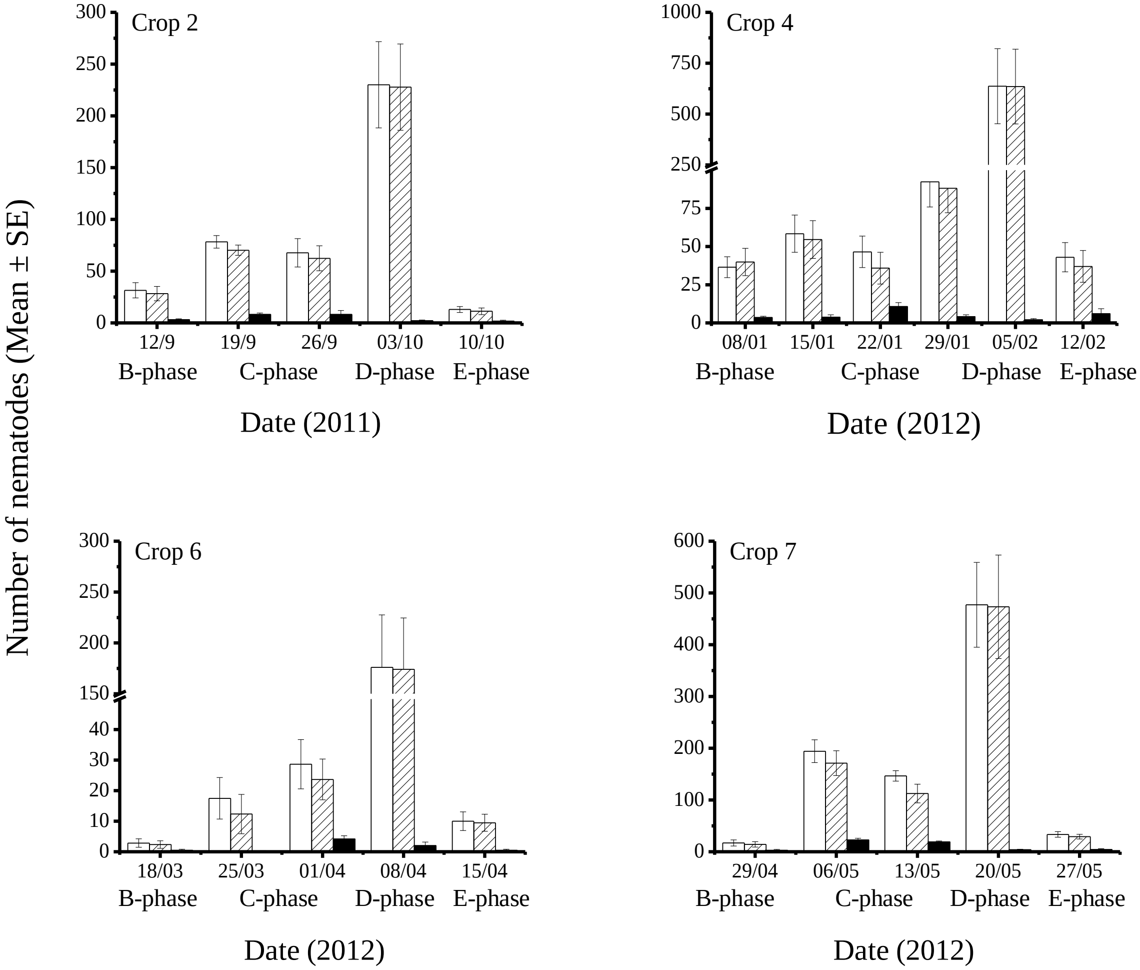


Fig. 4.18. Nematode densities per fig (all species) during development of four crops on male *F. hispida* trees during routine sequential sampling (open bars: total number of nematodes; hatched bars: number of juveniles; black bars: number of juvenile nematodes). Nematode numbers were obtained from extractions of whole figs. Note the varying scales of the Y-axes.

Table 4.2. Measurements of adult nematodes from late C-phase figs *of F. hispida* (Mean ± SE). (N= number of nematodes measured, L= length: from head to the tail tip, a = maximum width, Stylet = length of stylet: from head to the knob, b: length of aesophagous, c = length of tail: from anus to the tail tip, Spicule and Vulva: the distance from the head to the genitalia in males and females respectively, as a percentage of total body length). The terms used for measurement were adopted from Goodey (1963). *Caenorhabditis* sp. does not have a stylet.

Species	Sex	N	L (µm)	a (µm)	Stylet (µm)	b (µm)	c (tail) (µm)	Spicule (%)	Vulva (%)
Caenorhabditis sp.	Female	25	1870.7 ± 51.2	76.3 ± 3.7	-	319.1 ± 15.8	131.8 ± 4.4	-	61.2 ± 0.4
	Male	17	1740.3 ± 42.3	82.3 ± 2.5	-	326.8 ± 22.6	56.9 ± 0.9	90 ± 0.0	
Schistonchus centerae	Female	10	709.7 ± 51.5	44.0 ± 8.5	22.8 ± 2.5	51.8 ± 0.0	33.7 ± 3.2	-	75 ± 0.0
	Male	10	574.9 ± 17.2	21.2 ± 1.3	22.8 ± 2.1	59.6 ± 5.2	37.8 ± 1.0	95 ± 0.0	
Schistonchus guangzhouensis	Female	10	577.6 ± 40.3	32.9 ± 3.6	31.3 ± 2.3	75.6 ± 4.7	47.4 ± 7.2	-	75 ± 0.0
	Male	6	518.0 ± 8.2	19.2 ± 0.6	27.9 ± 2.4	90.6 ± 5.8	20.7 ± 1.1	95 ± 0.0	

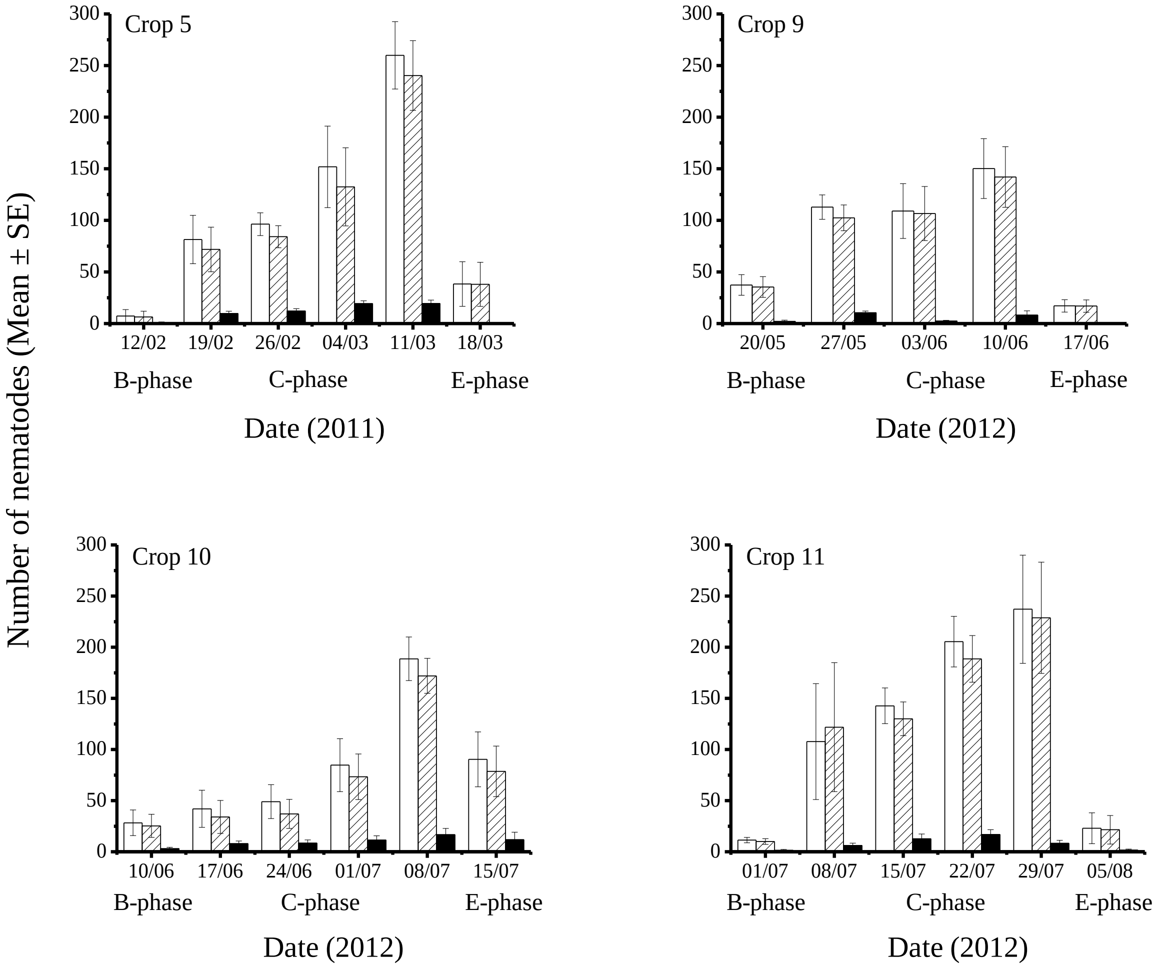


Fig. 4.19. Nematode densities per fig (all species) during development of four crops on female *F. hispida* trees during routine sequential samplings (open bars: total number of nematodes; hatched bars: number of juvenile nematodes; black bars: number of adult nematodes). Nematode numbers were obtained from extractions of whole figs. Female figs do not have a D-phase.

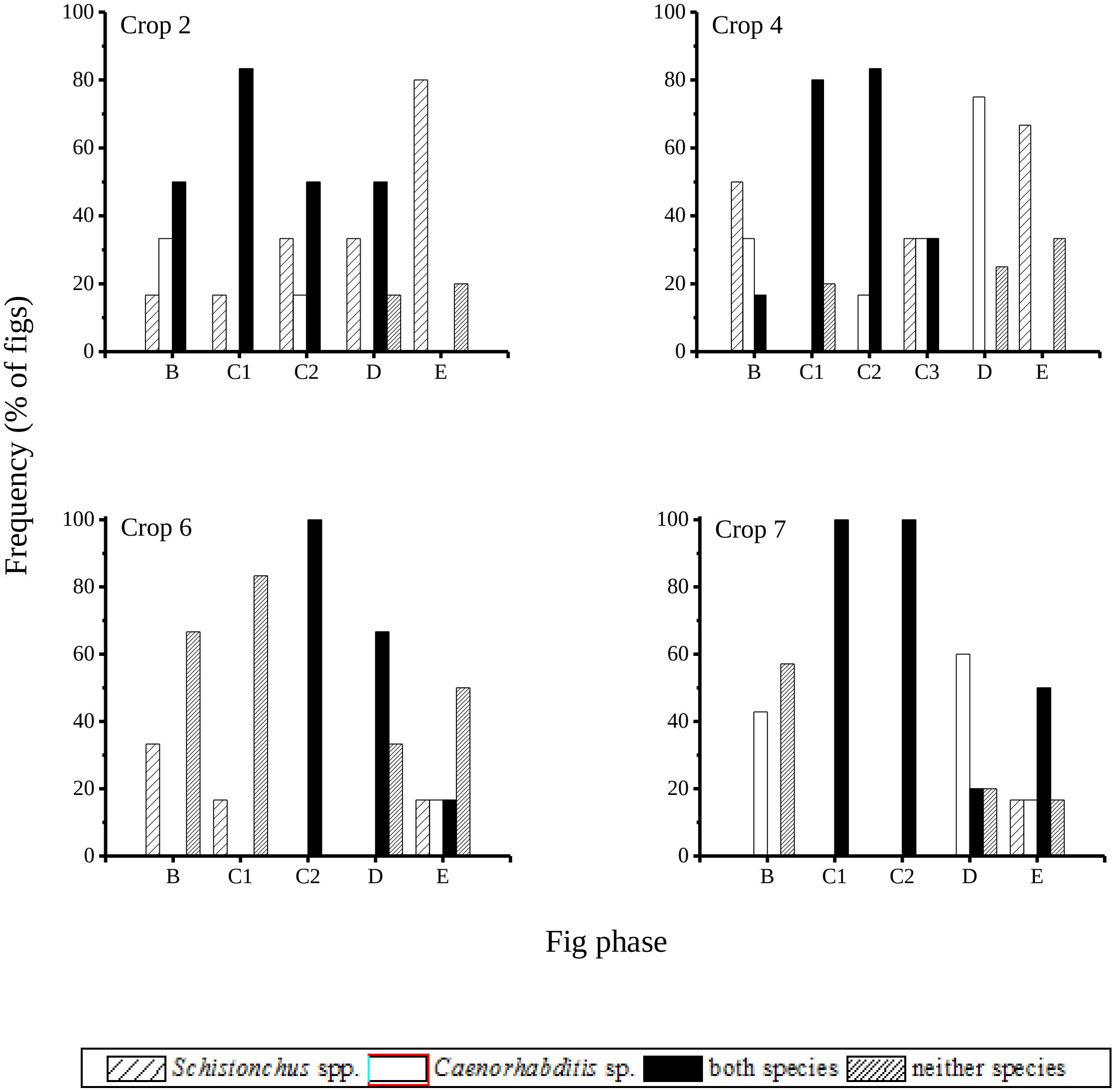


Fig. 4.20. Frequency of adults nematodes of each species present in each phase of male figs of *F. hsipida* from four crops with routine sequential sampling. N= 4-6 figs/ phase in each crop.

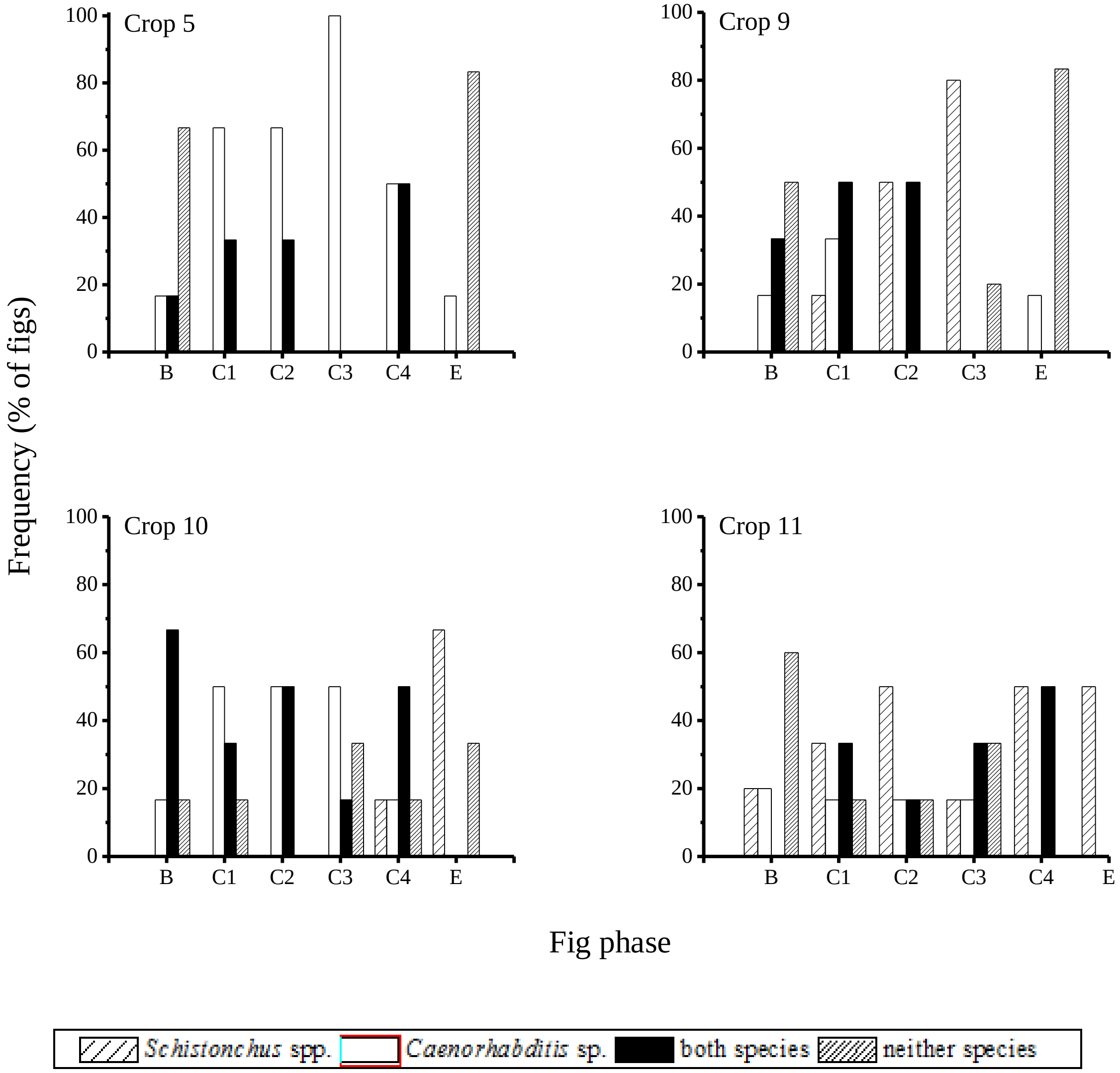


Fig. 4.21. Frequency of adults nematodes of each species present in each phase of female figs of *F. hsipida* from four crops during routine sequential samplings. N= 4-6 figs/ phase in each crop. Female figs do not have a D-phase.

Table 4.3. Nematodes from galls, male flowers and fig wasps sampled within early D phase figs of *F. hispida* male (N= 30 figs, collected from 6 crops, and 5 figs each crop). Male pollinators make holes in the side of galls of conspecific females, in order to mate with them. Zero counts were not included in calculations of mean number of nematodes attached per female pollinator inside the galls with mating holes.

Category	Gall condition condition	Number of each category	With nematodes	Range	Mean ± SE
Female pollinator		400	0	0	0
Female pollinator	With mating hole	100	88	1 – 11	2.86 ± 0.24
Emerged male wasp	-	50	0	0	0
Male wasp inside gall	-	75	0	0	0
Female NPFW	No hole	125	0	0	0
Male NPFW	-	55	0	0	0
Male flowers	-	250	0	0	0

4.5.4 Variation in the numbers of nematodes

Observations of the number of nematodes that emerged from female pollinators (either foundresses or newly offspring that had just emerged from figs) were initially done within one hour and then repeated 24 hours later. Within one hour however, all the nematodes regularly emerged from the wasps, so that the number of nematodes recorded within one hour and after 24 hours stayed the same. Therefore, the numbers of nematodes reported here are based on the first observation only.

The number of foundresses present in a B-phase fig varied from 0-5 in male trees with a grand mean of 1.8 ± 0.25 foundresses per fig (Mean ± SE, N = 31 figs, zero numbers of foundresses were included in the calculation). One foundress per fig was the most common number found in male trees. The number of foundresses that entered B phase figs of female trees ranged between zero and four, with a mean value of 1.4 ± 0.24 (Mean ± SE, N = 29 figs, zero numbers of foundress were included in calculation). Zero foundresses were commonly found in B-phase female figs, showing that more foundresses were likely to enter later so the number of foundresses present in B-phase figs will have been lower than the actual number that eventually entered the receptive figs, as more foundresses would have entered later.

Later observations on early C-phase figs showed that number of foundresses in C-phase figs ranged between one and five, with a mean value of 1.9 ± 0.2 (Mean ± SE, N = 30 figs, no zero pollinator counts) on male figs. In early C-phase female figs, the numbers of foundresses ranged between one and seven, with a mean value 2.5 ± 0.4 (Mean ± SE, N = 29 figs). There was no significant difference in the number of foundresses present in C-phase figs on male and female trees (lmer, z = -0.471, P = 0.638; Fig.4.22.). These results suggest that there is no preference among female pollinators to enter either male of female figs. All C-phase figs observed at least had one foundress inside (N = 30 for male figs, N = 29 for female figs). Out of 30 C-phase male figs observed, only one (3.3 %) had no nematodes while in 30 C-phase female figs observed, two of them (6.9 %) had no nematodes. This result showed that the probability that a foundress will carry and transfer nematodes into receptive figs was very high.

Observations and extractions of A-phase male and female figs during routine sequential sampling showed that there were no nematodes present at all at this stage, because no foundresses had entered into the figs. Number of nematodes per B-phase male fig ranged between 0 – 89 with an average of 31.58 ± 5.94 (Mean ± SE, N = 31). While in B-phase female figs the number of nematodes ranged from 0 – 114, with an average of 31.41 ± 5.73 (Mean ± SE, N=29). Number of foundresses entering a B-phase fig significantly affected the number of nematodes inside the B-phase male figs (lmer, z =

4.948, P <0.0001, and female figs (lmer, z = 5.257, P <0.0001). The more pollinators that entered a fig, the higher the number of nematodes present (Fig. 4.23.). There was no significant difference in the numbers of nematodes found in extractions from male and female B-phase figs (lmer, z = 0.233, P = 0.816).

Nematodes were observed inside one B-phase male fig where no foundresses were found. There were four B-phase male figs (out of 31) which had no foundress inside (12.9 %), and it was one of these that contained nematodes. On female trees, there were ten B-phase figs (out of 29) which had no foundress (34.5%), and seven of them had nematodes. This suggests that foundresses had re-emerged after entering the figs of *F. hispida* and that re-emergence may be more common from female figs (where the foundresses cannot oviposit). Re-emerging foundresses were observed several times in the field during routine sampling, when female pollinators were found crawling out of the ostioles after having lost their wings and antennae. A Fisher Exact Test was used (due to the relatively small sample size: 31male figs and 29 female figs and less than 10 in two cells of data table) to determine whether there was a significant difference between male and female figs in the number of figs having nematodes, but no foundresses inside. Only 3.2 % of male figs had nematodes with no foundresses, whereas 24.1% of female figs had nematodes with no foundresses. These results were significantly different (Fisher Exact test, P = 0.022). Thus, in B-phase figs, the number of foundresses that re-emerged from female figs was higher than those in male figs.

Numbers of nematodes extracted from B, C, D, and E-phase figs for each crop from male and female trees are presented in Tables 4.4. and 4.5. Nematodes within B-phase figs will mostly have been the ones that were transported into the figs by the pollinating wasps. The wasps were still intact, and sometimes were still alive inside the figs, showing that they had just entered them. The nematodes found in early C-phase figs may have represented overlapping generations, with the nematodes that had been transported and their offspring. Peak nematode population sizes were found in D-phase male figs (Fig. 4.18.) and late C-phase female figs (Fig. 4.19.). Nematode numbers in individual D-phase figs ranged between 123 and 981 in male figs with an average of 174 - 635 nematodes across the four different crops observed (Table 4.4.). While in late C-phase of female figs, numbers of juvenile nematodes ranged from 32 up to 890 in individual figs with an average of 142 – 240 across four different crops (Table 4.5.). The numbers of nematodes extracted from male and female figs in all phases of fig development were not significantly different (lmer, z = -0.619, P = 0.536).

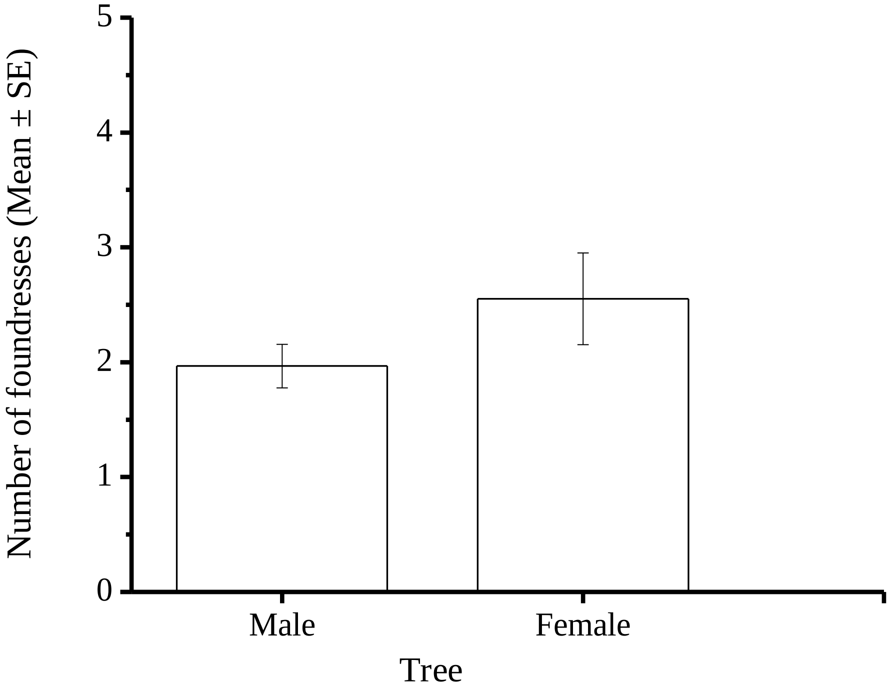


Fig. 4.22. Numbers of foundresses present per C-phase figs from male and female *F. hispida* trees (N = 30 for male figs, and N = 29 for female figs)

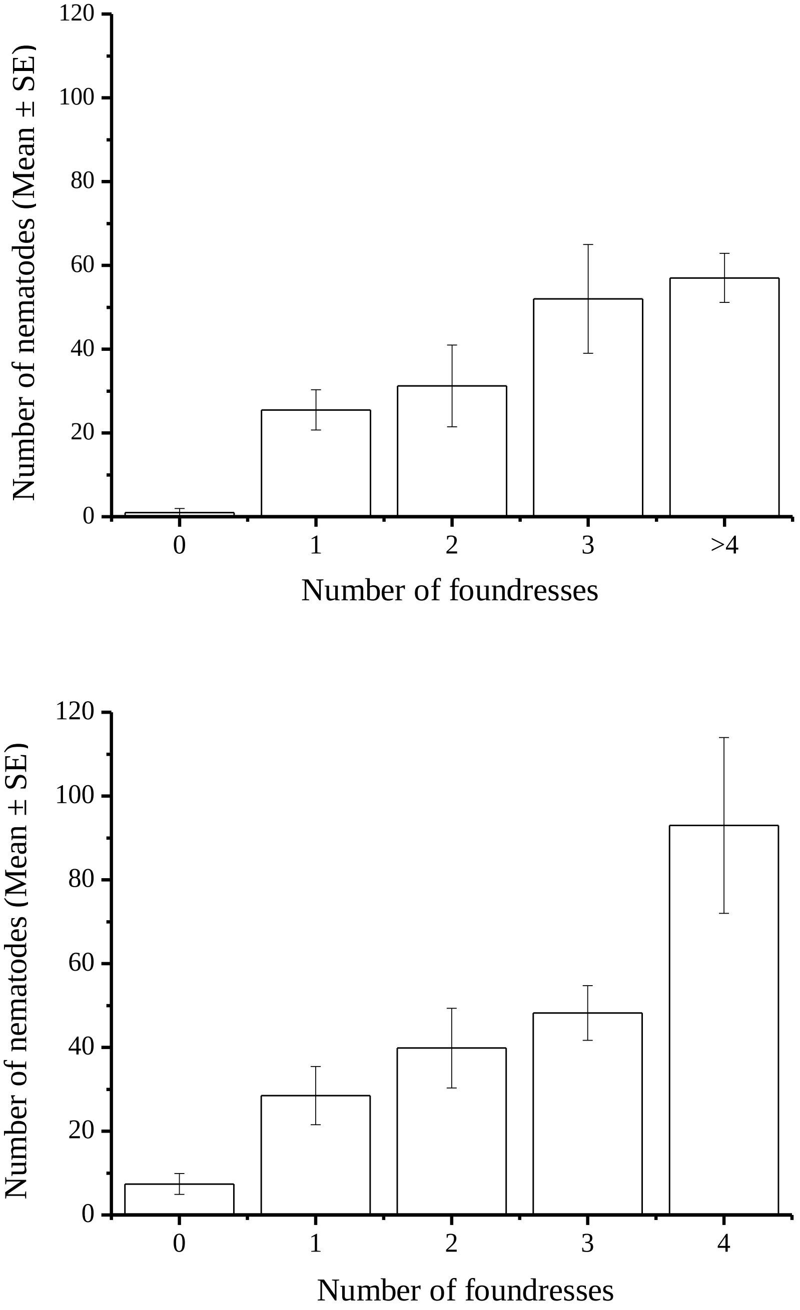


Fig. 4.23. Nematodes in B-phase male figs (top, N = 31) and in B-phase female figs (bottom, N = 29) of *F. hispida* in relation to the numbers of foundresses that had entered the figs.

Table 4.4. Number of nematodes per fig from *F. hispida* male trees during routine sequential sampling (N = number of figs extracted).

Date	Crop	Phase	N	Number of nematodes (Mean ± SE)		
				Total	Juveniles	Adults
12/09/2011	C2	B	6	31.50 ± 7.32	28.33 ± 6.95	3.50 ± 0.81
19/09/2011	C2	C	6	78.33 ± 6.06	70.17 ± 4.94	8.17 ± 1.33
26/09/2011	C2	C	6	67.67 ± 13.69	62.33 ± 12.06	8.17 ± 3.76
03/10/2011	C2	D	6	230.00 ± 41.65	227.83 ± 41.68	2.17 ± 0.60
10/10/2011	C2	E	5	13.00 ± 2.85	11.20 ± 3.22	1.80 ± 0.73
08/01/2012	C4	B	6	33.60 ± 7.55	38.60 ± 10.85	2.80 ± 0.49
15/01/2012	C4	C	6	58.40 ± 12.17	54.60 ± 12.36	3.80 ± 1.50
22/01/2012	C4	C	6	46.50 ± 10.30	35.83 ± 10.42	10.67 ± 2.60
29/01/2012	C4	C	6	92.33 ± 16.43	88.17 ± 16.01	4.17 ± 1.14
05/02/2012	C4	D	4	637.00 ± 184.37	635.00 ± 183.89	2.00 ± 0.82
12/02/2012	C4	E	6	43.00 ± 9.63	37.00 ± 10.44	6.00 ± 3.34
18/03/2012	C6	B	6	2.83 ± 1.38	2.33 ± 1.23	0.50 ± 0.34
25/03/2012	C6	C	6	17.50 ± 6.82	12.33 ± 6.44	0.17 ± 0.17
01/04/2012	C6	C	6	28.67 ± 8.07	23.67 ± 6.69	4.17 ± 1.08
08/04/2012	C6	D	4	176.00 ± 51.51	174.00 ± 50.50	2.00 ± 1.15
15/04/2012	C6	E	6	10.00 ± 3.07	9.50 ± 2.77	0.50 ± 0.34
29/04/2012	C7	B	7	17.43 ± 4.89	15.00 ± 4.40	2.43 ± 1.34
06/05/2012	C7	C	6	194.17 ± 22.01	171.17 ± 24.08	23.00 ± 2.94
13/05/2012	C7	C	6	146.50 ± 9.97	112.50 ± 17.97	19.00 ± 1.88
20/05/2012	C7	D	5	477.00 ± 122.15	473.20 ± 122.47	3.80 ± 1.50
27/05/2012	C7	E	6	33.50 ± 5.47	29.17 ± 4.50	4.33 ± 1.56

Table 4.5. Number of nematodes per fig of *F. hispida* female trees during routine sequential samplings. (N = number of figs extracted).

Date	Crop	Phase	N	Number of nematodes (Mean ± SE) Total	Juveniles	Adults
12/02/2012	C5	B	6	7.33 ± 6.18	6.50 ± 5.54	0.83 ± 0.65
19/02/2012	C5	C	6	81.50 ± 23.43	71.83 ± 21.60	9.67 ± 2.38
06/02/2012	C5	C	6	96.33 ± 11.01	84.17 ± 10.65	12.17 ± 2.27
04/03/2012	C5	C	6	151.83 ± 39.57	132.50 ± 37.88	19.33 ± 2.79
11/03/2012	C5	C	6	259.83 ± 32.62	240.33 ± 33.76	19.50 ± 3.31
18/03/2012	C5	E	6	38.33 ± 21.63	38.00 ± 21.39	0.33 ± 0.33
20/05/2012	C9	B	5	37.40 ± 9.96	35.40 ± 10.05	2.00 ± 1.38
27/05/2012	C9	C	7	112.86 ± 11.87	102.43 ± 12.54	10.43 ± 1.76
03/06/2012	C9	C	6	109.00 ± 26.56	106.60 ± 26.21	2.40 ± 0.68
10/06/2012	C9	C	5	150.17 ± 29.05	142.00 ± 29.41	8.17 ± 4.14
17/06/2012	C9	E	6	17.17 ± 6.00	17.00 ± 5.98	0.17 ± 0.17
10/06/2012	C10	B	6	28.33 ± 12.57	25.33 ± 11.37	3.00 ± 1.37
17/06/2012	C10	C	6	42.00 ± 18.23	34.00 ± 16.11	7.83 ± 2.73
24/06/2012	C10	C	6	49.00 ± 16.54	37.00 ± 14.25	8.50 ± 3.00
01/07/2012	C10	C	6	84.67 ± 25.86	73.33 ± 22.32	11.33 ± 4.20
08/07/2012	C10	C	6	188.67 ± 21.38	172.00 ± 17.05	16.67 ± 6.14
15/07/2012	C10	E	6	90.33 ± 26.78	78.67 ± 24.73	11.67 ± 7.51
01/07/2012	C11	B	6	11.40 ± 2.62	10.00 ± 2.83	1.40 ± 0.87
08/07/2012	C11	C	6	107.67 ± 56.67	121.80 ± 63.11	6.17 ± 2.21
15/07/2012	C11	C	6	142.67 ± 17.48	130.00 ± 16.50	12.67 ± 4.62
22/07/2012	C11	C	6	205.50 ± 24.74	188.67 ± 22.80	16.83 ± 4.70
29/07/2012	C11	C	6	237.17 ± 52.92	228.83 ± 54.37	8.33 ± 2.93
05/08/2012	C11	E	4	23.00 ± 15.03	21.50 ± 13.87	1.50 ± 1.19

Number of nematodes in D-phase (male figs) or late C-phase (female figs) represents the population of nematodes bred in male and female figs. Average number of total nematodes (juveniles and adults combined) in D-phase of male figs ranged from 176 up to 637 and in late C-phase of female figs ranged from 109 up to 260 across four crops with routine sequential sampling. Average numbers of *Caenorhabditis* sp. varied from 40 up to 120 in male figs and from 21.67 up to 124.33 in female figs. Populations of *Schistonchus* spp. ranged from 136.67 up to 530.75 in male figs and from 135.50 up to 267.33 in female figs. The means of total nematodes from male and female figs did not differ significantly (lmer, z = -1.874, P = 0.061), neither did mean numbers of *Caenorhabditis* sp. (lmer, z = -1.571, P = 0.116). Mean number of *Schistonchus* spp. in male figs, however, was significantly higher than those of in female figs (lmer, z = -3.001, P = 0.002) (Fig. 4.24.). These results showed that reproduction of *Schistonchus* spp. in female figs was not good as in male figs.

4.5.5 Do nematodes harm figs and pollinators?

Nematodes were found in some figs in all the routine samples of both male and female figs, with a high occupancy rate in each crop. Nematode occupancy ranged from 76-100% in male figs, and between 86 to 97 % in female figs (Fig. 4.25.). Pollinator wasp offspring consistently emerged from figs occupied by nematodes. Impacts of nematode occupancy were assessed using early D-phase figs for male trees, and late C-phase for female trees. On male trees, the number of nematodes present within a fig did not affect the number of galls containing female pollinators in the same fig (lmer, z = -0.73, P = 0.463). They also had no effect on female trees, where the number of seeds that developed was not affected by the number of nematodes inside the same fig (lme, t-value = 0.548, df = 29, P = 0.588). Female pollinators that were newly emerged from D-phase figs of male trees (obtained from 29 figs from five different crops and trees) were examined for nematodes, as were female pollinators captured when they were arriving at B-phase figs on male trees (29 sweeps from 5 crops on 5 trees). Nematodes were present in some wasps from all 10 crops. The percentage of newly emerged females that had nematodes ranged from 20 % to 100 % in different figs, with an average of 62-85 % across the five different crops (n = 290 females). Among femle pollinators arriving at B-phase figs, the percentage of females that had nematodes ranged between 30 and 90 % in each sweep sample, with an average of 60-80 % across the five different crops (Table 4.6., Fig. 4.26). The grand mean percentage of female pollinators with nematodes was 64.83 ± 4.34 % (Mean ± SE, N = 29 figs, 290 pollinators) for emerging females, and 72.41 ± 3.24 % (Mean ± SE, N = 29 traps, 290 pollinators) for arriving females. There was no significant difference between emerging and arriving pollinators in the proportion with any nematodes (lmer, z = 1.100, P = 0.271). This suggests that the presence of nematodes in the dispersing female pollinators does not harm them, because if this was the case then the proportion of pollinators with nematodes attached in females arriving on B–phase figs should have been significantly lower than the percentage of pollinators with nematodes attached on emerging females from D-phase figs.

The most common number of nematodes carried was two per female pollinator both in emerging and arriving females. Increasingly, fewer females had increasing numbers of nematodes (Fig. 4.27). Across the five crops observed, the average number of nematodes per female pollinator was between 0.22-3.4 for emerging females (N = 29 figs) and 1.25-5.20 for arriving females (N = 29 traps) (Fig. 4.28). The mean number of nematodes per female pollinator was slightly higher in arriving females than emerging females (range = 0 - 21 nematodes, mean ± SE = 2.39 ± 0.17, N= 290 pollinators, for emerging females; and range = 0 - 22 nematodes, mean ± SE = 2.65 ± 0.20, N= 290 pollinators, for arriving females), but the difference between those two means was not significant (lmer, z = -0.314, P = 0.754).

The number of nematodes per female pollinator was also very similar among emerging and arriving individuals when only infected females were considered (range = 1-21, mean ± SE = 3.7 ± 0.21, N = 188 for emerging females, and range = 1- 22, mean ± SE = 3.7 ± 0.24, N = 210 for arriving females). There was no significant difference between the grand means of nematode loads among infected females when emerging and arriving (lmer, z = 0.037, P = 0.971). Across the five different crops, the number of nematodes per infected female was between 1.9 and 4.4 (N = 188 females from 29 figs) for emerging females, and between 1.9 and 5.9 (N = 210 females from 29 traps) for arriving females. The number of nematodes per infected female nonetheless seemed to be more varied among arriving females (Fig. 4.29.). This is understandable since the female pollinators arriving on the B-phase figs are likely to have come from a large number of different natal crops, where infestation levels would have been varied. The percentage of arriving females containing nematodes was not correlated with the number of nematodes per infected female (Spearman's ρ = 0.20, P = 0.29, N = 29 sweeps) (Fig. 4.30.). This supports the idea that the female pollinators had arrived from many different crops with different numbers of nematodes present.

Among emerging female pollinators there was a positive correlation between the proportion of infected females emerging from each fig and the number of nematodes present in each infected female (Spearman's ρ = 0.62, P = 0.0004, N = 29 traps) (Fig. 4.31.). Between crop differences were evident. Number of nematodes per emerging wasp was much lower in Crop 21 (C21) than those in other crops. Presumably it is because nematodes were relatively rare in its figs (Fig. 4.28.). Overall, among female pollinators sampled from 29 figs and 5 different crops, 36 % of the emerging wasps (103 out of 290 females) had no nematodes present, compared with an equivalent figure of 27 % for arriving females (79 out of 290 females). Again, this indicates that there was no cost to those female pollinators carrying nematodes, since the percentage of females having no nematodes was lower among the arriving females. Had the nematodes caused any harm for the female pollinators, the percentage of emerging females having nematodes would have been higher.

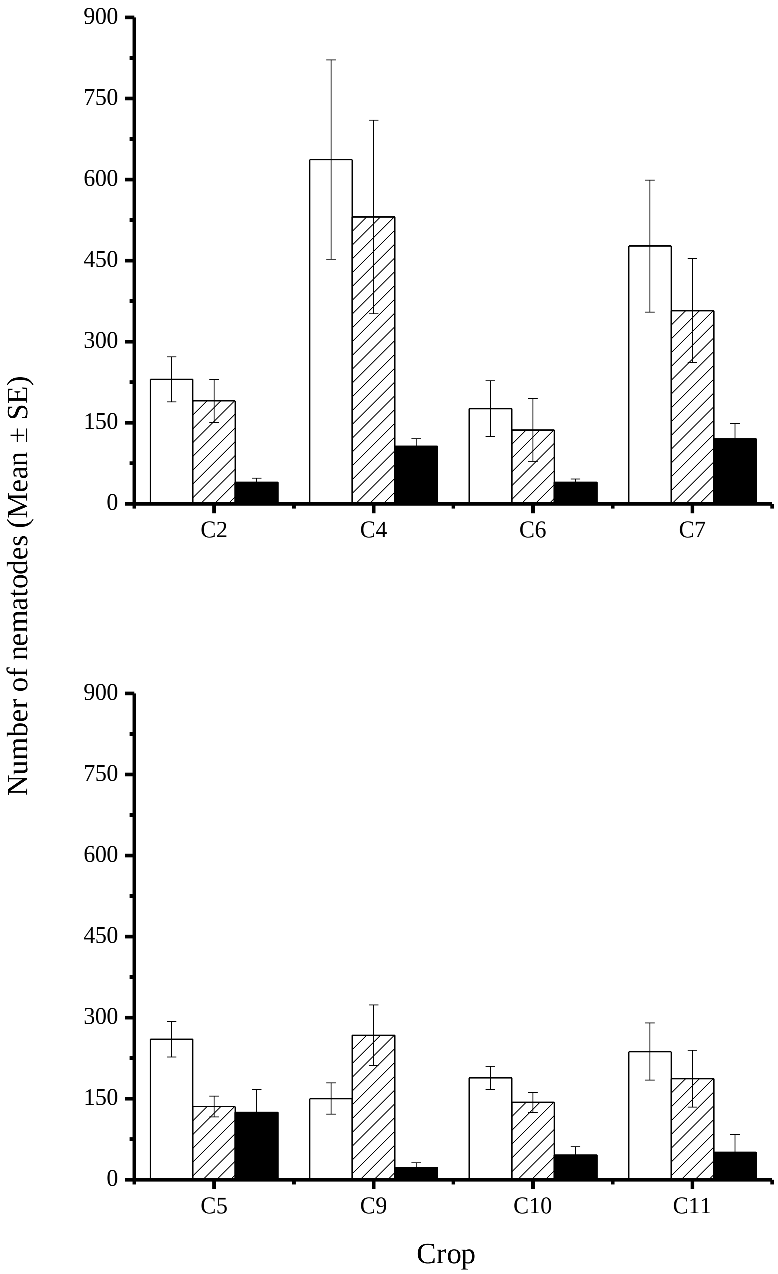


Fig. 4.24. Nematode densities (juveniles and adults combined) per D-phase male (top) and per late C-phase female (down) figs of *F. hispida* during routine sequential samplings (open bar = total nematodes; hatched bars: number of *Schistonchus* spp.; closed bars: number of *Caenorhabdities* sp). (N = 4-6 figs/crop for both male and female figs).

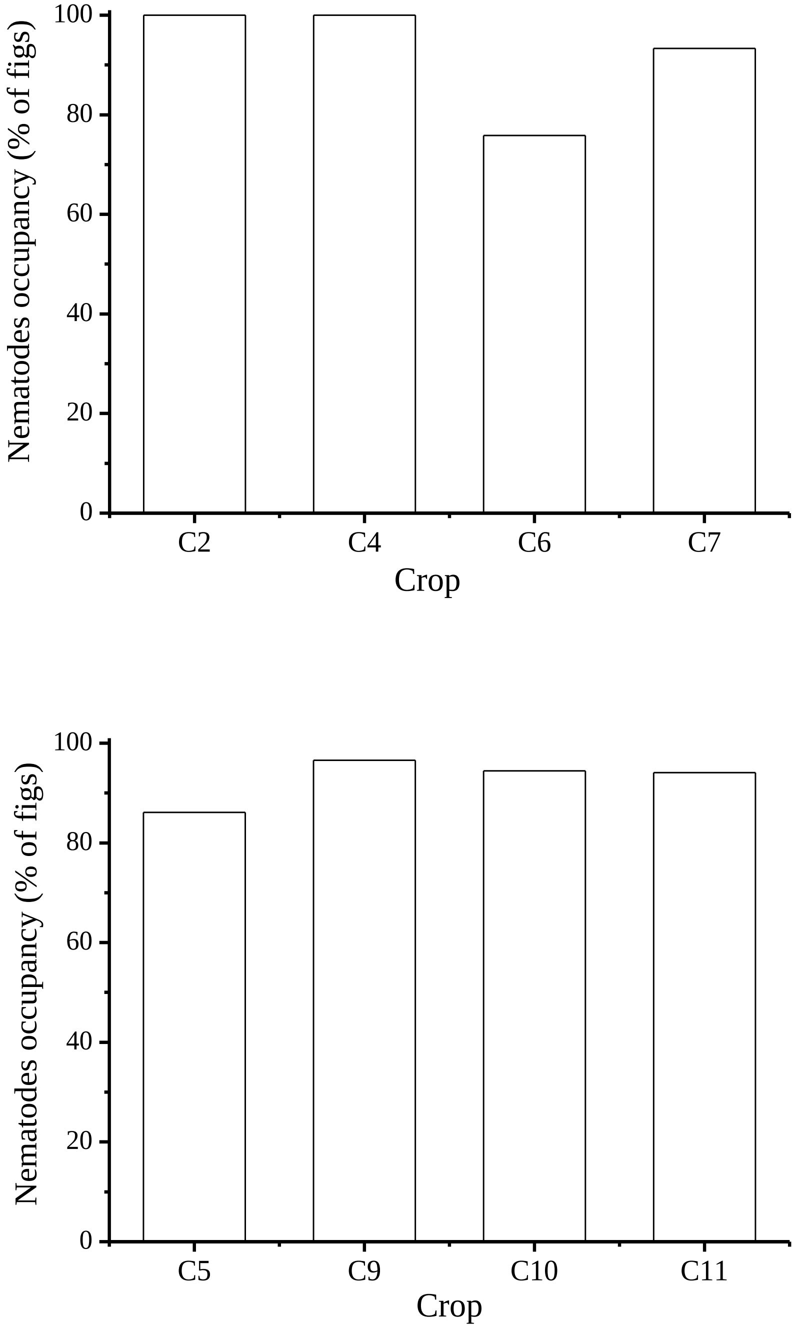


Fig. 4.25. The proportion of figs where nematodes were present (fig phases pollinated B-E combined) in four crops of male (top) and female (bottom) figs of *F. hispida* during routine sequential sampling.

Table 4.6. Nematodes on emerging female pollinators from D-phase figs and on arriving female pollinators around B-phase figs of *F. hispida* (N1 = number of figs or sweeps, N2 = number of female pollinators sampled per each fig or trap).

Source of pollinators	Crop	N1	N2	Wasps with nematodes (%) (Mean ± SE)	Number of nematodes per wasp (0 included) (Mean ± SE)	Number of nematodes per infected wasp (Mean ± SE)
emerging	C12	6	10	73.33 ± 6.67	2.92 ± 0.55	3.82 ± 0.44
	C15	5	10	62 ± 12.41	2.83 ± 0.85	4.39 ± 1.08
	C17	6	10	85 ± 7.19	3.32 ± 0.88	3.71 ± 0.75
	C19	6	10	60 ± 11.08	1.95 ± 1.04	3.08 ± 0.88
	C21	6	10	70 ± 4.28	1.03 ± 0.12	1.90 ± 0.23
arriving	C13	6	10	70 ± 3.42	2.25 ± 0.92	3.23 ± 0.58
	C14	6	10	60 ± 3.16	1.25 ± 0.51	1.99 ± 0.17
	C16	6	10	78.33 ± 3.14	5.20 ± 2.12	5.97 ± 1.70
	C18	6	10	80 ± 4.0	3.43 ± 1.41	4.37 ± 1.22
	C20	5	10	75 ± 3.16	1.72 ± 0.77	2.18 ± 0.33

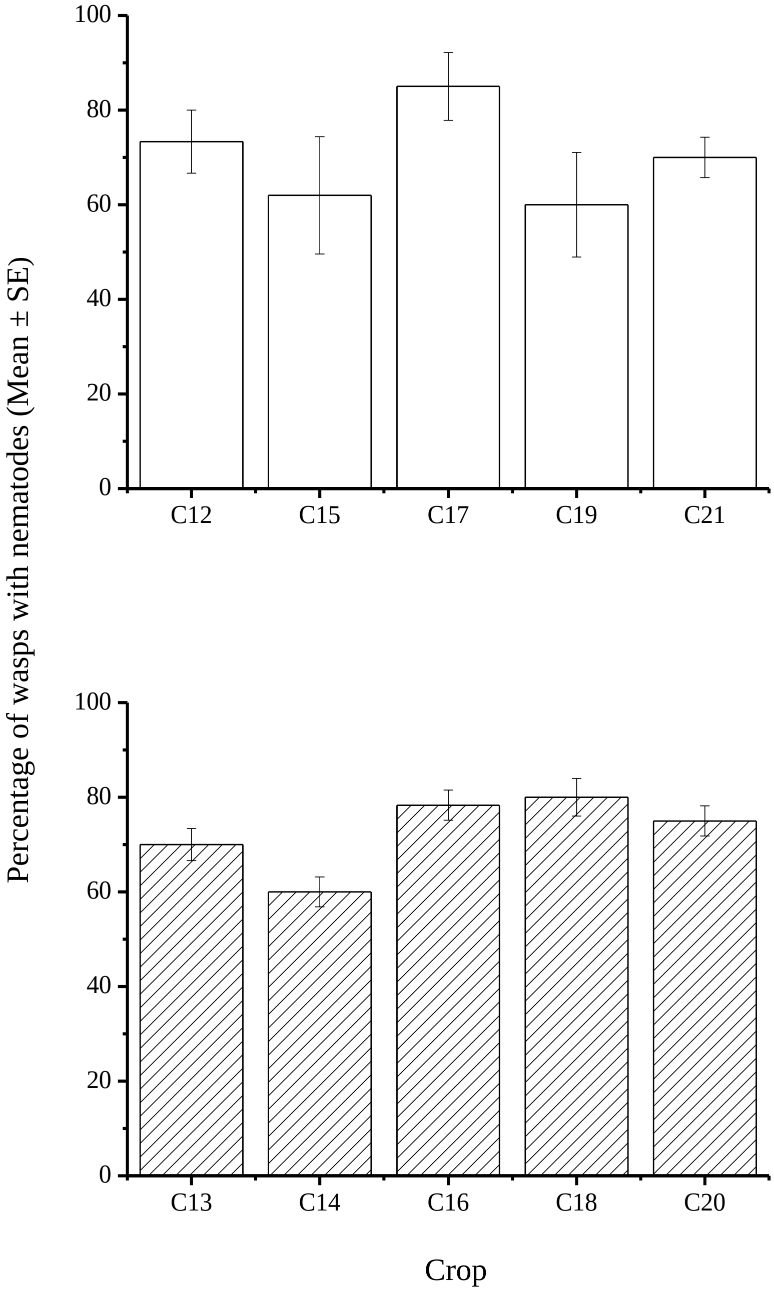


Fig. 4.26. Percentage of pollinator females with nematodes that were newly-emerged from *F. hispida* figs (top) or were arriving at B phase male figs (bottom) (N1 = 10 female wasps/ fig, 5-6 figs/crop, total 290 female wasps observed for emerging female pollinators , N2 = 10 wasps/trap, 5-6 traps/crop (29 traps), total 290 female wasps observed for arriving female pollinators).

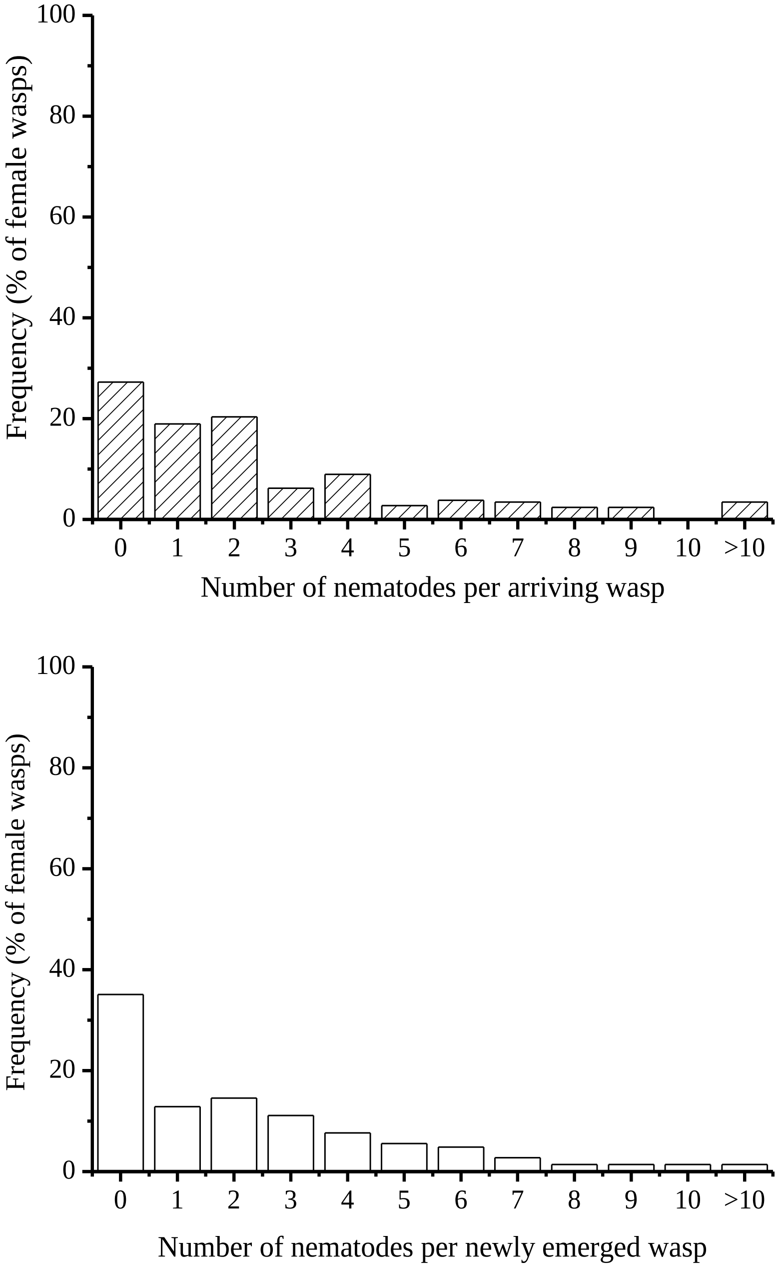


Fig. 4.27. The frequencies of newly emerged female pollinators (top) and arriving female pollinators at B-phase male figs (bottom) of *F. hispida* with different numbers of nematodes attached (N = 290 female pollinators each for emerging and arriving pollinators).

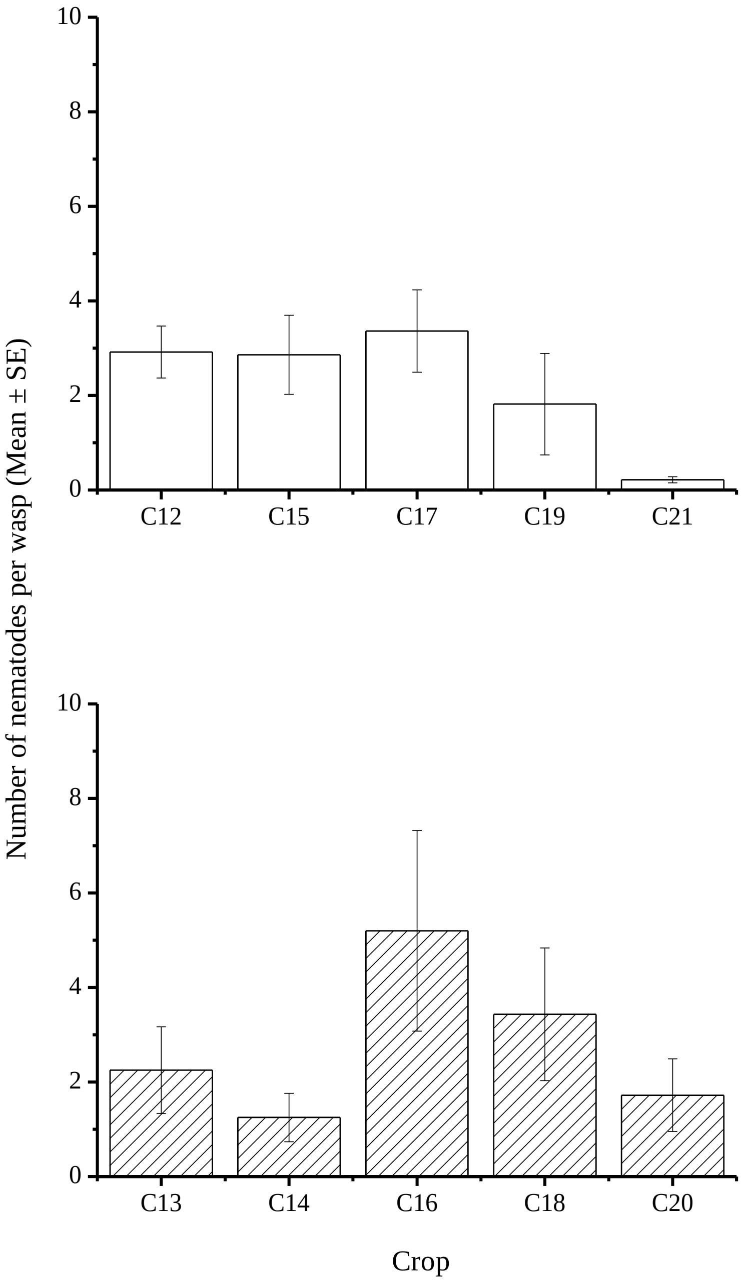


Fig. 4.28. Numbers of nematodes associated with newly-emerged female pollinators of *F. hispida* (top) and females arriving at B-phase figs (bottom) (pollinators having no nematodes were included in the calculation) (N1 = 10 female wasps/ fig, 5-6 figs/crop, total 290 female wasps observed for emerging female pollinators, N2 = 10 wasps/trap, 5-6 traps/crop (29 traps), total 290 female wasps observed for arriving female pollinators).

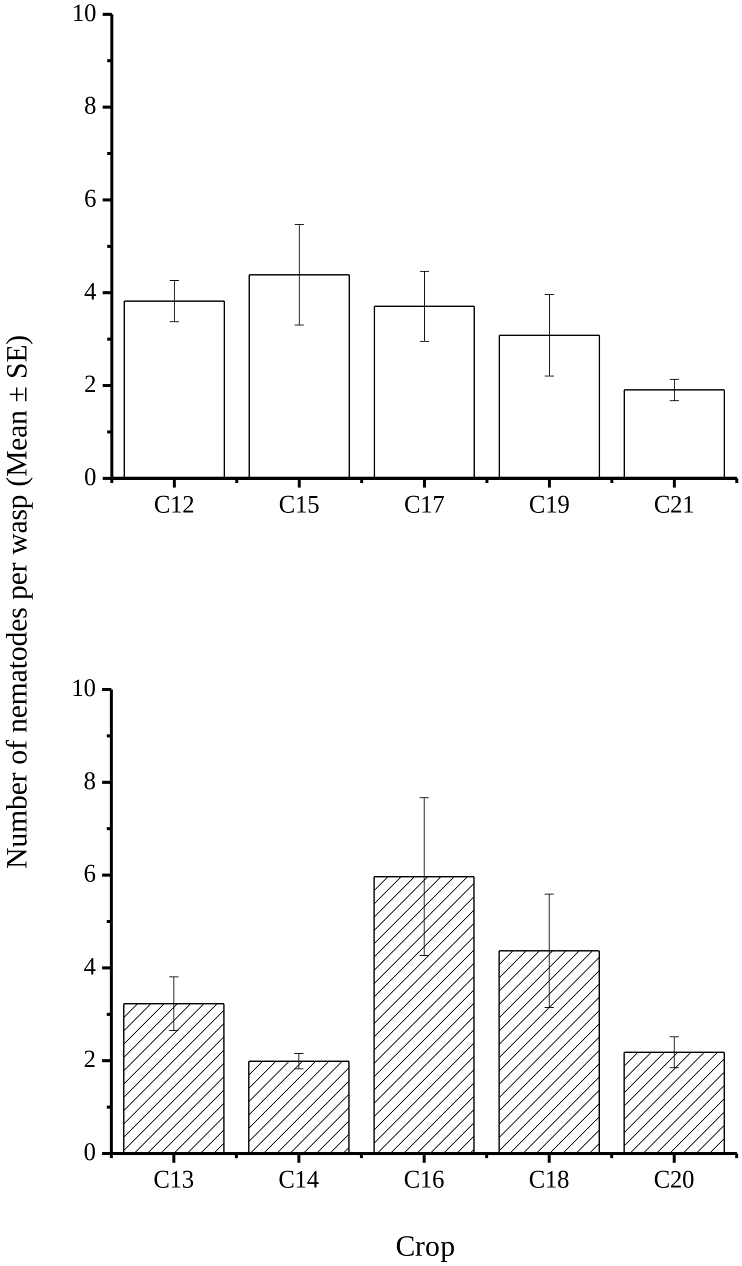


Fig. 4.29. Number of nematodes on newly-emerged female pollinators of *F. hispida* (top) and females arriving at B-phase of male figs (bottom). Only infected females are included in the calculations. (N1 = 10 female wasps/ fig, 5-6 figs/crop for emerging female pollinators, N2 = 10 wasps/trap, 5-6 traps/crop (29 traps) for arriving female pollinators. In total 188 out 290 wasps infected in emerging females, and 210 out of 290 wasps infected in arriving females).

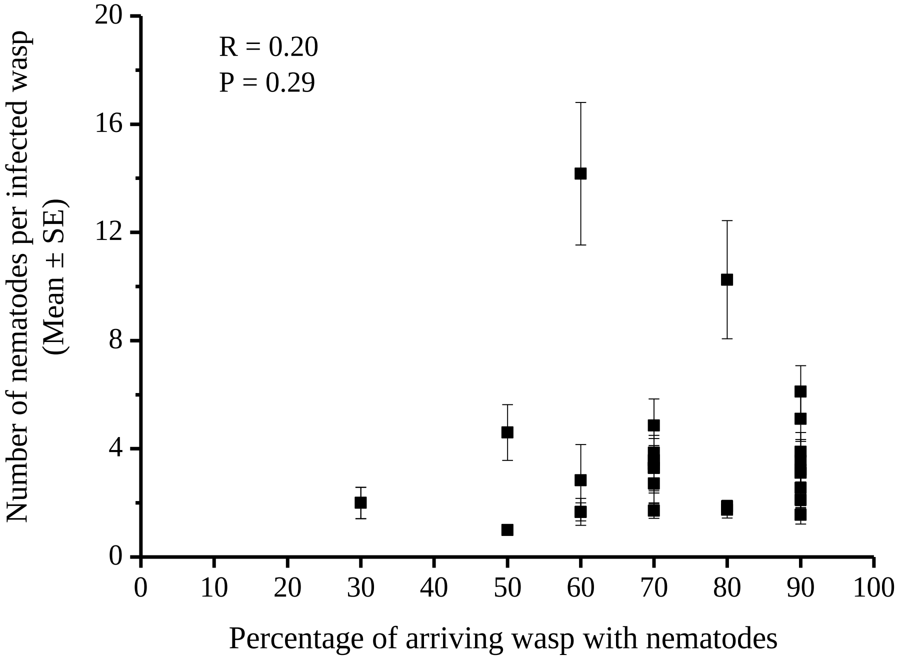


Fig. 4.30. The relationship between the percentage of arriving females on B-phase figs of *F. hispida* with nematodes and number of nematodes attached to each infected female. (N = 10 female pollinators/trap, 5-6 traps/crop (29 traps), total females sampled was 290).

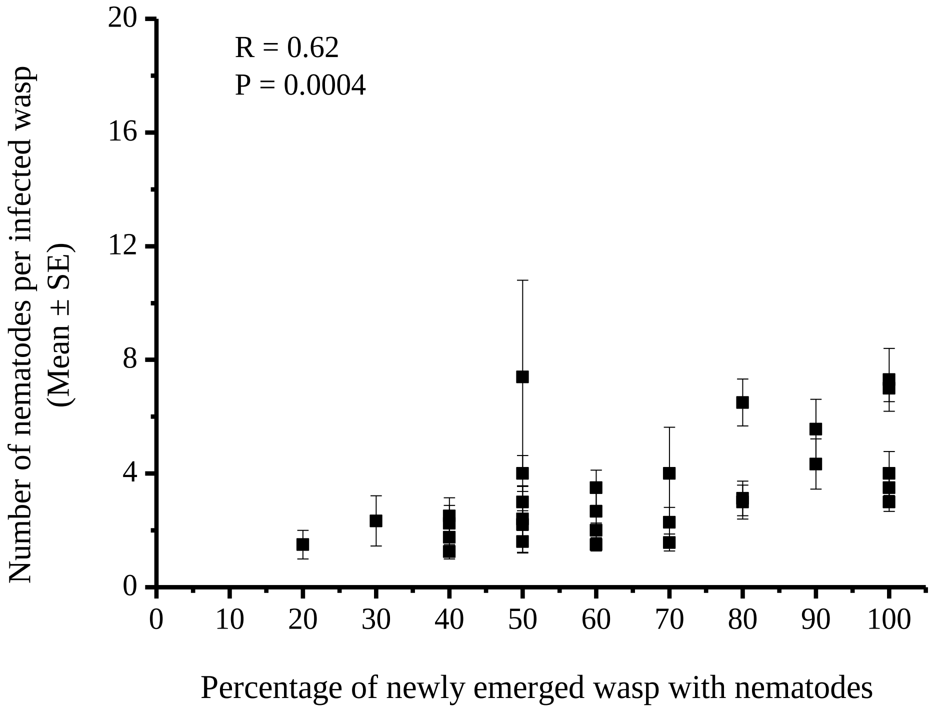


Fig. 4.31. The relationship between the percentage of newly emerged female pollinators from figs of *F. hispida* that had any nematodes attached and the number of nematodes attached per infected pollinator. (N = 10 female pollinators/fig, 5-6 figs/crop (29 figs), total females sampled was 290).

4.5.6 Nematode transportation from fig to fig

In addition to the pollinating fig wasp (*C. solmsi marchali*), male figs of *F.hispida* were also host to two species of non- pollinating wasps which develop together with the pollinator within the figs, *Apocrypta* sp. and *Philotrypesis* sp. (Table 4.7.). These two species laid eggs into the figs by inserting their long ovipositors from outside the figs, several days after the female pollinators entered the figs through the ostiole. Based on what is known of the biology of congeneric species, they are likely to be parasitoids of the pollinator. The offspring of these non-pollinating wasps emerge from the figs at the same time as those of the pollinators.

In D-phase male figs, the male pollinating wasps emerged first and chewed holes in galls containing females to mate. Some nematodes might use these mating holes to enter the galls and attach themselves to the females before they emerge. Male non-pollinators then emerge into the lumen, together with the females of all the species. None of the male pollinators or non-pollinating wasps had nematodes attached (Tables 4.3. and 4.8).. Nematodes were found attached to *Philotrypesis* sp. females, but this was a very small proportion (three out of 211 *Philotrypesis* wasps) (Table 4.8.). The non-pollinators were unlikely however to have been actively chosen to transport the nematodes, since none of the non-pollinator species found in this study enter the figs to oviposit. Furthermore, samples of 320 fig wasp larvae (species unknown) extracted from mid C-phase figs (from 30 figs, five crops) found none that had nematodes. These results confirm that the main way that nematodes are transported from fig to fig is by attaching themselves to a female pollinator (Table 4.8.) at the time when these females are ready to leave the figs.

Table 4.7. Community structure among fig wasps emerging from D-phase of *F. hispida* male figs (N = number of figs sampled)

Crop	N	Diameter (cm) (Mean ± SE)	Emerging wasps (Mean ± SE)				
			C. solmsi marchali females	*C. solmsi marchali* males	*Apocrypta* sp. (female)	*Philotrypesis* sp. (female)	Non-pollinator (males)
C22	6	3.13 ± 0.08	169.50 ± 63.65	38.67 ±17.89	51.50 ±14.77	5.67 ± 0.92	6.17 ± 1.25
C23	6	3.17 ± 0.08	234.83 ± 47.21	34.50 ± 8.46	6.67 ± 2.26	4.50 ± 0.62	2.17 ± 0.17
C24	6	3.28 ± 0.09	157.12 ± 60.24	34.00 ± 4.49	4.77 ± 1.33	1.50 ± 0.76	2.17 ± 0.40
C25	5	3.12 ± 0.04	364.00 ± 65.23	42.00 ±14.95	6.20 ± 1.33	4.20 ± 1.67	3.20 ± 0.18
C26	6	3.17 ± 0.05	301.83 ± 15.69	31.67 ± 6.57	15.50 ± 2.25	4.33 ± 1.36	6.50 ± 0.76
C27	6	3.12 ± 0.08	293.00 ± 17.49	30.67 ± 5.78	16.17 ± 2.96	4.83 ± 1.22	5.8 3 ± 1.01

Table 4.8. Nematodes on adult fig wasps that had emerged recently from figs of *F. hispida* (N = 26 figs, collected from 5 crops).

Fig wasp species	Sex	N (wasps)	Number with nematodes	Percentage with nematodes (%)
Ceratosolen solmsi	F	260	156	60.0
	M	202	0	0.0
Apocrypta sp.	F	258	7	2.7
	M	137	0	0.0
Philotrypesis sp.	F	211	3	1.4
	M	59	0	0.0

Observations of the contents of early D-phase figs showed that most nematodes at that time were ‘swimming around’ actively within the liquid in the lumen of the fig, waiting to attach to female pollinators when they emerged from the galls into the lumen before leaving the figs. The number of nematodes found in the liquid from late C and early D-phase figs from male trees ranged from 14 to 240 in each fig, with a grand mean of 64.2 ± 16.03 nematodes per fig (Mean ± SE) across five different crops. In the liquid of female figs, the nematodes ranged between 35 and 351 per fig, with a grand mean of 73.47 ± 15.97 per fig (Mean ± SE, Table 4.9.). It should be noted that number of nematodes found in the liquid will underestimate the true number of nematodes in each fig, since some nematodes would be left on the surface of the flowers. The high numbers of nematodes found in the liquid indicates that nematodes mostly waited in the lumen of fig to come into contact with the female pollinators as the wasps emerge from the figs.

On contact with female pollinators, the nematodes entered the female’s bodies through inter-segmental membranes and then stood a chance of being carried to suitable B-phase figs, if the pollinators entered male figs. In D-phase male figs, the nematodes were not found to be associated with the male flowers (which pollinator females visit to collect pollen), only within the fig liquid and on the surface of the female flowers.

Table 4.9. Nematodes extracted from the liquid inside C-phase male and female *F. hispida* figs.

Tree sex	Crop	N (figs)	Diameter (cm) (Mean ± SE)	Volume (ml) (Mean ± SE)	N nematodes (Mean ± SE)
Male	C28	6	3.50 ± 0.05	0.29 ± 0.04	40.67 ± 8.89
	C29	6	3.35 ± 0.06	0.46 ± 0.04	86.50 ± 10.02
	C30	6	3.52 ± 0.04	0.42 ± 0.05	43.50 ± 11.84
	C31	6	3.33 ± 0.18	0.38 ± 0.05	88.50 ± 35.78
	C32	6	3.68 ± 0.10	0.38 ± 0.05	61.83 ± 13.61
Female	C33	6	2.97 ± 0.02	0.46 ± 0.04	60.00 ± 6.09
	C34	6	3.37 ± 0.02	0.71 ± 0.14	121.00 ± 46.74
	C29	6	3.27 ± 0.07	0.96 ± 0.16	92.00 ± 19.66
	C35	6	3.10 ± 0.05	0.63 ± 0.12	40.00 ± 2.62
	C36	6	3.25 ± 0.08	0.62 ± 0.12	54.33 ± 4.75

4.6 Discussion

4.6.1 Plant phenology

Ficus hispida is a fig tree species where individual trees can fruit almost continuously, with one crop after another. This sometimes resulted in unsynchronized fruiting within plants, as is seen in many other dioecious fig tree species (Patel 1996; Kuaraksa, Elliott and Hossaert-Mckey 2012) that could allow fig wasps to sometimes cycle on one male tree. In the study area, the trees were fruiting all year around, as was found in North Thailand, where an individual male tree can produce up to eight crops a year, while female tree can bear up to 12 crops (Kuaraksa, Elliott and Hossaert-Mckey 2012). The unsynchronized fruiting and the year around fruiting of *Ficus* spp. trees may favour the pollinating wasp in relation to the time needed for the female wasps to search the receptive figs to enter, thus ensuring survival despite the short life-span associated with pollinating wasps (Janzen 1979a; Compton, Ross and Thornton 1994; Anstett, HossaertMcKey and Kjellberg 1997a; Kuaraksa, Elliott and Hossaert-Mckey 2012). The asynchrony of fruit production in individual male *F. hispida* trees should reduce pollinator mortality associated with flight between trees (Jia et al. 2008), and thus increase their ability to maintain the pollinator populations all year-round, even with small tree populations (Kuaraksa, Elliott and Hossaert-Mckey 2012).

Several studies have reported that development times of figs (crop duration) on female trees are longer than those of male trees, as in *F. fistulosa* (Corlett 1987), *F. aurantiaca* (Chou and Yeh 1995), *F. exasperata* (Patel and McKey 1998; Patel and Hossaert-McKey 2000), *F. vulva* (Harrison, Yamamura and Inoue 2000; Kuaraksa, Elliott and Hossaert-Mckey 2012) and *F. variegata, F. oligodon, and F. fulva* (Kuaraksa, Elliott and Hossaert-Mckey 2012). In south India, *F. hispida* has also been reported previously to have longer crop durations on female trees compared to male trees (Patel and McKey 1998). However in this present study, *F. hispida* showed similar crop durations on male and female trees. The same was also found with *F. hispida* studied in North Thailand (Kuaraksa, Elliott and Hossaert-Mckey 2012).

Seasonal variation in temperature is known to affect the development time of figs (Zhang, Song and Yang 2006; Pereira, Teixeira and Kjellberg 2007; Jauharlina et al. 2012), but seasonal changes in the tropics are much less evident than those in temperate regions. Thus the effect of seasonal changes on crop duration may not be as significant, as was evident from this present study where changes in daily temperature and rainfall had no significant effect on crop duration of *F. hispida* figs on both male and female trees. *Ficus hispida* growing in the northen part of Thailand were similar (Kuaraksa, Elliott and Hossaert-Mckey 2012).

4.6.2 Nematodes and *Ficus hispida*

Several species of nematodes are known to interact intimately with fig trees and their pollinating wasps. Many studies have concluded that only female pollinating wasps are chosen by the nematodes to transport them between receptive figs of the same species (Vovlas, Inserra and Greco 1992; Giblin-Davis et al. 1995; Zeng, Giblin-Davis and Ye 2007; Zeng et al. 2010; Krishnan et al. 2010; Jauharlina et al. 2012). Occasionally nematodes are found also on females of non-pollinating wasps (Jauharlina et al. 2012), as also was found in this present study. The nematodes will however not be transferred into the figs, since the non-pollinating fig wasps, *Apocrypta* sp. and *Philotrypesis* sp, do not enter the figs to lay their eggs.

The routine sequential sampling from both male and female trees of *F. hispida* presented here found that there are two genera of nematodes confirmed to be transferred by the pollinating wasp that occupy the figs, namely *Schistonchus* (Aphelenchoida : Aphelenchoididae) and *Caenorhabditis* (Rhabditida : Rhabditidae). Nematode species from the genus *Schistonchus* are commonly found in figs of *Ficus* spp. Potentially, most *Ficus* species may have associated *Schistonchus* species (Giblin-Davis et al. 1995). *Schistonchus* spp. are known to live and develop at the expense of male and/or female flowers within the figs. *Schistonchus* feeds on floral tissue and also induces tissue hypertrophy. Infection by this nematode is usually associated with hypertrophied cells, tissue necrosis, and the presence of exudate in flowers within the figs (Vovlas, Inserra and Greco 1992; Giblin-Davis et al. 1995; Center et al. 1999).

There were two species of *Schistochus* found in the *F. hispida* trees studied here, *S. centerae* and *S. guangzhouensis*. Both species has been reported to be present in male figs of *F. hispida* in China (Zeng, Giblin-Davis and Ye 2007). These two species are however the only nematodes to associate with *F. hispida* in China, whereas in this study, another species (*Caenorhabditis* sp.) is commonly found together with *Schistonchus* spp. in the same figs. It was already known that more than one species of *Schistonchus* can be associated with the same species of *Ficus* (Zeng, Giblin-Davis and Ye 2007; Zeng et al. 2010; Center et al. 1999; Davies et al. 2010; Davies et al. 2013). Comparisons between several *Schistonchus* species have shown that particular species can infest particular parts of the figs (Center et al. 1999). It means that more than one species of *Shistonchus* may occur simultaneously without any direct competition inside the figs as they inhabit different niches (Davies et al. 2013). Both species of *Schistonchus* presented here are assumed to feed on the female flowers externally since there were no nematodes found in the galls or inside male flowers, which parts of the figs each species occupy is however unknown.

Both species of *Schistonchus* reported here on *F. hispida* did not develop and reproduce within developing larvae within the galls of female flowers (neither did *Caenorhabditis* sp.). They did not come into contact with the female fig wasps until the males chewed holes through their galls for mating. Similar observations were made on another *Schistonchus* sp which is associated with *Pegoscapus* sp. on *F. laevigata*, but in this species, after being transferred into young figs, the nematodes seek out and feed on mainly male flowers (Center et al. 1999). Different findings were reported from *S. caprifici* which is known to develop and reproduce in immature pollinating wasps as well as in fig flowers. *Schistonchus caprifici* takes nutrients from the wasp's haemocoel in order to develop and reproduce (Vovlas, Inserra and Greco 1992; Vovlas and Larizza 1996).

Previous studies have proven that *Schistonchus* spp. can be carried by the pollinating wasps both externally in abdominal folds, and internally in the abdominal haemocoel (Davies et al. 2010). The *Schistonchus* spp. in this study were transferred mostly as juveniles, and occasionally as female adults within the haemocoel of the pollinator fig wasp *Ceratosolen solmsi marchali*. The stages of *Schistonchus* nematodes that attach to fig wasps vary between the different fig trees, pollinating wasps and nematodes species involved. Second stage juveniles of *S. racemosa* were found in the abdominal folds of the *Ceratosolen* sp. pollinator of *F. racemosa* in India (Reddy and Rao 1986). Conversely, female *Blastophaga psenes*, pollinating *F. carica sylvestris,* are known to be infected by both juveniles and adults of *S. caprifici* which penetrate into the haemocoel (Vovlas, Inserra and Greco 1992). Mated adult females of another species of *Schistonchus* are the only stage known to be transported in the haemocoel of the female *Pegoscapus* sp, which pollinates *F. laevigata* in Florida (Giblin-Davis et al. 1995)

The presence of a *Caenorhabditis* species in the *F. hispida* figs and their transfer by pollinating wasps into figs was confirmed by DNA sequencing (N. Kanzaki, personal communication). This is a new finding for this widely studied nematode genus. The presence of rhabditid nematodes from figs in Australia has however been mentioned recently (K.A. Davies and R.Giblin-Davis, unpublished data) and also recorded in *F. septica* in Taiwan (Kanzaki et al. 2012b). Whether the rhabditid nematodes they found were also *Caenorhabditis* has not yet been confirmed. The ecological role of *Caenorhabditis* in the pollinating wasps and figs of *F. hispida* is still unclear, despite its frequent presence in the figs. *Caenorhabditis* was transferred exclusively into figs of *F. hispida* as juveniles, whereas adults were always found only inside figs. The stage when *Caenorhabditis* attached to the insects is called dauer juveniles or dauer larvae. This is often the stage for nematode dispersal in response to unsuitable environments (Kiontke, Hironaka and Sudhaus 2002; Kiontke and Sudhaus 2006; Okumura et al. 2013)

Nematodes carried by insects that never disembark and use their carrier's decomposing cadavers as their source of development are said to display necromeny

(Kiontke and Sudhaus 2006). In contrast, phoresy is an association where nematodes use the insects only for transportation to another suitable place for their development (Kiontke, Hironaka and Sudhaus 2002; Okumura et al. 2013). *Caenorhabditi*s *japonica* for example, is known to have facultative necromeny association with its insect carrier female *Parastrachia japonensis* Scott. (Heteroptera: Parastrachiidae).

All known *Caenorhabditis s*pecies are colonizers of nutrient and microorganism-rich organic material (Kiontke and Sudhaus 2006). *Caenorhabditis japonica*, which is a relative of the model organism *C.elegans*, is known as a bacteriophagous nematode species (Kiontke and Sudhaus 2006; Okumura et al. 2013). Juveniles of the *Caenorhabditis* species encountered in this study left the wasps and transferred into figs to develop and reproduce. However, whether this nematode is purely phoretic (using the wasps as vehicle only) or facultatively necromenic (also feeding on the wasp's cadaver then disembarking into the figs to develop) is not clearly understood. Larvae and adults of *Caenorhabditis* in this study were found within the lumen of the figs. This nematode species may have more than one generation within the figs. In this case *Caenorhabditis* may have fed on the insect remains while carried by the pollinator, and fed on bacteria or other microorganisms while developing and reproducing within the fig. The presence of protozoa within the lumen of the figs was quite common, particularly inside C-phase figs when the liquid was most likely to be present. They are a potential food source, as are *Schistonchus* larvae.

The number of nematodes inside the B- phase figs was related to the number of foundresses entering the figs and the average number of nematodes carried per foundress. Not all newly emerged female pollinators had nematodes attached, and when they had nematodes the number of nematodes carried per female was quite varied. However, the more foundresses that enter a fig and the number of nematodes carried per foundress, are both likely to determine the size of the next generation of nematodes inside the fig. This results in more nematodes being carried out by newly emerged female pollinators when they exit their natal figs. Herre (1993) suggested when only one foundress enters a fig, it could be used to estimate a lifetime reproductive success of the wasp as a function of presence or absence of nematodes attached to the wasp. In this study, one foundress is the most common number found inside the early C-phase of male figs. This finding could however ,not be used to assess the above suggestion since the re-emergence of foundresses was observed on several occasions, so the counts of wasps remaining inside figs is an underestimate of how many had entered.

Studies of nematodes within the figs of dioecious fig tree species have focussed on the figs of male trees, because it is only in this sex that nematodes can complete the dispersal stages of their life cycles (Vovlas and Larizza 1996; Zeng, Giblin-Davis and Ye 2007; Davies et al. 2013). Pollinating wasps and the nematodes that go inside female figs with them face a dead end. Tthe present study has shown however, that the nematodes transported into female figs of *F. hispida* can develop and reproduce within the figs, with similar population trends to those in the figs of male trees. There are no fig wasps produced in the female figs (a small number of non-pollinating fig wasps are known to reproduce in female figs of other fig tree species), and therefore there is no way for the nematodes that bred in the female figs to be transported to other figs to develop and reproduce.

Despite the high occupancy rates and large numbers of nematodes within the figs of *F. hispida* they do not seem to have any effect on the development of pollinating wasps inside the galls on male trees, and the development of seeds on female trees. In male figs, the reproductive success of the pollinator is very important for nematode survival. Any reduction in the wasp's reproductive success due to the presence of nematodes will result in the reduction in the number of vectors available to the nematodes for transfer to other suitable figs, and so would reduce nematode survival (Herre 1995). In female figs, all nematode offspring will always be eliminated, since there are no pollinators produced to transport them between the figs.

Individual female pollinator fig wasps carrying a higher number of nematodes may suffer greater mortality and have a lower possibility of reaching suitable figs (Herre 1993; Herre 1995). The numbers of nematodes attached to female wasps emerging from *F. hispida* figs were similar to those found on fig wasps that had successfully arrived at young figs that were ready to be entered. The same was also found for the proportion of female pollinators with nematodes attached. Therefore, there is evidence to believe that the presence of nematodes on female pollinators of *F. hispida* does not cause any harm to the pollinators themselves during their dispersal phase. There is no cost for the wasps if they carry nematodes from natal figs and no apparent cost to the reproductive success of male trees. Development and reproduction of nematodes inside female figs does not have a detectable negative impact to the seeds production either. The reproductive success both of male and female figs seems to be not affected by nematodes occupancy inside the figs.

Chapter 5 Fig wasps, fig nematodes and their common host *Ficus racemosa*

5.1 Abstract

Nematodes are routinely transported by pollinating fig wasps (Agaonidae) into receptive figs when the wasps enter to lay their eggs. Previous studies have focused mainly on nematode taxonomy while their ecology and relationships with the fig wasps and the figs are largely unknown. In this chapter I investigated the nematodes carried by female *Ceratosolen fusciceps* Mayr into figs of *Ficus racemosa,* a monoecious fig tree, in Sumatra, Indonesia. Eight species of nematodes were recorded from the figs, two of which are still unidentified. The species found were (1) *Teratodiplogaster fignewmani,* (2) *Teratodiplogaster* sp., (3) *Parasitodiplogaster* sp., (4) *Schistonchus* sp1. (5) *Schistonchus* sp2. (6) *Mononchoides* sp., (7) and (8) two undescribed Diplogastridae species ('umbrella-like' species 1 and species 2). This is the most diverse fig nematode community recorded. Most nematodes were transported into figs only as juveniles except the *Schistonchus* spp. which were occasionally transported as adults. The highest nematode populations were routinely found in D-phase figs, when the new generations of wasps were about to emerge. The nematodes attach themselves to newly-emerged female pollinators, which then carry them out of the figs when they fly in search of receptive figs. Details of the ecology of each nematode species are likely to differ, but as a group they did not seem to significantly affect seed and wasp development in *F. racemosa* figs.

5.2 Introduction

The mutualism between fig trees and their pollinating wasps has been well documented in many previous studies (Janzen 1979a; Compton 1993; Weiblen 2002). The mutualism has persisted for more than 60 million years and involves more than 750 fig tree species with their associated fig wasps (Ronsted et al. 2005; Cook and Segar 2010). Increasing numbers of studies have shown that some fig tree species are pollinated by two or more fig wasp species. The interactions between the fig trees and their pollinating wasps tend to be species-specific, because very few fig wasps colonise more than one species of fig tree (Berg 1989; Wiebes 1979; Compton, Grehan and van Noort 2009).

Mutualisms provide resources that also attract other species to exploit the interaction between the mutualist species. These exploiter species may influence the net benefits of the mutualism for one or both mutualist partners (Bronstein 1991; Bronstein 2001a; Bronstein 2001b). Nematodes have been recognised as associates with figs since the last century (Gasparrini, 1864 cited in Vovlas, Inserra and Greco (1992). The nematodes' relationship with fig wasps was however, not further explained until recently (Reddy and Rao 1986; Vovlas, Inserra and Greco 1992; Vovlas and Larizza 1996; Krishnan et al. 2010).

From over 800 of fig trees species worldwide, only a few have been checked for the presence of nematodes. Nematodes from the genus *Schistonchus* (Aphelenchoididae) are found associated with many different fig tree species and exhibit strong host-specificity (Vovlas, Inserra and Greco 1992; Davies et al. 2010; Davies et al. 2013). These nematodes are transferred by pollinating wasps into figs as juveniles and/or adults when female fig wasps enter receptive figs (B-phase) to lay their eggs and pollinate the flowers inside the fig. Nematodes then develop and reproduce within the figs. Subsequent generations of nematodes are ready to be transported out of the figs at the same time as the fig wasps' offspring emerge from the galls (early D-phase). The nematodes can then be transferred into another receptive fig (Vovlas, Inserra and Greco 1992; Vovlas and Larizza 1996; Giblin-Davis et al. 1995).

Nematodes belonging to the genus *Schistonchus* have stylets that they use when feeding on the tiny flowers inside the figs. The *Schistonchus* sp. associated with figs of *F. laevigata* is known to feed only on male flowers (Giblin-Davis et al. 1995), while other species of *Schistonchus* feed on both male and female flowers (Vovlas, Inserra and Greco 1992; Center et al. 1999). Feeding by *Schistonchus* on the fig flowers result in development of hypertrophied epidermal cells (Giblin-Davis et al. 1995). In a heavily nematode-infested fig of *F. laevigata*, feeding by *Schistonchus* sp. on male flowers may cause a significant reduction in pollen availability for emerging female pollinator (Giblin-Davis et al. 1995), but the location of the nematodes in the male flowers means that they are readily picked up by female pollinators when they attempt to fill their thoracic pollen baskets. In a study on *F. aurea*, *F. maxima*, *F.poponoei*, and *F. trigonata*, the effects of feeding by *Schistonchus* spp. appear to be superficial, without real damage to the plant or their pollinating wasps (Center et al. 1999). Similarly, there is no evidence of any harmful effect on *Blastophaga psenes*, the pollinating wasps of *F. carica sylvestris*, when *S. caprifici* enter inside developing wasp larvae within their galls (female flowers) (Vovlas, Inserra and Greco 1992).

Parasitodiplogaster (Diplogastridae) is another genus of nematodes that is recorded regularly in many species of fig trees (Giblin-Davis et al. 1995; Giblin-Davis et al. 2006; Compton 1993; Bartholomaeus et al. 2009; Jauharlina et al. 2012). The nematodes belonging to this genus are carried by female pollinating wasps and transferred as third stage (J3) (dauer larva) juveniles into B-phase figs, when the wasps enter the figs to pollinate and oviposit into available flowers. The nematodes increase greatly in size as they moult and develop into fourth stage (J4) inside the haemocoel of the wasps. The nematodes then emerge from the fig wasp cadaver into the fig lumen. The nematodes develop into adults and produce a new generation of J3 which infect the new generation of pollinator females as they emerge from their galls (Giblin-Davis et al. 1995; Jauharlina et al. 2012).

Parasitodiplogaster nematodes are known to feed on the bodies of the wasps that carry them between figs. The short longevity of female wasps (maximum 48 hours) forces them to locate and enter another B-phase fig in a short time. Most successful wasps are likely to have found a suitable fig within a few hours (Compton 1993), leaving nematodes little time to damage the wasps during transportation (Jauharlina et al. 2012). It would be a disadvantage for the nematodes to damage the fig wasp before or during transportation, since the nematodes need the wasps to transfer them into the next fig. In cases where only one female pollinator (foundress) enters a B-phase fig, any damage caused by the nematodes will ultimately result in the reduction (or even elimination) of opportunities for the next generation of nematodes to be transported. If however, more than one foundress enters a fig, nematodes may more safely increase the extent of their damage to the host wasps before they have finished egg-laying, as more fig wasp offspring will be available to carry the new generation of nematodes and disperse them between figs. Consistent with this, there is evidence for a link between the extent of *Parasitodiplogaster* damage to fig wasps and foundress numbers (Herre 1993; Herre 1995). Nonetheless, any increase in rapid damaging activity of the nematodes should generally occur after the female wasp entry into the B-phase figs (Giblin-Davis et al. 1995).

Some other nematodes are also associated with figs, including the diplogastrid *Myctolaimus racemosa* (Anand, 2005), and *Ceratosolenus hyderabadensis* sp. n that are found in the fig pollinating wasp *Ceratosolen* sp. and the figs of *Ficus racemosa*, in India (Anand 2006). Other species belong to the genus *Teratodiplogaster* (Diplogastridae), which is phylogenetically close to *Parasitodiplogaster*, with *T. fignewmani* found in *Ficus racemosa* figs in Australia (Kanzaki et al. 2009). A recent study, using materials collected from Africa (Martin, Owen and Way 1973), described *T. martini* from an unidentified *Ficus* sp, (Kanzaki et al. 2012a).

Despite many nematode species being found to be associated with fig trees and their pollinating wasps, the relationships between the three of them and in particular how nematodes affect the mutualism have rarely been clearly defined. This study investigated the nematode species associated with figs of *F. racemosa* and their relationship with the fig tree and its pollinating wasp. This chapter addresses the following questions.

1. How many nematode species are present in figs of *F. racemosa*, in Sumatra and how does their abundance vary?
2. How does fig wasp foundress number affect the number of nematodes within a fig?
3. What is the impact of nematodes on seed and pollinator production?
4. What is the structure of the broader fig wasp community?
5. Do the nematodes only seek out female pollinators for transmission between figs?

5.3 Study species

Ficus racemosa L., known as the cluster fig, is the only species in Asia that belongs to the monoecious section of the subgenus Sycomorus (Fig. 5.1.). The other monoecious Sycomorus species occur in Africa (Galil and Eisikowich 1968). This fig tree species is distributed from India to Australia and grows commonly in Southeast Asia (Corner 1965).

Ficus racemosa is a large-sized tree species, reaching 25-40 m in height and 60-120 cm in trunk diameter (at breast height) (Corner 1965; Zhang, Song and Yang 2006). It grows in evergreen forests, moist localities and the banks of streams in deciduous forests up to an elevation of 1800 m above sea level. The tree usually grows in groups of five to ten (Corner 1965). The tree is often cultivated for shade and its edible fruits. In India, the tree is known as one of the sacred plants (*Nalpamara*) and is commonly planted around houses and temples (Paarakh 2009). *Ficus racemosa* has also been known as a source for traditional medicine. The leaves of *F. racemosa* are reported to have antifungal, anti-inflammatory and analgesic activities, the stem bark has hypoglycaemic activity, and the figs are used for the management of diabetes (Jahan et al. 2009; Paarakh 2009; Sirisha et al. 2010).

The tree has unisexual male and female flowers inside its figs that mature asynchronously, which prevents self-pollination (Wang et al. 2008). The figs exist in large clusters on short and leafless branches emerging from the trunk and the main branches. The tree produces 5-6 fig crops each year with the phenology of the figs from their early stages until the figs mature and abort lasting about 60 days. The figs become bright red in colour when they ripen (Paarakh 2009; Ranganathan, Ghara and Borges 2010) (Fig. 5.2.).

The developmental stages of figs are generally divided according to the terminology formulated by Galil and Eisikowich (1968b), starting from the A-phase, then B, C, D and E phases (Fig. 5.3.). The A-phase of the fig is a ‘pre-receptive’ phase before the wasp’s entry. This phase may last up to three weeks. The wasps enter the fig in the ‘receptive’ phase (B-phase) which may last for three to six days, or more. The subsequent C-phase is the ‘inter-floral’ phase in which the wasps and seeds develop. This phase may last between two and six weeks, depending on the fig tree species. In *F. racemosa*, the C-phase mostly lasts around two weeks (personal observation). The mated female wasps leave the figs, loaded with pollen in the D-phase or ‘emission’ phase. This phase may last three to four days. Upon the wasps’ departure, the figs then ripen in E- phase or ‘post-floral’ phase. At this phase, the figs become soft and ready to be eaten by vertebrates or eventually abort. During C- and early D-phase, figs of *F. racemosa* contain liquid within the lumen, which is a characteristic feature of *Sycomorus* figs.

Ficus racemosa bears its figs synchronously within the tree in very large numbers. Pollination depends on its pollinating wasp *Ceratosolen fusciceps* Mayr (Hymenoptera: Agaonidae), and the wasps depends on the fig to lay eggs and complete their life cycle (Zhang, Song and Yang 2006; Wang et al. 2008; Wang and Sun 2009). The number of pollinating wasps that enter a receptive fig (foundresses) of *F. racemosa* is between five and 30, but sometimes it can reach more than 70 (Wang et al. 2008; Wang and Sun 2009). The figs of monoecious species such as *F. racemosa* produce both seeds and wasps. Stability of mutualisms between fig trees and pollinating wasps is thought to be influenced by the highly variable lengths of flower styles inside the figs (Nefdt and Compton 1996; Yu et al. 2004). This leads to the figs of *F. racemosa* mainly having fig wasp larvae developing in shorter styled flowers and seeds in flowers with longer styles.

5.4 Methods

5.4.1 Study site

The study was undertaken at the same sites as those of *F. hispida* (Chapter 4). This study was conducted during a period of 19 months, from July 2011 until February 2013. Along with *F. hispida*, *F. racemosa* were also very common in the area. In comparison to *F. hispida*, all the *F. racemosa* trees grew in more difficult positions to reach. Most grew on cliffs heading down from the road to the ocean, but with their canopy and upper parts of the trees above the side of the roads. Most of the trees grew near rivers or small streams. Meteorological information for the area was summarized in Chapter 4 (Figs. 4.9. and 4.10.)

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Fig. 5.1. *Ficus racemosa* tree (R1). The tree grows on the river banks.

Fig. 5.2. Mature figs of *F. racemosa* showing synchrony fruit stage within the tree

Fig. 5.3. Phases of *F. racemosa* fig development

5.4.2 Routine sequential sampling procedures

The routine sequential sampling methods employed for *F.racemosa* trees were the same as those on *F. hispida* described in the previous chapter (Section 5.4.2). The trees marked for sampling were all much bigger than taller than *F. hispida*. Some of the *F. racemosa* trees were located close to *F. hispida*, some others were further away. There were 20 *F. racemosa* trees that were marked (Table 5.1., Fig. 5.4.), some of which were used for weekly routine sampling, and others were used for additional data collection. There were also several trees that were not sampled at all, because they did not have figs at appropriate times.

5.4.3 Fig extractions

The methods applied for fig extraction were the same as those on *F. hispida* figs, as described in Chapter 4 (Section 4.4.3, Fig. 4.11.)

5.4.4 Identification of nematodes

Preliminary identification of nematodes was done by observing the presence of a stylet and/or bulb, the shape of the mouth and the shape of the tail. The developmental stages of the nematodes found were also recorded. Identification of nematodes was done morphologically by comparing the nematodes with a previous investigation of nematodes found on *F. racemosa* in China (Zeng, Giblin-Davis and Ye 2007). Additional help with nematode identifications was obtained from the Forestry and Forest Products Research Institute, Tsukuba, Japan (Kanzaki, Personal communications).

5.4.5 Community structure of fig wasps

In addition to routine sequential sampling, six early D-phase figs from six crops from six different *F. racemosa* trees were sampled. The methods employed to investigate the community structure were the same as used for *F. hispida* figs, as described in Chapter 4 (Section 4.4.9). All the wasps from each fig were identified and counted accordingly.

5.4.6 Nematodes on newly emerged fig wasps

Nematodes of each species of emerging wasp were observed by additional sampling of six to seven D-phase figs from different crops of *F. racemosa*. The methods were the same as employed on Section 4.4.10 in Chapter 4. The observations were done on six crops from six different trees.

5.4.7 Impact of nematodes presence on number of female pollinator offspring and seeds

Impact of the presence of nematodes on the numbers of female pollinator offspring and seeds was investigated with the same methods employed in Chapter 4 (Section 4.4.7). Six to seven more early D-phase figs were sampled from each crop of *F. racemosa* figs. The investigation was done on five crops which came from five different trees.

Table 5.1. Locations and characteristics of *Ficus racemosa* trees.

No	Tree number	Circumference (cm)	Diameter (cm)	Height (m)	GPS location	
					East	North
1	R1	153	48.7	15	95° 15’ 48.60”	05° 22’ 56.28”
2	R2	136	43.3	17	95° 15’ 30.54”	05° 22’ 43.98”
3	R3	118	37.6	17	95° 15’ 30.48”	05° 22’ 43.98”
4	R4	135	43.0	25	95° 14’ 31.20”	05° 20’ 40.02”
5	R5	80	25.5	20	95° 15’ 13.44”	05° 19’ 59.34”
6	R6	90	28.7	23	95° 15’ 05.28”	05° 19’ 40.80”
7	R7	132	42.0	25	95° 15’ 06.48”	05° 19’ 23.52”
8	R8	60	19.1	20	95° 15’ 05.22”	05° 19’ 45.06”
9	R9	200	63.7	30	95° 14’ 58.74”	05° 19’ 30.72”
10	R10	60	19.1	15	95° 14’ 57.36”	05° 19’ 29.64”
11	R11	82	26.1	25	95° 15’ 05.34”	05° 19’ 22.92”
12	R12	150	27.7	10	95° 15’ 01.38”	05° 19’ 7.92”
13	R13	150	27.7	30	95° 15’ 0.78”	05° 19’ 6.84”
14	R14	150	27.7	35	95° 14’ 15.60”	05° 18’ 33.24”
15	R15	150	27.7	20	95° 14’ 20.34”	05° 18’ 28.86”
16	R16	135	43.0	25	95° 14’ 25.89”	05° 18’ 37.00”
17	R17	200	63.7	25	95° 14’ 09.36”	05° 18’ 22.50”
18	R18	40	12.7	10	95° 15’ 02.82”	05° 18’ 51.36”
19	R19	300	95.5	40	95° 15’ 0.66”	05° 18’ 53.16”
20	R20	82	26.1	20	95° 15’ 26.10”	05° 19’ 15.60”

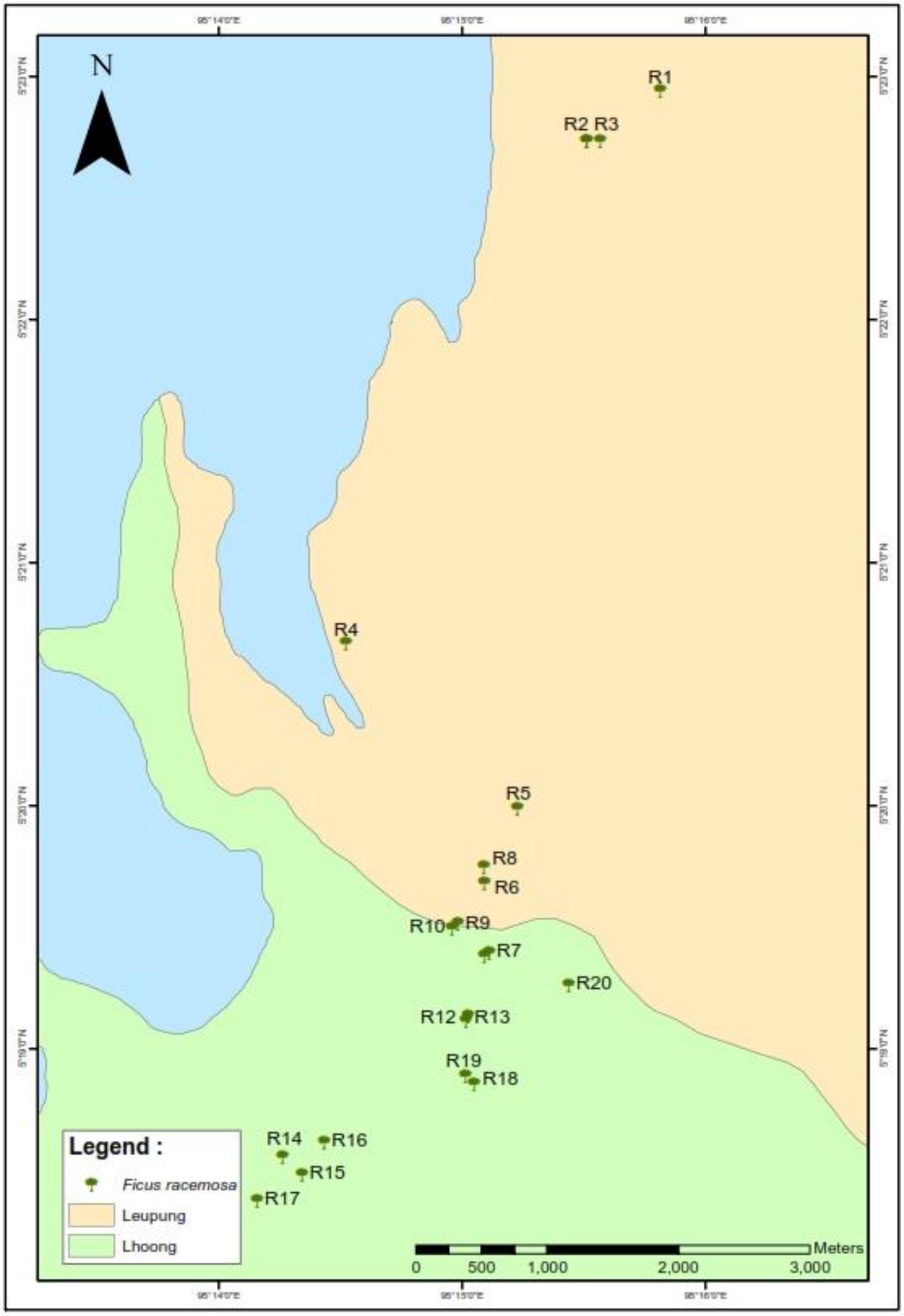


Fig. 5.4. Sampling locations for *F. racemosa* in the districts of Leupung and Lhoong, Great Aceh Regency, Banda Aceh.

5.4.8 Measurements of nematodes

Measurements of nematodes were done on adults of each nematode species found within the figs. The measurements were done on the last three crops when each species of nematode was recognized. Additional samples of late C-phase figs on each of the last three crops were taken for this purpose. Late C-phase figs were chosen due to the large numbers of adult nematodes at this phase. Figs were wiped clean and cut into several pieces, soaked in water inside a Petri dish. After two hours, adult nematodes were hand- picked individually from the dishes and placed onto slides. Each slide was then run above a container containing hot water (60-70 ^{0}C) to make the nematode relax but not damaged, so that measurements could be made under microscope. The measurements done were simpler than those on *F. hispida* in previous chapter due to the large number of observation in any one day. The measurements included only the length of the nematodes, from head to the tail tip (Length), the widest part of nematodes (width), and the distance from the head to the genitalia of male and female nematodes respectively (spicule or vulva). Measurements were taken using a measuring eye piece attached to a compound microscope having 100 times magnification.

5.4.9 Data analysis

Crop duration was analysed by linear models since only one crop was observed in each tree. Number of wasps and number of seeds were treated as continuous data because their means were high and analysed by linear mixed effect models using *nlme* package. Assumptions were checked by looking at the normality of residuals. For count data (number of nematodes), analysis was done using lme4 package, with Poisson errors added into the models. Dispersion was checked and over dispersion in the model was corrected by including fig identity as individual level random effect. Linear mixed effect models were applied, because the crops were nested within the trees, generating both fixed and random factors. Temperatures and rainfall were treated as fixed effects for crop duration analysis. Numbers of foundresses present in B-phase figs was treated as a fixed effect for analysing the number of nematodes in B-phase figs. Crops were included in the models as random effects. The effects were considered to be significant at P-values <0.05 and are reported together with t-values and degrees of freedom (df) when analysed using nlme or z-values when analysed using lme4.

5.5 Results

5.5.1 Seasonality

The maximum and minimum mean daily temperatures in the area during the period of study were presented in Chapter 4 (Fig. 4.12.), as were the relative humidity (Fig. 4.13.) and rainfall (Fig. 4.14.). The extreme daily temperatures during the 19 months of the study contrast with the average minimum and maximum daily temperatures, which were quite different. The average minimum temperatures recorded daily ranged from 22.0 to 23.2.0 ^{0}C, while the average daily maximum temperatures were between 30.6 and 34.2 ^{0}C.

5.5.2 Fruiting phenology

Eight crops were sampled throughout their development during the 19 months of study. Development of each crop was followed by weekly sampling from the young figs (A-phase) until the figs started to mature and fall to the ground (E-phase). There were 244 figs observed and their contents extracted during this routine sampling. Another 195 figs from 34 different crops were observed for specific purposes. As in other monoecious fig tree species, the fruiting development of each crop within a tree tended to be synchronous with mostly the same phase at any one time. There was usually a gap of 1-2 weeks between two consecutive crops on each tree, however there were cases when new figs from the next crop had already appeared while some E-phase figs from the previous crop were still on the tree. These cases were usually found on the trees which had relatively small numbers of figs in each crop. The figs were green in colour during A-phase and became brightly red in colour when mature in E-phase. The size of the figs ranged between 0.9 and 1.4 cm in diameter in A-phase, and grew bigger between 2.5 and 3.4 cm in diameter in E-phase.

The duration of crop development from A-to E-phase was similar for each crop, and ranged between 41 and 42 days (note that sampling intervals were every seven days, so these values are approximate). On average, it took 41.63 ± 0.18 days (Mean ± SE, N = 8 crops) for each crop to complete development from A to E phase. The duration of C-phase was the longest (Fig. 5.5.). Crop duration was not affected by the (relatively small) variation in daily temperatures during fig development (lm, $F_{(1,5)} = 3.078$, P = 0.139, nor by monthly rainfall (lm, $F_{(1,5)} = 0.289$, P = 0.614) (Fig. 5.6.).

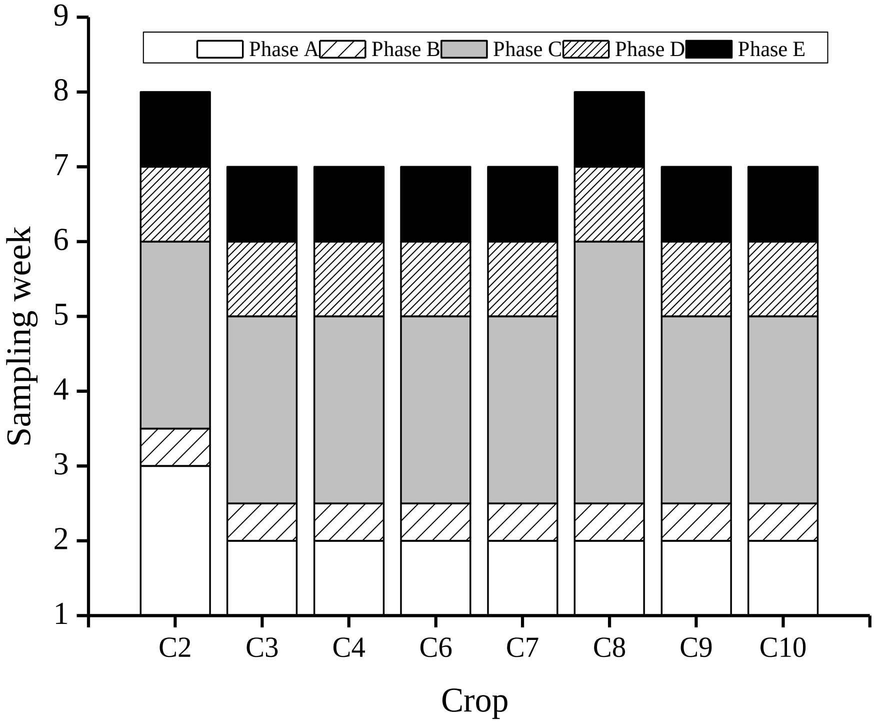


Fig. 5.5. Duration of each fig developmental phase of crops from eight *F. racemosa* trees

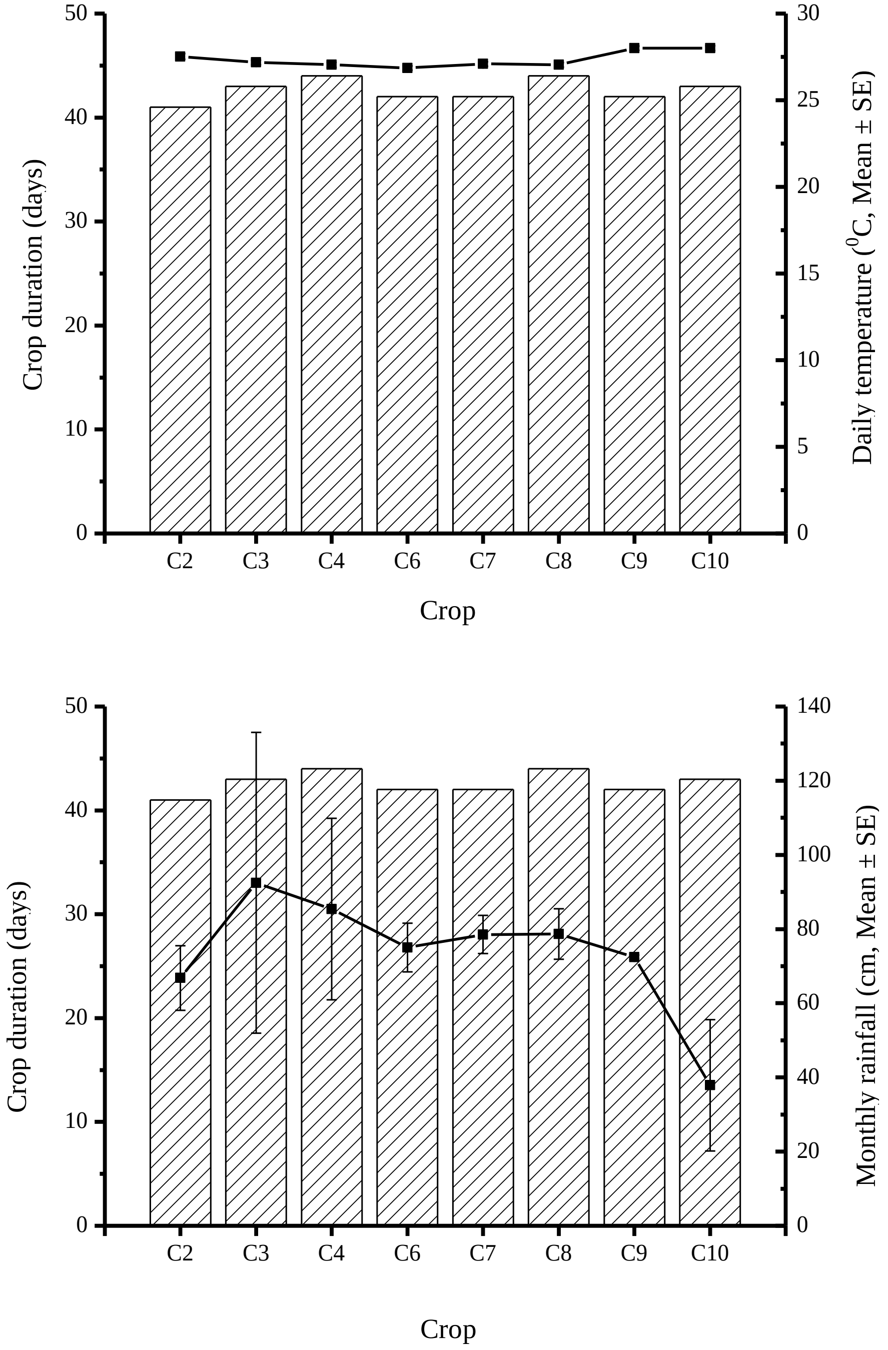


Fig. 5.6. The time taken to reach maturity by crops of *Ficus racemosa* in relation to temperature (top) and rainfall (bottom). Standard errors for the mean temperatures were too small to be seen in the top figure. Crop durations were recorded from A-phase figs up to when the first E-phase figs were found in each crop.

5.5.3 Species of nematodes and their population sizes

The communities of nematodes found in *F. racemosa* figs were more complicated than those in *F. hispida* figs. Eight species of nematodes were recorded, all transferred by the pollinating wasp *Ceratosolen fusciceps* into figs of *F. racemosa* (Table 5.2). They were (1) *Teratodiplogaster fignewmani*, (2) *Teratodiplogaster* sp., (3) *Parasitodiplogaster* sp., (4) *Schistonchus* sp1. (5) *Schistonchus sp2*. (6) *Mononchoides* sp., (7) and (8) two undescibed Diplogastridae species (umbrella-like species 1 and species 2) (Appendix 4-11). Among the species, *Teratodiplogaster* spp. were observed as the most mobile nematodes, swimming very fast within the liquid found on the lumen of late C-phase figs. It was also found that the fig lumen contained other microorganism including protistans.

Table 5.2. Nematode species transported by pollinating wasps into figs of *F. racemosa.*

No.	Nematodes species	Family	Ecological role
1.	*Teratodiplogaster fignewmani*	Diplogastridae	Bacterial-feeding (?)
2.	*Teratodiplogaster* sp.	Diplogastridae	Bacterial-feeding (?)
3	*Schistonchus* sp 1	Aphelechoididae	Plant parasite
4	*Schistonchus* sp. 2	Apelenchoididae	Plant parasite
5.	*Parasitodiplogaster* sp.	Diplogastridae	Parasite of pollinating wasp
6.	*Mononchoides* sp.	Diplogastridae	Unknown
7.	Umbrella-like sp. 1	Diplogastridae	Unknown
8.	Umbrella-like sp. 2	Diplogastridae	Unknown

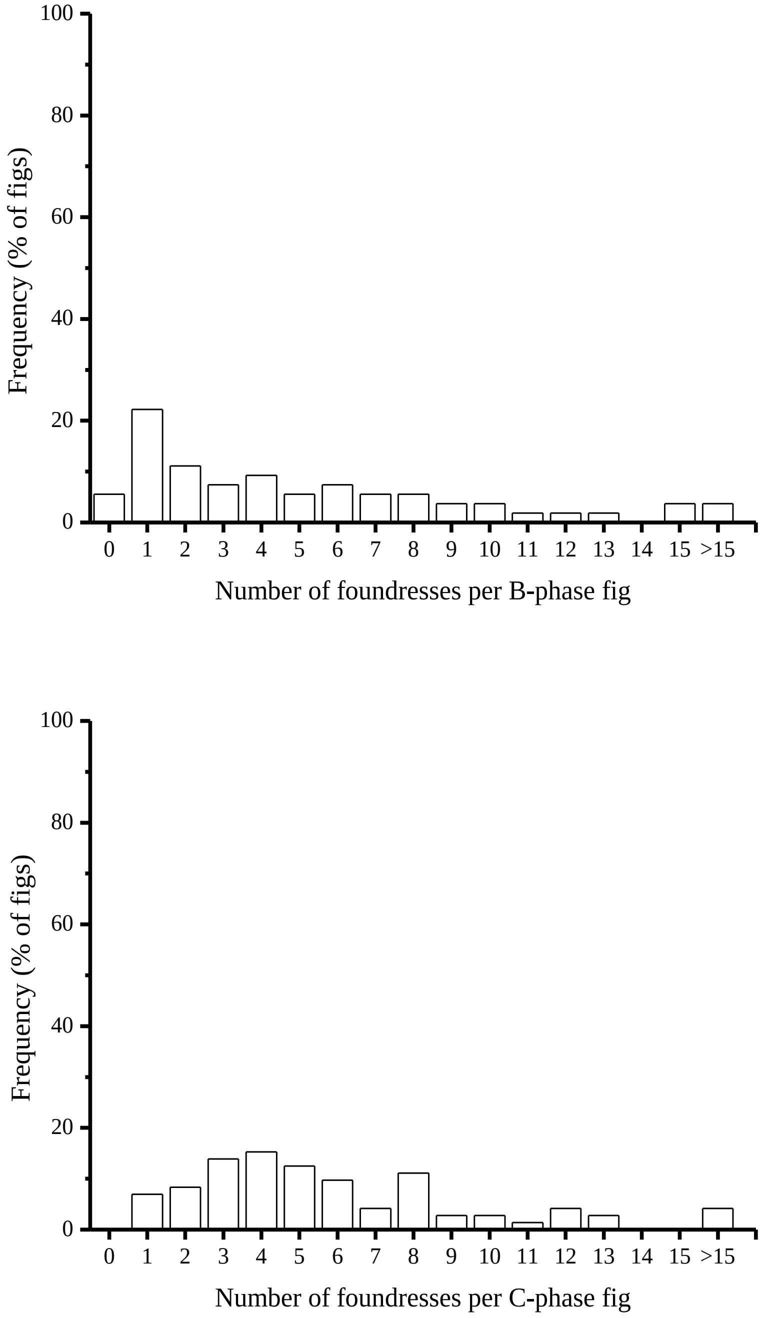


Fig. 5.7. Frequency of foundresses entering a fig of *F. racemosa*, calculated when the figs were still in B-phase (top) and already in early C-phase (bottom) (N = 54 B-phase figs, and 72 C-phase figs).

The nematodes were transferred into figs as juveniles, except for the *Schistonchus* spp., which were occasionally transferred as adults. Dissection of recently-dead female pollinators that had entered inside B-phase figs showed that sometimes there were no nematodes found within the wasps. Extractions from the figs however proved that the wasps had transferred nematodes into the figs, showing how quickly the nematodes emerged from the pollinators once they were inside the figs. On the other hand, there was a case when there was no foundress present in a fig, but nematodes were still found by fig extraction, showing that the foundress may have re-emerged through the ostiole after initially entering the fig.

Numbers of female pollinators present inside B-phase figs (foundresses) ranged between 0 and 23 per fig with a grand mean of 5.35 ± 0.70 (Mean ± SE, N = 54 figs and 289 fig wasps). One foundress per fig was the most common number present inside the B-phase figs. Further observation on early C-phase figs indicated that numbers of foundresses counted in B-phase might not the true number of foundresess entering a fig and that entry can occur over extended periods. More foundresses had entered the figs after the time of B-phase figs sampling, with the number of foundresses present in early C-phase figs ranging between 1 and 34 with a grand mean of 6.20 ± 0.60. Four foundresses per fig was the most common number found inside early C phase figs (Fig 5.7.). There was a significant relationship between the numbers of foundresses present in B-phase figs and the number of nematodes found within the figs by extraction (lmer, $z = 7.415$, $P<0.0001$). The more foundresses present inside the B-phase figs, the higher number of nematodes were extracted from the same figs (Fig. 5. 8.). Nematodes on B-phase figs were presumed to be the ones being transferred into the figs along with the foundresses. Nematodes on C-phase figs could not be related directly to number of foundresses, since some nematodes found at this stage might have been the new generation nematodes developing in the figs.

Nematodes developed and produced their offspring inside the figs along with the development of the figs. Numbers of nematodes peaked in D-phase figs on each crop. Numbers of nematodes in D-phase figs ranged from 53 up to 2040 per fig, with a grand mean of 684.89 ± 76.09 (Mean ± SE, N = 48 figs) across all D-phase figs extracted from the eight crops (Fig. 5.9.). They mostly consisted of nematode larvae from new generations. Numbers of juvenile nematodes at this stage were much higher than numbers of adults (Fig. 5.10.). These nematode offspring were ready to attach themselves to the new generation of female pollinating wasps which emerged from their galls about the same time. The female fig wasps then emerged from the figs carrying nematodes with them in search of B-phase figs on another tree to enter.

Adult nematodes were identified to species in the last three crops included in the routine sequential sampling. The number of adults of each nematode species was calculated from these three crops of C-phase figs, the stage that was thought to have the highest populations of adult nematodes. The number of diplogastrid adults altogether was higher than *Schistonchus* spp. adults in each of the three crops observed (Fig. 5.11.). Not all nematode species were found at the same time in any one fig (Fig. 5.12.), but T*eratodiplogaster* spp.*,* *Parasitodiplogaster* sp, and *Schistonchus* spp. were quite common (Fig. 5.13.). Among all species nematodes present, the adult size of *Schictonchus* spp. were smaller than others (Table 5.3.).

Identification of nematode species could not be done when they were in juvenile stages. For juveniles, I only distinguished two separate families based on their stylet presence or absence. Aphelenchoididae (for the genus *Schistochus*) have stylets and the other was Diplogastridae (the rest of the nematode species) that did not have stylets. In D-phase, when the juvenile nematodes population peaked, juveniles of Diplogastrid nematodes consistently dominated juveniles *Schistonchus* in each crop observed (Fig. 5.14.).

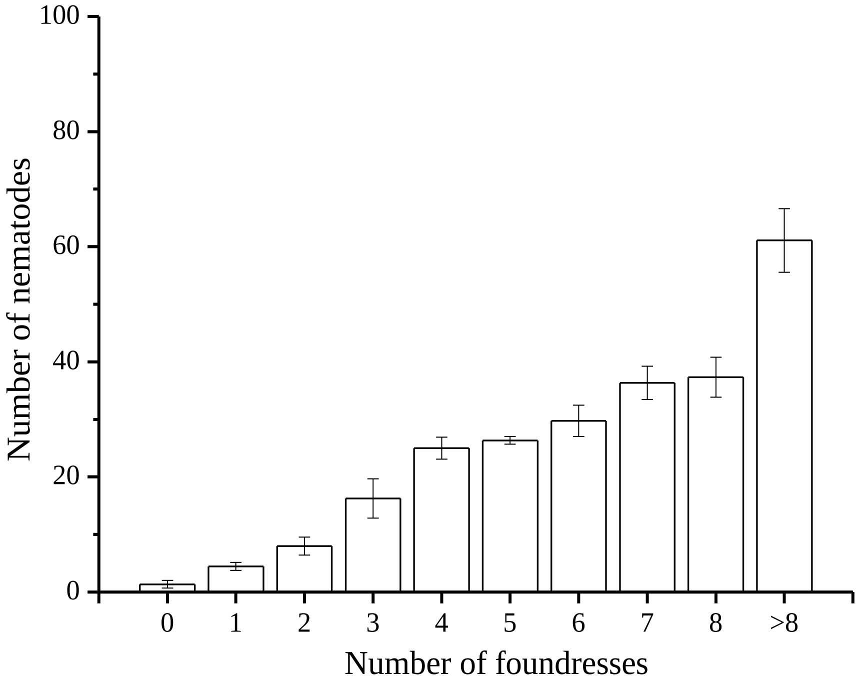


Fig. 5.8. Numbers of nematodes in figs containing different numbers of foundresses (N = 54 figs) in B-phase figs of *F. racemosa.*

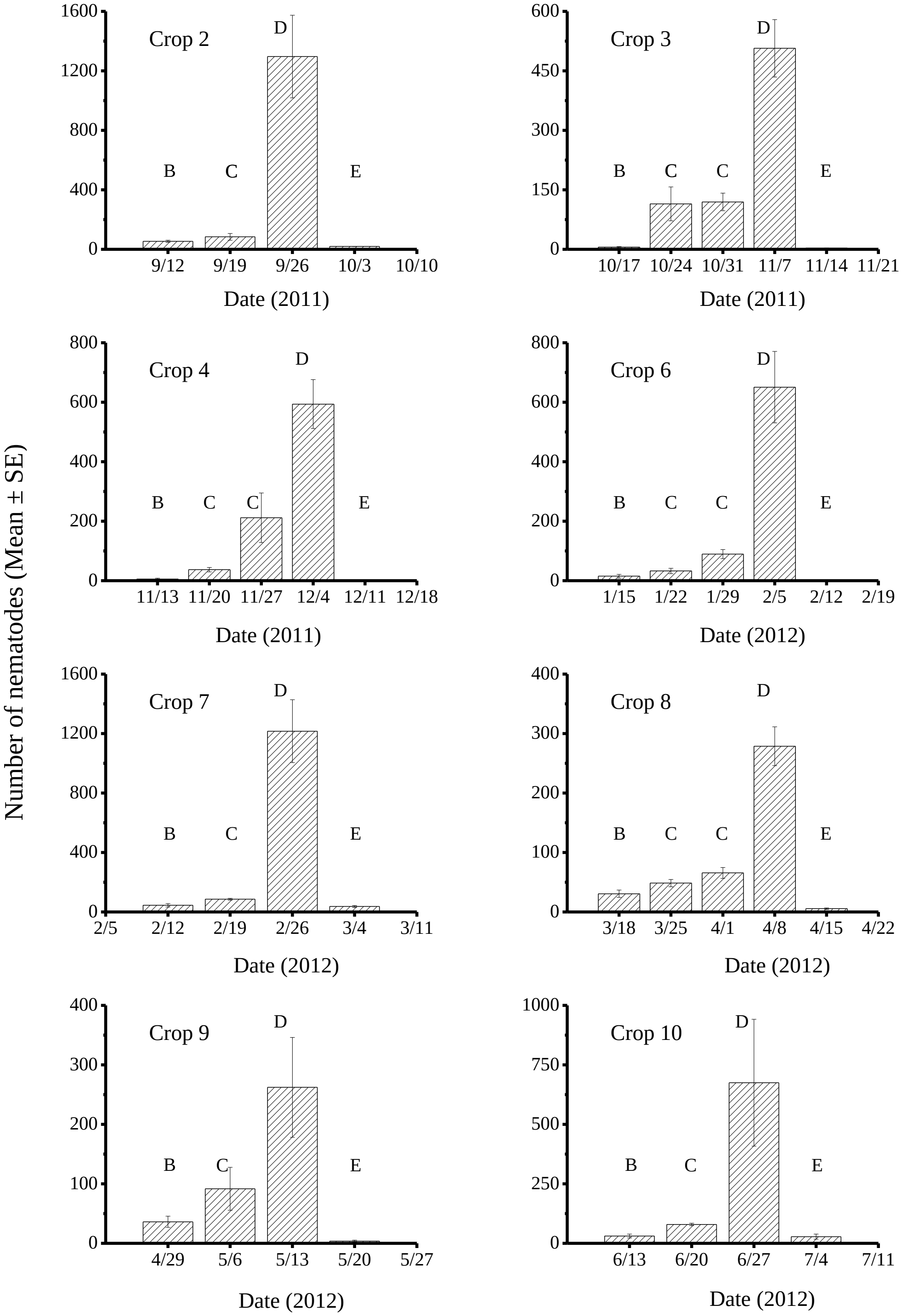


Fig. 5.9. Nematode populations per fig (all species) during the development of eight crops of *F. racemosa* in routine sequential samplings. Numbers were obtained from extraction of whole figs (note the varying scale of the Y-axis).

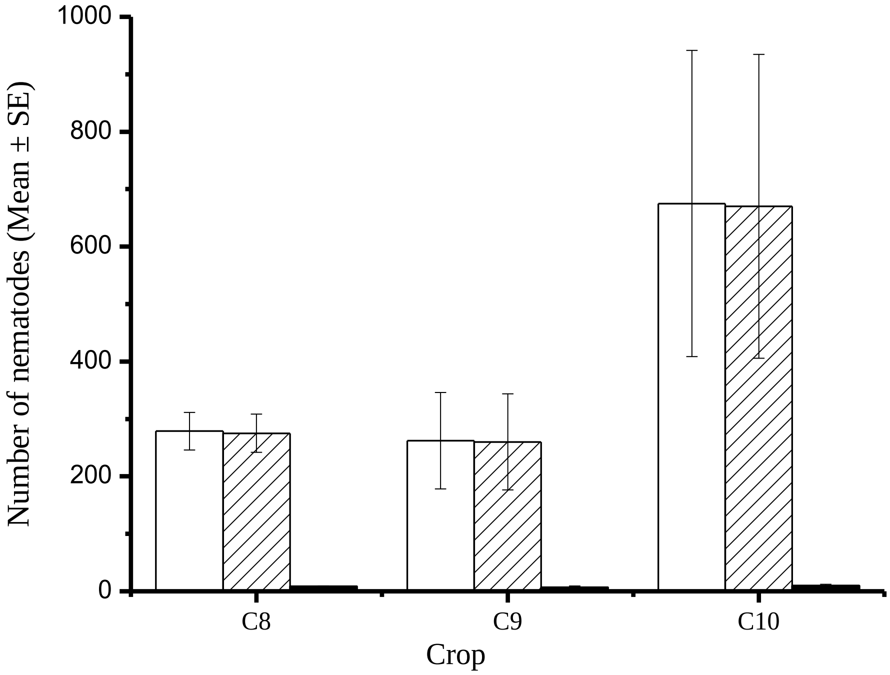


Fig. 5.10. Nematode populations (all species) per D-phase fig on three crops of *F. racemosa* figs from routine sequential sampling. Open bars = total nematodes; hatched bars = juveniles nematodes; and closed bars = adult nematodes (N = 6 figs/crop).

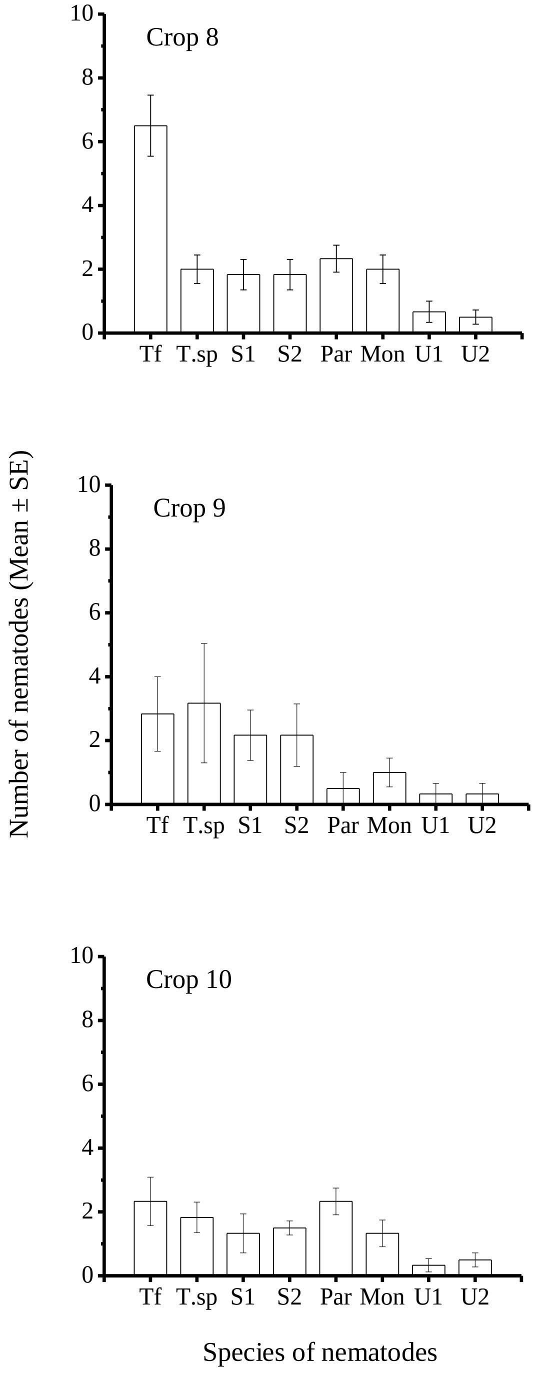


Fig. 5.11. Number of adult nematodes per C-phase fig in three crops of *F. racemosa* (N = 6 figs/crop) (Tf = *Teratodiplogaster fignewmani*, T.sp = *Teratodiplogaster* sp., S1 = *Schistonchus* sp.1, S2 = *Schistonchus* sp.2, Par = *Parasitodiplogaster*, Mon = *Mononchoides*, U 1= umbrella-like sp.1, U 2 = Umbrella-like sp. 2) (N = 6 C-phase figs/crop).

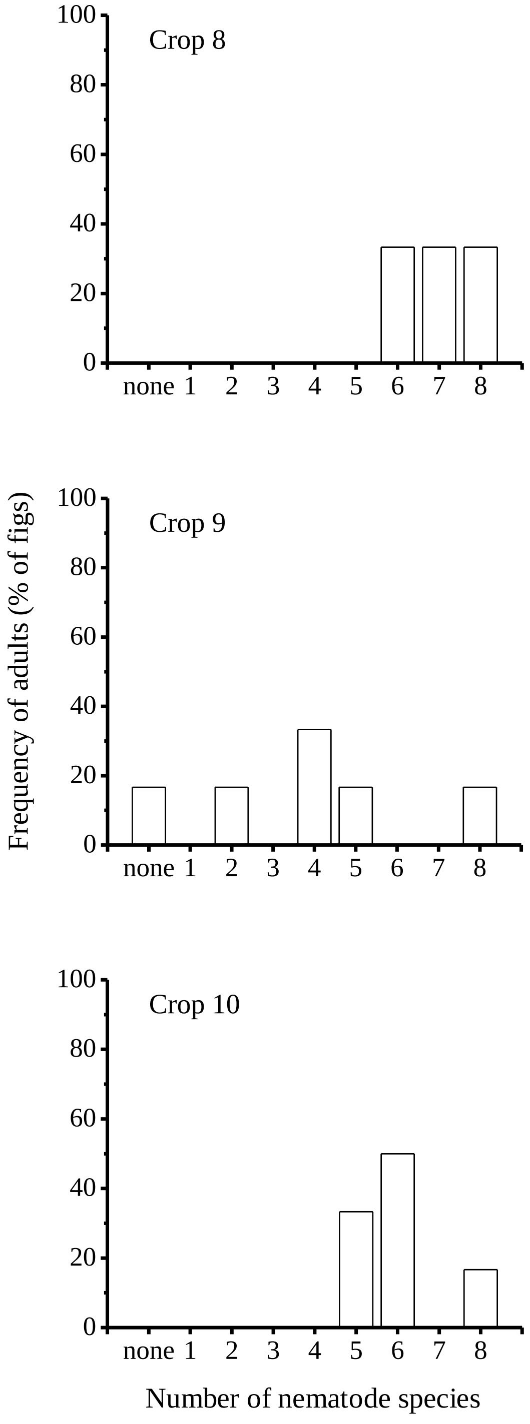


Fig. 5.12. Frequency of adult nematodes based on species found separately or altogether in the same fig in C-phase of *Ficus racemosa* figs from three crops during routine sequential sampling (N = 6 figs/phase/crop, None = no adult of nematodes found in the figs).

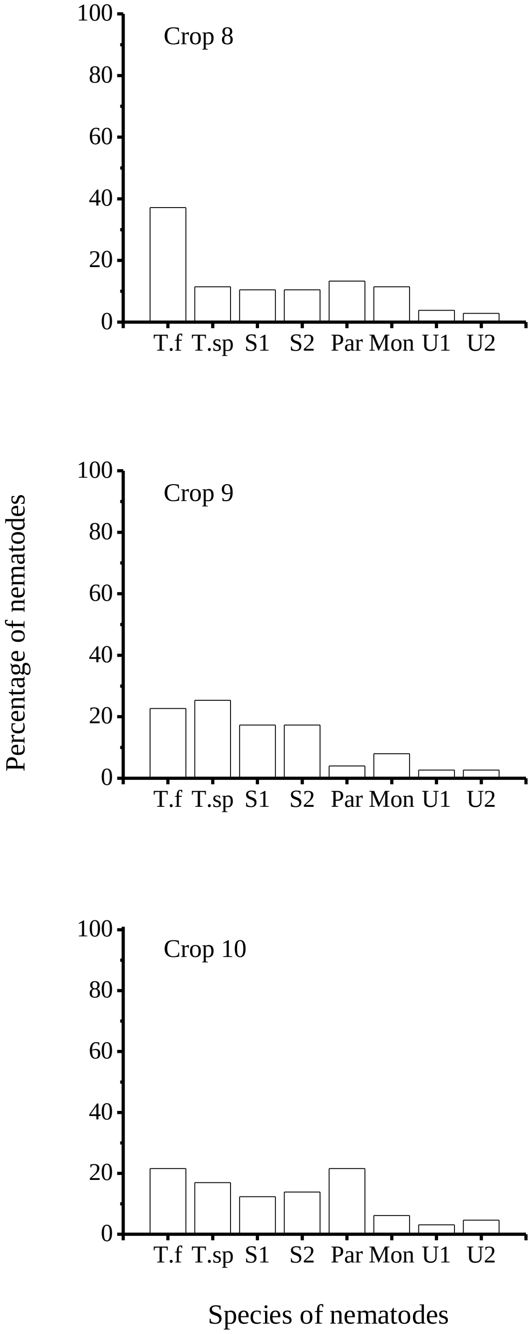


Fig. 5.13. Percentage of adult nematodes per C-phase fig in three crops of *F. racemosa* (N= 6 figs/crop) (Tf = *Teratodiplogaster fignewmani*, T.sp = *Teratodiplogaster* sp., S 1 = *Schistonchus* sp.1, S 2 = *Schistonchus* sp.2, Par = *Parasitodiplogaster*, Mon = *Mononchoides*, U 1= umbrella-like sp.1, U 2 = Umbrella-like sp. 2) (N = 6 C-phase figs/crop).

Table 5.3. Measurements of adult nematodes from late C-phase figs of *F. racemosa* (Mean ± SE). Length: the distance from head to the tail width = maximum width of the body, spicule and vulva: the distance from the head to the genitalia in males and females respectively, as a percentage of total body length).

Species	Sex	N (figs)	Length (µm)	Width (µm)	Spicule (%)	Vulva (%)
Teratodiplogaster fignewmani	male	15	1797.13 ± 38.70	21.32 ± 0.09	95	-
	female	16	1941.64 ± 102.96	22.73 ± 1.00	-	55
Teratodiplogaster sp.	male	13	1755.50 ± 26.39	23.43 ± 0.79	95	-
	female	11	2173.07 ± 135.32	23.29 ± 1.52	-	50
Schistonchus sp.1	male	15	692.36 ± 24.98	23.50 ± 1.93	95	-
	female	8	715.44 ± 17.25	26.03 ± 1.69	-	60.
Schistonchus sp.2	male	14	648.09 ± 24.72	21.43 ± 1.95	95	-
	female	9	634.47 ± 14.23	26.12 ± 1.50	-	60
Parasitodiplogaster sp.	male	9	1707.22 ± 91.83	27.37 ± 1.71	95	-
	female	15	2251.27 ± 106.82	27.53 ± 1.66	-	50
Mononchoides sp.	male	15	1453.55 ± 60.82	25.44 ± 1.11	95	-
	female		1298.73 ± 39.42	30.55 ± 1.21	-	50
Umbrella like sp.1	male	11	1011.58 ± 101.16	24.78 ± 1.01	85	
	female	8	1023.37 ± 41.43	27.37 ± 1.72	-	60
Umbrella like sp.2	male	9	1081.86 ± 34.65	20.30 ± 0.79	90	-
	female	6	961.33 ± 60.45	19.71 ± 1.13	-	55

Table 5.4. Community structure of emerging fig wasps from D phase figs of *F. racemosa*.

			Mean number of emerging wasps (5-7 figs/crop) (Mean ± SE)					
			Male	Female				Male
Crop	N (figs)	Diameter (cm)	Pollinator	Pollinator	*Platyneura fusca*	*Platyneura agraensis*	*Apocrypta* sp.	Non-pollinator
C11	6	2.52 ± 0.04	231.00 ± 29.66	49.83 ± 7.86	62.17 ± 15.07	0.17 ± 0.17	30.67 ±11.90	1.83 ± 0.60
C12	6	2.55 ± 0.02	278.83 ± 42.48	104.17 ± 27.67	45.83 ± 20.77	1.83 ± 1.64	6.83 ± 3.53	3.67 ± 0.61
C13	6	2.55 ± 0.06	282.17 ± 28.54	132.83 ± 18.24	1.33 ± 0.67	0.33 ± 0.33	2.17 ± 0.91	0.83 ± 0.48
C14	6	2.72 ± 0.03	430.00 ± 88.85	124.33 ± 23.91	26.33 ± 9.63	0.00 ± 0.00	17.50 ± 4.17	1.83 ± 0.31
C15	6	2.50 ± 0.07	258.50 ± 35.06	134.67 ± 21.82	0.33 ± 0.33	0.00 ± 0.00	1.67 ± 0.67	0.50 ± 0.22
C16	6	2.68 ± 0.05	416.83 ± 26.67	130.83 ± 3.12	1.17 ± 0.65	6.17 ± 4.28	22.50 ± 6.69	4.83 ± 1.60

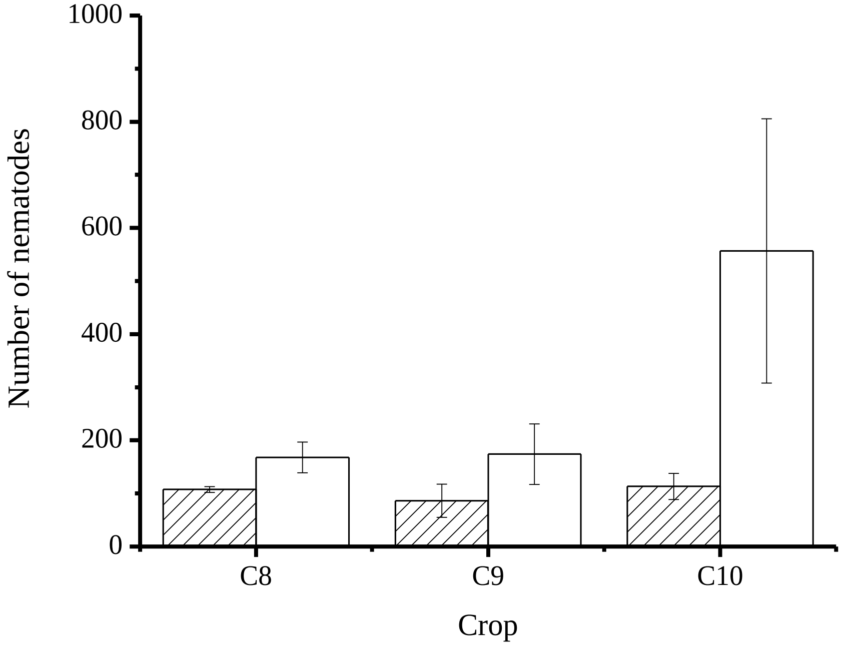


Fig. 5.14. Numbers of juvenile nematodes per D-phase fig in three crops of *F. racemosa* figs from routine sequential sampling (N = 6 figs/crop) (Hatched bars: Aphelechoididae, open bars = Diplogastridae).

5.5.4 What damage do nematodes do to figs and to pollinators?

Regular sampling of the eight crops showed that nematodes were found in each crop during B to E phases. There were no nematodes found in A- phase figs, since they were not yet entered by female pollinating wasps. The occupancy of nematodes in each crop was very high, and ranged from 82.61 to 100 % of the extracted figs (Fig. 5.15.).

In monoecious fig tree species such as *F. racemosa*, each gall inside the fig can contain a developing fig wasp (pollinator or non-pollinator) or seed. In early D-phase figs, the contents of each gall could be observed and recorded. Observations of non-routine samples of early D-phase figs showed that the number of nematodes did not significantly affect the number of galls containing female pollinator offspring (the sex that carries pollen) (lme, df = 25, t-value = -0.670, P = 0.509) or the number of seeds produced in the same figs (lme, df = 25, t-value = -0.736, P = 0.469) (Fig. 5.16.). Furthermore, number of nematodes did not significantly affect the number of galls containing all fig wasps combined (pollinators and non-pollinators) either (lme, t-value = -0.444, df = 25, P = 0.661). These results showed that there was no evidence to believe the presence of nematodes within the figs caused any harm to the female pollinators, the fig wasps or the seeds in the same figs. Each species of nematode found in the figs of *F. racemosa* may have a different ecological role within the figs, but as a group they did not seem to significantly affect the development of the seeds and the fig wasps.

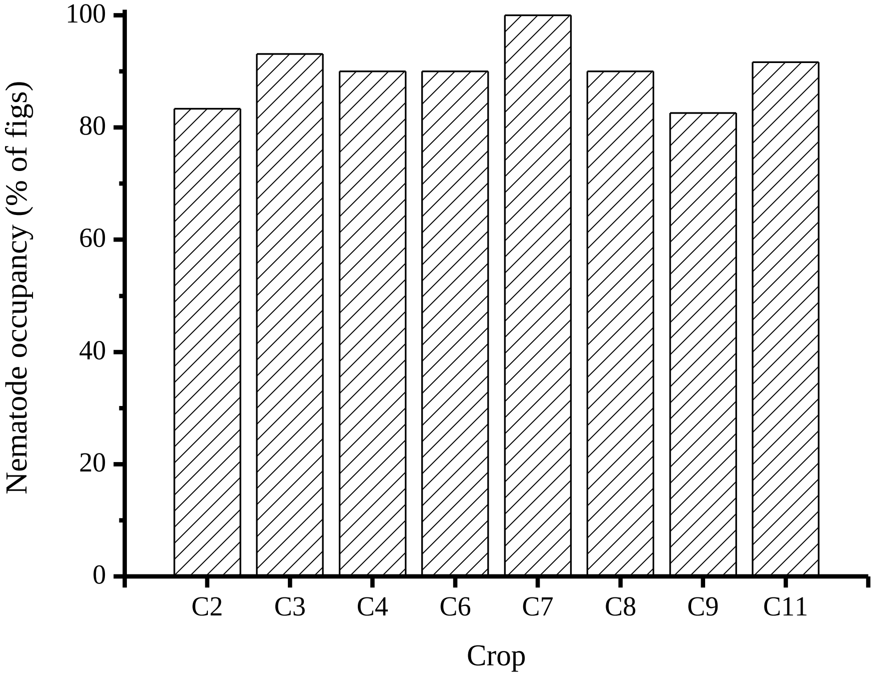


Fig. 5.15. Proportion of figs where nematodes were present (figs phases B-E combined) in eight *F. racemosa* fig crops from routine sequential sampling.

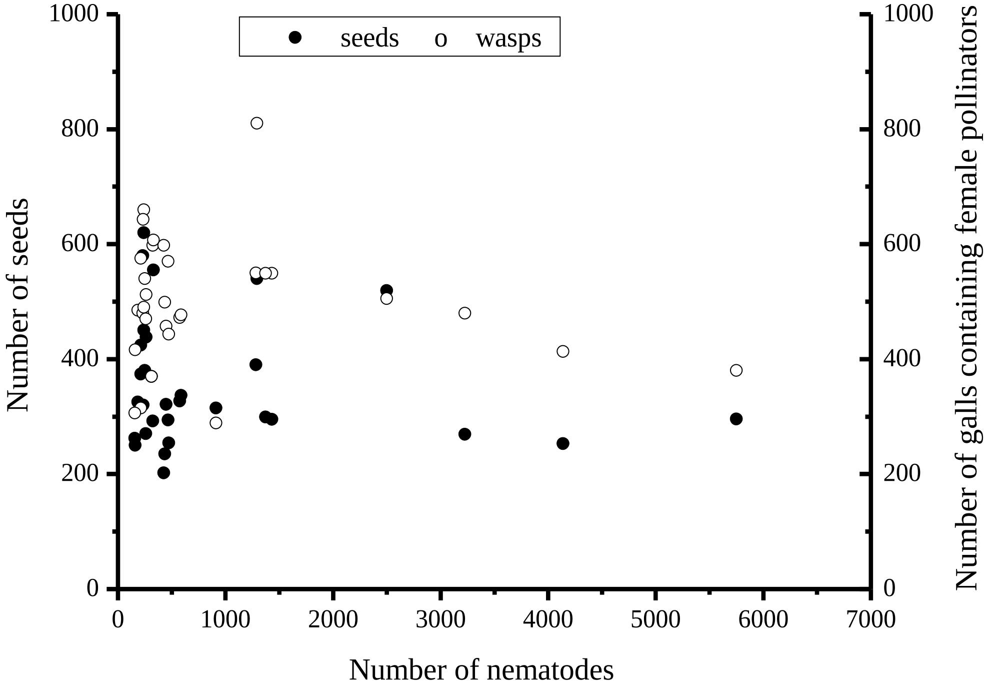


Fig. 5.16. The effect of numbers of nematodes on the number of seeds and the number of galls containing adult female pollinators in early D-phase figs of *F. racemosa* (N = 31 figs, from 5 different crops).

5.5.5 Nematode transportation from fig to fig

Figs of *F. racemosa* host several non-pollinating wasp species which develop at the same time as pollinating wasps within the figs. The fig wasp community comprised one species of pollinating wasp (*Ceratosolen fusciceps*) and three species of non-pollinating wasps namely *Platyneura fusca*, *P. agraensis*, and *Apocrypta* sp. (Table 5.4.). All three species of non-pollinating wasps laid eggs from outside of the figs using their long ovipositors. The eggs are laid at different times but the adults left the figs together.

Observations of fig wasps emerging from D-phase figs showed that nematodes chose the female pollinator to attach themselves to for dispersal to suitable figs to enter and start the life cycle again. The result showed that 319 out of 380 (83.95 %) of newly emerged female pollinators had nematodes with them. Only one from 167 (0.6 %) female *Apocrypta* sp had nematodes attached, and no males of any wasp species had nematodes attached (Table 5.5.).

Observations on early D-phase figs (Table 5.6.) when most fig wasps were still inside their galls (the fig wasps had not yet emerged from the figs) found that no female or male pollinators, female or male non-pollinators, and male flowers had nematodes with them. This result revealed that none of the nematodes present develop inside the galls. Contact between nematodes and the wasps occurred later, when the wasps went out of the galls or the nematodes entered through mating holes in galls in cases where male pollinators have been out and mated with the females inside the galls. Mid C-phase figs were also examined, when the wasps were still at their larval stage. There were no nematodes inside the galls or attached to the larvae inside the galls in these mid C-phase figs. From 300 larvae observed (five crops, six figs/crop, 10 galls/fig), none of them had nematodes attached. These results show that nematodes developed only within the lumen and then waited for the female wasps to emerge from the galls or went inside the galls through mating holes made by male pollinators.

The present of adults in certain crops may be used to predict how many generations of nematodes occurred inside the figs. *Schistonchus* spp. adults seem to occur in each fig phase observed (Figs. 5.17., 5.18., and 5.19.). Occasionally, the adults of this species also occurred in B-phase figs. They might have been the ones being transported by female pollinators, as these nematodes were also transferred as adults. The adults found on C, D, and E-phases figs were the nematodes from the next generation which developed inside the fig. Based on their presence in each fig-phase, it seems that *Schistonchus* spp. may have several generations developing inside the figs. No adults from other species were present in B-phase figs. In B-phase figs, other nematodes (Diplogastridae) were still the juveniles that were transferred into the figs. Adults of diplogastrid nematodes were mostly found in C (Figs. 5. 18. and 5.19.) or late C-phase figs (Fig. 5.17.). Several late-emerging adults were also found in D-phase figs. These diplogastrid nematodes may have only one generation developing inside the figs, which is also the case for the *Parasitodiplogaster* species that feed on agaonid cadavers in the figs of other species. What other Diplogastrid nematodes species feed on was however, undefined.

Table 5.5. Nematodes on newly emerged adult fig wasps from figs of *F. racemosa* (N=38 figs, collected from 6 crops, F = female, M = male).

Fig wasps species	Sex	Number of wasps sampled	Number with nematodes	Percentage with nematodes (%)
Ceratosolen fusciceps	F	380	319	83.95
	M	380	0	0.00
Platyneura fusca	F	186	0	0.00
P. agrae	F	91	0	0.00
Apocrypta sp.	F	167	1	0.60
Non-pollinator species	M	114	0	0.00

Table 5.6. Nematodes from different locations within early D phase figs of *F. racemosa* (N = 30 figs, collected from 6 crops, five figs each crop). Male pollinators make holes in the side of galls of conspecific females, in order to mate with them. Zeros were not included in the calculation of mean numbers of nematodes attached per female pollinator inside the galls with mating holes.

Samples observed	Gall condition	Number observed	With nematodes	Range	Mean ± SE
Female pollinator	No hole	495	0	0	0
Female pollinator	With mating hole	105	92	1-25	3.12 ± 0.24
Emerged male wasp	-	275	0	0	0
Male wasp inside gall	-	25	0	0	0
Female NPFW	No hole	300	0	0	0
Male NPFW	-	300	0	0	0
Male flowers	-	250	0	0	0

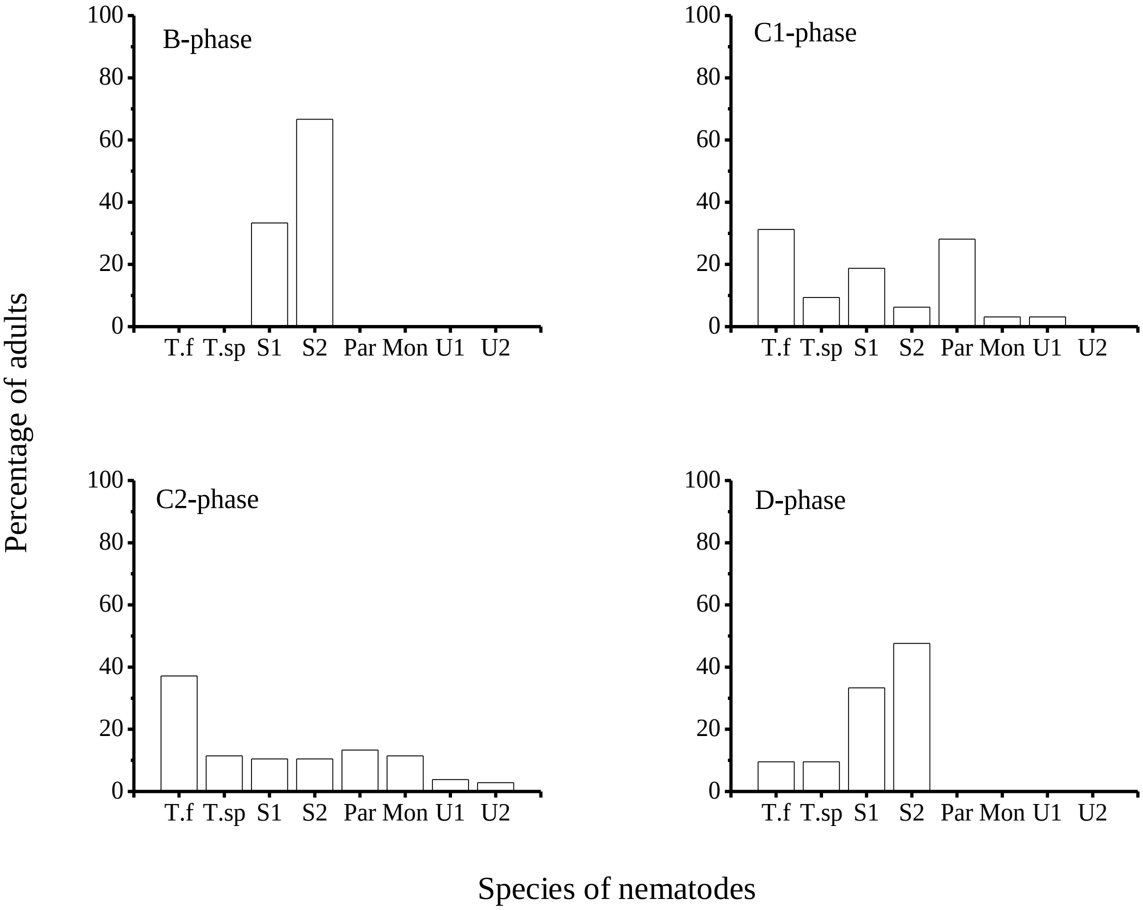


Fig. 5.17. Percentage of adult nematodes of each species in B-D phase from Crop 8 of *Ficus racemosa* figs from routine sequential samplings. Percentage from E-phase was not included since there were no adult nematodes found in E-phase figs on this crop (N= 6 figs for each phase) (Tf = *Teratodiplogaster fignewmani*, T.sp = *Teratodiplogaster* sp., S1 = *Schistonchu*s sp. 1, S2 = *Schistonchus* sp.2, Par = *Parasitodiplogaster* sp., Mon = *Mononchoides* sp., U1 = umbrella like sp. 1, U2 = Umbrella like sp.2).

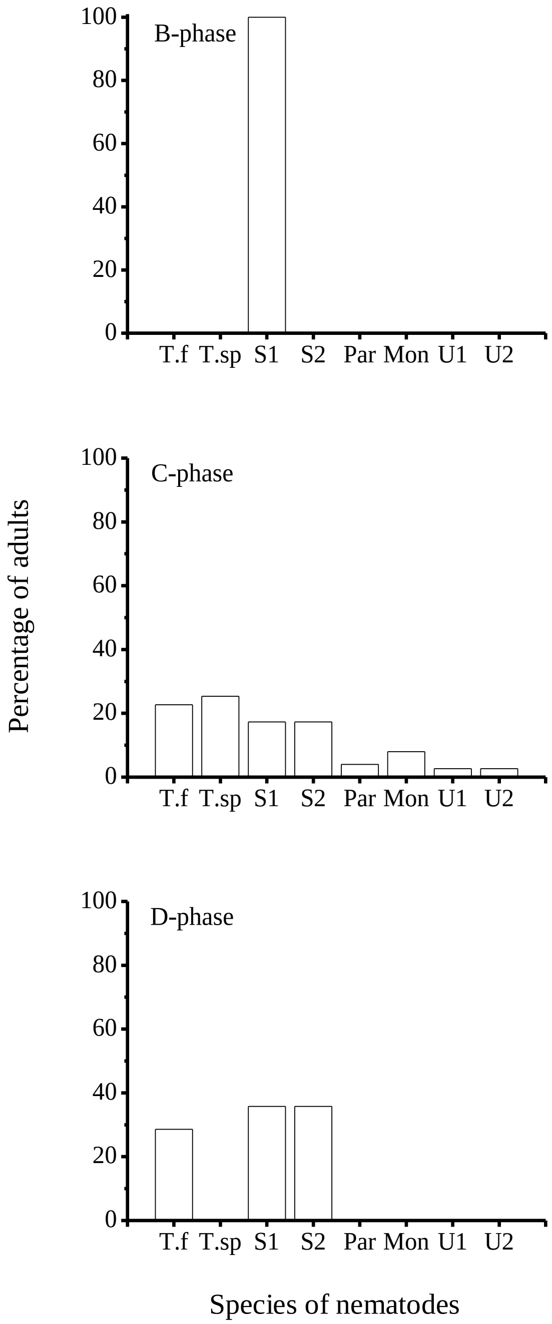


Fig. 5.18. Percentage of adult nematodes of each species in B-D phase from Crop 9 of *Ficus racemosa* figs from routine sequential samplings. Percentage from E-phase was not included since there were no adult nematodes found in E-phase figs on this crop (N= 6 figs for each phase) (Tf = *Teratodiplogaster fignewmani*, T.sp = *Teratodiplogaster* sp., S1 = *Schistonchus* sp. 1, S2 = *Schistonchus* sp.2, Par = *Parasitodiplogaster* sp., Mon = *Mononchoides* sp., U1 = umbrella like sp. 1, U2 = Umbrella like sp.2).

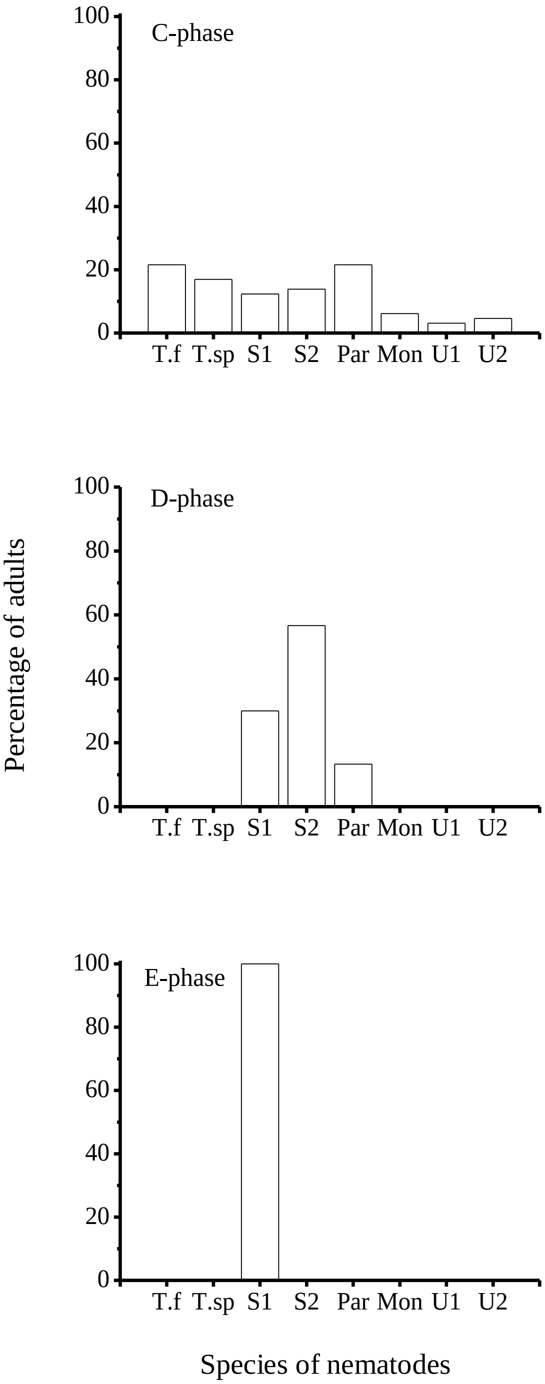


Fig. 5.19. Percentage of adult nematodes of each species in C-E phase from Crop 10 of Ficus racemosa figs from routine sequential samplings. No adult nematodes found in B-phase figs on this crop (N= 6 figs for each phase) (T.f = *Teratodiplogaster fignewmani*, T.sp = *Teratodiplogaster* sp., S1 = *Schistonchus* sp. 1, S2 = *Schistonchus* sp.2, Par = *Parasitodiplogaste*r sp., Mon = *Mononchoides* sp., U1 = umbrella like sp. 1, U2 = Umbrella like sp.2).

5.6 Discussion

5.6.1 Fruiting phenology

The *Ficus racemosa* trees sampled in this study were all large trees bearing a very large number of figs in each crop. Fruiting tended to be synchronous within each crop in one particular tree as seen in many other monoecious fig tree species (Zhang, Song and Yang 2006; Wang and Zheng 2008). At a population level, the fruiting of *F. racemosa* in the study site was asynchronous among different trees. This allows the newly emerged female pollinators to have receptive figs available on other trees.

Development of figs within a crop (crop duration) is usually affected by the season of their development (Zhang, Song and Yang 2006). A study on figs of *F. burtt-davyi* in Grahamstown (South Africa) showed that crop duration was significantly longer during winter months and shorter during summer months (results from previous chapter). In Xishuangbanna, China, the figs of *F. racemosa* only needed around 30 days to complete their development in the rainy season. In the cool-dry season, it took around 60 to 90 days to complete their development (Wang and Sun 2009). However, seasonal changes in the tropics, as shown in this present study, are less evident than those in sub-tropical regions. Thus, it is understandable why the crop durations of *F. racemo*sa in this study were not affected by temperature and rainfall during their development, as the seasonal changes in the study site were less pronounced. All the crops developed in a similar length of time (within one week of 41-42 days) during the course of this study.

5.6.2 Nematodes and their ecology

The nematode community observed in figs of *F. racemosa* in this study comprises more species than those on any other species of fig trees recorded earlier anywhere (Anand 2006; Kanzaki et al. 2009; Anand 2002; Kanzaki et al. 2012a; Zeng et al. 2011b; Davies et al. 2013). *Mononchoides* species have never been described before from *F. racemosa* figs, even though its presence has been noticed elsewhere recently (N. Kanzaki, pers.comm). The other two undescribed species with a very interesting stomatal shape (umbrella-like species) will need further identification related to their taxonomy and biology, but probably represent an undescribed and highly specialised genus. They may feed on bacteria or protistans in the liquid that partly fills the lumen of *F. racemosa* figs.

Nematode populations peaked in D-phase figs when the new generation of fig wasps were ready to emerge from their galls and leave the figs. Nematodes found in D-phase figs were mostly juveniles which were ready to attach to the fig wasps to be transported outside of the figs. At this stage, conditions within the figs are packed. Hundreds of galls containing pollinating and non-pollinating wasps are ready to emerge. Nematodes, therefore, need to quickly choose the right 'vehicle' to transport them (Krishnan et al. 2010). As shown in this present study, nematodes choose female pollinators to carry them outside the figs and transport them to another suitable fig, where they can disembark and the life cycle starts again. Female pollinators are a reliable choice for the nematodes to continue their life cycles. This is due to the fact that males of pollinating and non-pollinating wasps usually are wingless and die within their natal figs and most females of non-pollinating wasps do not enter another fig after they emerged from their natal fig. Most females of non-pollinator species lay eggs from the outer surface by inserting their long ovipositors through the fig wall (Proffit et al. 2007). Nematodes attached to female non-pollinators would face a dead-end, since the wasps would not enter the figs, and nematodes usually do not transfer through the fig wasp's ovipositor.

Nematodes are known to attach inside abdominal folds or enter the haemocoel of fig wasps (Reddy and Rao 1986; Giblin-Davis et al. 1995). A choice assay conducted by Krishnan et al. (2010) has shown that *Schistonchu*s spp. nematodes from *F. racemosa* were able to distinguish between female pollinator and female non-polliantors using cuticular hydrocarbons and volatiles released by the wasps. When whole wasps and volatile cues were offered to the nematodes, they were also able to differentiate between male and female pollinators. These findings supported the previous studies as well as those here, that nematodes purposely choose the female pollinating wasps to disperse them.

Numbers of foundresses (female pollinator that enter a fig) per receptive B-phase fig of *F. racemosa* controlled the number of nematodes inside the figs. The more foundresses that enter a fig, the higher the possibility for nematodes to be transported inside the fig, leading to higher populations of nematodes developing there. Large numbers of foundresses are quite common in *F. racemosa*, and can reach up to 70 foundresses (Wang et al. 2008). The maximum number of foundresses found in this study was not that high (the maximum was 34 foundresses in one C-phase fig). The number of nematodes developing within a fig was however still quite high, reaching up to more than 2000 nematodes in D-phase figs, showing the high capability of nematodes to develop and reproduce within the figs.

As shown in the previous chapter on *F. hispida* figs, the nematodes found in figs of *F. racemosa* did not develop within the developing wasps when they were inside the galls, nor inside male flowers. Observations on cut open D-phase figs showed that nematodes were swimming around in the lumen of the fig waiting to be picked up by the new emerged female pollinators. Some nematodes also performed a unique behaviour by standing up on their ‘tails’ and waving their bodies as previously observed in nematodes from the same or different fig tree species (Krishnan et al. 2010; Jauharlina et al. 2012). This behaviour is performed to increase the chance in contacting mobile insects that can transfer the nematodes (Croll and Mathews 1977).

The high numbers of nematodes inside D-phase figs increased the chances that nematodes would be picked up by each female pollinator, and infection rates were found to be high. One female pollinator was found to be able to carry up to 55 nematodes out of a fig. Given the high population of nematodes inside D-phase figs, the number of nematodes carried by some female pollinators is likely to be higher than this. Any nematodes that fail to attach to female pollinator will stay in the figs and eventually will die.

Both this study of *F. racemosa* figs and the previous chapter with *F. hispida* figs have shown that the development of nematodes within a fig has no clear negative impact on the female pollinating wasps and on the seeds. In the dioecious figs of *F. hispida*, production of wasps and seeds is separated between male and female trees. In monoecious figs like *F. racemosa*, each fig will produce wasps and seeds. Nevertheless, the presence of nematodes within a fig did not reduce the development of the wasps as well as the seeds in the same fig. This is quite interesting, since occupancy of nematodes of *F. racemosa* figs was consistently high. One possibility is that figs entered by more pollinators initially produced more seeds and pollinator offspring, but also introduced more nematodes. The negative impact of nematodes might then be masked, if it occurs at all.

Nematodes are non-mutualist species that interact with both fig tree and pollinating fig wasp mutualists. Bronstein (2001b) used the term ‘exploiters’ for the organisms that exploit the mutualisms between other organisms or species. These exploiters use the mutualist species to obtain goods and services that they cannot produce or acquire by themselves. The exploiters may use mutualists to transport them to the places that they cannot reach, as with all the nematodes presented here. Nematodes in this case are the exploiters of the mutualism between fig tree and its pollinating wasp. They use the pollinating wasps as a ‘vehicle’ to transport them into figs where they develop and reproduce. The nematodes use the resources offered by both mutualist species but provide no benefit in return to the mutualist partners. This condition may cause significant fitness costs on either mutualist species. However, this impact may range from high cost to no cost at all, as seen here, to the mutualist species (Bronstein 2001b).

Parasitodiplogaster sp. nematodes enter females of the pollinating fig wasp *Elisabethiella baijnathi*, and eat their cadavers after they enter into the figs of *F. burtt-davy*i in South Africa (Jauharlina et al. 2012). *Parasitodiplogaster* sp. may delay its development until the female wasp successfully enters a B-phase fig. It is not known whether this nematode feeds before the fig wasp dies or survives for one or two days only on reserves attained during parasitism on the female pollinator (Giblin-Davis et al. 1995). Competition between nematodes in the same fig wasp may drive feeding to start as soon as possible, even though the early death of the fig wasp would be problematic. The same feeding behaviour is assumed for the *Parasitodiplogaster* sp. found in *F. racemosa* figs. When more than one foundress enters a B-phase fig, which commonly occurs in figs of *F. racemosa*, higher number of nematodes are likely to be carried into the figs. This will lead to higher population sizes of nematodes in the next generation which results in a higher possibility for some nematode offspring to be dispersed by female pollinators to another receptive fig. Herre (1993) suggested that when more foundresses enter a fig, parasitism rates by *Parasitodiplogaster* increase. In this case the new generation of nematodes would have more chances to be carried by the new generation of female wasps from other foundresses (horizontal transmission). The damage to the foundresses due to the presence of *Parasitodipogaster* was not assessed in this study, however it was revealed that the presence of nematodes did not significantly affect the development of the seeds and the wasps.

Teratodiplogaster spp. in this study were highly active, especially during the adult stages in the lumens of C-phase figs. At this stage, the fig lumen contained fluid in which the nematodes were swimming around very fast. *Teratodiplogaster fignewmani* is also reported to occur in the figs of *F. racemosa* in Australia (Kanzaki et al. 2009). The stomatal structure (the scoop-like lips) of this nematode is thought to be an adaptation to a free-living aquatic or semi-aquatic phase (Martin, Owen and Way 1973; Kanzaki et al. 2009). *Schistonchus* spp. are known worldwide as plant feeding species, being found in many *Ficus* spp. including *F. racemosa* figs previously (Vovlas, Inserra and Greco 1992; Vovlas and Larizza 1996; Vovlas et al. 1998; Center et al. 1999; DeCrappeo and Giblin-Davis 2001; Davies et al. 2013). *Schisctonchus* spp. found in *F. racemosa* in this study presumably have more than one generation developed within the figs, as shown earlier with another *Schistonchus* sp. transferred by the pollinating wasp *Pegoscapus* sp. into *Ficus laevigata* figs (Giblin-Davis et al. 1995).

The ecological roles of the other nematodes found in this present study are still unclear, but those with modified umbrella-like heads may feed on the bacteria and protists that swim in the liquid inside *F. racemosa* figs, and are presumably also transported by pollinator females. Protists have not been reported from figs before. Together with the yeasts and possibly bacteria that are transported by foundress fig wasps, also with mites in some fig tree species, a diverse community appears to travel with the wasps as they disperse, with unknown consequences for the mutualists.

Chapter 6 Do the first fig wasps to emerge from figs carry more nematodes?

6.1 Abstract

Emergence of female pollinating fig wasps from their natal figs occurs in a sequence because of the small size of the hole(s) cut through the fig wall by their males. Nematodes often develop inside figs, using the female pollinators to transport them between figs. The effect of position in the female emergence sequence on nematode loads was investigated using *Ceratosolen fusciceps* Mayr, a pollinator of *Ficus racemosa* figs. Although there was a variation between crops in nematodes carried, there were usually more nematodes on the first fig wasps to emerge. Entry into figs by early-emerging foundresses also resulted in higher total numbers of the next generations of nematodes within the figs. *Parasitodiplogaster* sp. was recorded more frequently among figs entered by late-emerging foundresses. The nematodes associated with *C. fusciceps* appear to have little impact on pollinator fecundity and their ability to pollinate, but for other fig pollinating wasps that encounter more damaging nematodes there may be significant fitness costs for those fig pollinating wasps that emerge later.

6.2 Introduction

The interaction between fig trees (Moraceae) and their pollinating wasps is a striking example of an obligate mutualism that has been evolving for more than 60 million years (Ronsted et al. 2005; Cook and Segar 2010). Each *Ficus* species is pollinated by a single (or few) species of fig wasp (Hymenoptera: Agaonidae). The agaonid female wasps are the only pollen vectors for the figs, while the wasp offspring feed and develop inside the figs. Fig trees have a unique enclosed inflorescence (Syconium or the fig), an urn-shaped structure which forms a hollow ball and is lined with numerous tiny flowers. The female wasps (foundresses), loaded with pollen, enter the receptive figs through a narrow often bract-lined ostiole. Inside the figs the foundresses either actively or passively pollinate the flowers and lay eggs in some of them, and the foundresses then die (Weiblen 2002; Herre, Jander and Machado 2008). The wasp offspring and the seeds develop over a period of a few weeks. The wingless male wasps emerge first from the galls and search for the galls containing female wasps to mate by inserting their genitalia through the hole that is made by the males. The female fig wasps then emerge into fig cavity, and at the same time the male flowers mature and are releasing pollen. In actively pollinated figs, the female wasps deliberately collect pollen before they come out from the fig through an exit hole (or holes) chewed by the male wasps through the fig wall. Females emerge in response to a fall in carbon dioxide levels within the figs, after the hole cut by the males lets air come inside (Galil, Zeroni and Bogoslav 1973). The male wasps generally die inside the fig or on its outer surface. The winged, mated, pollen-bearing female wasps disperse and search for new receptive figs to enter. The pollen brought by the wasps will be deposited deliberately onto the female flowers when pollination is active (Kjellberg et al. 2001; Jousselin, Rasplus and Kjellberg 2003).

Agaonid pollinating wasps are pro-ovigenic, having females that emerge from their natal figs with all their eggs already mature. This condition allows the female wasps to lay eggs over a short period of time as the females only live a short time (Nefdt and Compton 1996; Ghara and Borges 2010). Female pollinating wasps display morphological adaptations to allow them to enter the figs. They have a flattened head, hooked antennae, mandibular appendages and robust legs to push them through the ostiole (van Noort and Compton 1996) .

The figs of each fig tree species are known to also host a community of non-pollinating fig wasp (NPFW) species. Several NPFW species enter the fig in the same ways as pollinating females and lay eggs from inside the figs. Most of the NPFW species however, lay their eggs from the outer surface of figs by inserting their extra-long ovipositors (Cook and Segar 2010; Chen et al. 2013). In addition to NPFW's, figs are also host to a variety of other species including mites and nematodes (Compton 1993; Walter 2000; Krishnan et al. 2010; Jauharlina et al. 2012). Several species of nematodes have been reported to be transferred into receptive figs by female pollinating wasp when the wasps enter through the ostiole. The nematodes develop and reproduce inside the figs along with the development of seeds and wasp offspring. The new generation of nematodes attach themselves to the female wasp offspring when they are ready to exit from the natal fig and disperse in search for new receptive figs (Vovlas et al. 1998; Giblin-Davis et al. 2003; Kanzaki et al. 2009; Davies et al. 2010; Jauharlina et al. 2012). The figs where the nematodes and wasps develop provide an ideal situation for the development of a 'passenger-vehicle' (nematodes and mite-fig wasps) relationship, since the nematodes need to disperse to enter another suitable fig as well as the fig wasps (Krishnan et al. 2010).

The diversity of biology present in fig nematodes is illustrated by two genera of nematodes, *Parasitodiplogaster* (Diplogasteridae) and *Schistonchus* (Aphelenchoidae), both of which are exclusively associated with figs and their fig wasp pollinators. *Parasitodiplogaster* species are parasitic/predatory on the fig wasps that carry them. The nematodes belonging to this genus develop at the expense of adult female pollinators, eating them either shortly before or after they have died. The nematodes mate and produce infective juveniles inside the figs that infect the next generation of female wasps as they leave to find new figs (Poinar and Herre 1991; Giblin-Davis et al. 2006; Jauharlina et al. 2012). *Schistonchus* species, on the other hand, are plant-parasitic nematodes that feed on the epidermis of flowers inside the figs (Vovlas, Inserra and Greco 1992; Vovlas and Larizza 1996). Other nematodes associated with figs include the diplogastrid *Myctolaimus racemosa* (Anand 2005*)* and *Ceratosolenus hyderabadensis* (Cylindrocorporidae) associated with the pollinating fig wasp *Ceratosolen* sp. and figs of *Ficus racemosa* in India (Anand 2006). Another genus, *Teratodiplogaster* (Diplogastridae) is found in *Ficus racemosa* figs in Australia (Kanzaki et al. 2009). All these nematodes typically attach themselves onto the offspring of pollinator females to transport them from natal figs (Vovlas and Larizza 1996; Giblin-Davis et al. 1995; Gulcu et al. 2008; Kanzaki et al. 2009; Davies et al. 2013). Often their feeding behaviour is poorly known.

About half of the 800 described fig tree species have a monoecious breeding system, in which seeds and wasps develop within the same figs. Each fig then will produce wasp offspring and seeds, as well as pollen. In functionally dioecious fig tree species there is specialization in reproduction, with male trees that only produce fig wasps, and female trees produce only seeds (Wiebes 1979; Herre, Jander and Machado 2008). Any nematodes that are brought into the female figs will then face a dead end, since no fig wasp offspring are produced, and the new generation of nematodes have no means to transfer them outside the natal figs (Chapter 4).

Monoecious figs contain flowers with a wide range of style lengths. Because male eggs are often concentrated at the beginning of oviposition sequences, preferential oviposition into shorter styled flowers with longer pedicels and the laying of mostly male eggs at the start of oviposition bouts leads to male offspring tending to be concentrated in galls located towards the centre of figs, with female offspring distributed in progressively longer styled flowers as foundress number increases ((Compton, Ross and Thornton 1994; Jousselin and Kjellberg 2001). The major natural enemies of pollinator fig wasps are non-pollinating fig wasps (NPFW), which either destroy pollinator larvae or compete with them for oviposition sites. Most NPFW oviposit from the outside of the figs, making more peripheral flowers more vulnerable to attack. This can result in greater pollinator mortalities among more peripheral galls, and changes in their adult offspring sex ratios because a higher proportion of females are killed (Pereira and do Prado 2005). Dioecious figs display a much smaller variance in style and pedicel length and location relative to the fig wall. This condition changes after pollination and in at least one dioecious species, differential growth of the pedicels leads to the galls containing male offspring becoming concentrated towards the centre of figs, where mortalities are reduced (Yu and Compton 2012). This produces the same effect as seen in monoecious species.

Ceratosolen solmsi marchali Mayr is a pollinator of dioecious *Ficus hispida* L. in China. Female pollinators that emerge from galls located towards the centre of figs mate more often, and with more males, and produce more adult offspring when they are subsequently introduced into receptive figs (Yan-Qiong Peng, personal communication). They also tend to be mated first, and are more likely to emerge successfully from their galls. However, it is not known whether they are also the first females to emerge from their natal figs through the exit holes cut through the fig wall by male pollinators.

The timing of fig wasps emergence varies between species, particularly between species of fig wasps that disperse during the day or at night (Zachariades, Schatz and Compton 2010). Ants (Hymenoptera : Formicidae) are regularly found to prey on the pollinating wasp *Ceratosolen capensis* Grandi, and other fig wasps as they are exiting from the figs of the monoecious fig tree *F. sur* in Southern Africa (Cushman et al. 1998). The male pollinators that emerge first from the fig are ten times more likely to be captured and killed by the ants that are waiting on the fig surface. Predation on male pollinators helps decrease predation on the female pollinators that emerge later (Zachariades, Schatz and Compton 2010).

The above study found that earlier female wasps that emerged together rapidly as a group were less likely to be taken by ants than later-emerging stragglers. The earliest female pollinators to emerge from the figs may however also have a higher risk of being captured by ants because not all the waiting ants will be occupied by other fig wasps. The emergence sequence of female pollinators may also influence the number of nematodes carried by them, as nematodes need to be transported outside the figs by the females to search for other suitable figs. In this chapter, I investigated whether the emergence sequence of female pollinating wasps is correlated with the number of nematodes carried. I also examined whether the introduction into figs of foundresses from different positions in the emergence sequence would affect the population size of the new generation of nematodes within the figs. The questions addressed are as follows:

1. Do the first fig wasps to emerge carry the same number of nematodes as those that emerge later?
2. Does their position in the emergence sequence influence nematode population sizes when the fig wasps are introduced into receptive figs?
3. Do figs entered by foundresses that are early in the emergence sequence contain different nematode species than figs entered by late-emerging foundresses?

6.3 Study species

In this chapter, the study was focused on *Ficus racemosa* L., a monoecious fig tree belong to the subgenus Sycomorus. *Ficus racemosa* is the only monoecious Sycomorus species occurs outside Africa, and grows commonly along rivers in Southeast Asia (Corner 1965). The figs are produced in large clusters on short and leafless branches emerging from the trunk and the main branches. During development, they change from small, thin-walled figs in A-phase to large, thick walled figs in later phases. The figs grow from 1 cm in diameter during A-phase up to more than 3 cm at maturity in E-phase (Pers. Obs). The colour changes from green in younger figs becoming bright red in colour when they are ripe (Paarakh 2009). The figs of *F. racemosa* are pollinated by the fig wasp *Ceratosolen fusciceps* Mayr (Zhang, Song and Yang 2006; Wang et al. 2008; Wang and Sun 2009).

6.4 Methods

6.4.1 Study site

The site for the studies in this chapter was the same as location as with the last two previous chapters (Chapter 4 and Chapter 5). The studies were done at the end of 2012, when each species of nematode found in the figs of *F. racemosa* was already recognizable as a result of the sequential routine sampling (Chapter 5).

6.4.2 Nematodes on female pollinators from different times in their emergence sequence

This study was done to investigate whether the position in the emergence sequence of female pollinating fig wasps had an effect on the number of nematodes they carried. Six mature D-phase figs from a crop were removed and placed separately inside net bags of 12 cm x 9 cm in size and tightened with a string to prevent the emerging wasps flying out. All the figs were brought to the laboratory for further observation. Any fig that released the wasps before arriving in the laboratory was excluded from further observation. Each bag containing a fig was labeled and placed onto the Laboratory bench, so that it could easily be recognized when the first wasp emerged. The foundresses' emergence sequence was divided into three categories: (1) the females that emerged during the first five minutes after the emergence of the first male, (2) the females that emerged from 5 to10 minutes after the first emergence, and (3) the females that emerged more than 10 minute after the first emergence. The three categories of emergence were chosen based on preliminary observations of the emerging process of the fig wasps from their natal figs. The female fig wasps emerged as soon as the male pollinators exited the figs from the exit hole made by the males. During the first five minutes, the female fig wasps emerge at a high rate leaving almost no time lag between emergence of each female. In the next five minutes, the emergence is a bit slower with lags between two consecutive emerging fig wasps. After 10 minutes the emergence of female wasps is much slower than those in the first emergence. All fig wasps that will emerge have usually left the figs one hour after the first emergence of male pollinators.

Using a stop watch, I monitored the female pollinator fig wasps that emerged from the figs during the first five minutes. Then the fig was quickly removed from the initial bag and placed in the second bag. Again, using a stopwatch the fig was left in the second bag for another five minutes. Each fig was then removed from the second net bag and placed inside the third bag where the fig was left for one hour to let all the wasps emerge. Ten female pollinators were sub-sampled from each bag. Each wasp was placed individually onto glass slide with a drop of sugar water (40%). Emerging nematodes from the wasps were counted within one hour. Figs from five crops which came from five different trees were monitored (Table 6.1).

Table 6.1. *Ficus racemosa* trees used to observe numbers of nematodes in fig wasps at different positions in their emergence sequence (the tree identification codes are the same as the ones used in previous chapters, however the crops used for the present study were different). Trunks were measured at one metre above the ground.

Tree	Crop	Circumference (cm)	Diameter (cm)	Height (m)	Location (from GPS)	
					East	North
R3	C1	118	37.6	17	95° 15’ 30.48”	05° 22’ 43.98”
R2	C2	136	43.3	17	95° 15’ 30.54”	05° 22’ 43.98”
R10	C3	60	19.1	15	95° 14’ 57.36”	05° 19’ 29.64”
R1	C4	153	48.7	15	95° 15’ 48.60”	05° 22’ 56.28”
R4	C5	135	43.0	25	95° 14’ 31.20”	05° 20’ 40.02”

6.4.3 Experimental work

In this study, I had to find trees that just produced new A-phase figs. Individual figs were bagged with a net bag of 12 cm x 9 cm in size and tightened with a string to prevent any fig wasps from being able to enter. Some of the trees used in this study were tall and I had to use a ladder to climb the tree and work with the figs. Each bagged fig was left to develop until B-phase. While waiting for the fig to develop to B-phase I searched for potential foundresses from other trees that had figs in C-phase that could be expected to develop into D-phase (with fig wasps ready to emerge) at about the same time as the bagged figs reached B-phase on the experimental trees.

When the trees started to have D-phase figs, I collected several figs and placed them individually in net bags with the same size as before. The D-phase figs then were brought to the site of the experimental trees with bagged B-phase figs. When pollinator females started to emerge, I separated the female pollinators into three categories of emergence, as before: (1) within the first 5 minutes, (2) from 5 to10 minutes after the first emergence, and (3) more than 10 minutes after the first emergence. Five female pollinators from each category were introduced into each B-phase fig by placing the females near the ostiole. The female would enter the fig through the ostiole soon after being placed on it. The figs then were re-bagged to prevent any other fig wasps from entering. There were ten B-phase figs introduced with five foundresses from any particular category of foundresses emergence. So, in total there were 30 B-phase figs in one tree for this purpose. Five foundresses were introduced per fig after several earlier attempts using one and three foundresses. Most of the figs with one and three foundresses aborted from the trees. All foundresses used for introduction into figs from each experimental tree came from one D-phase fig obtained from another tree.

The bagged figs were left to develop into late C-phase, when they were collected and brought to the laboratory for nematode extraction. The same extraction process as described in the previous chapters (Chapter 4 and 5) was applied to count total nematodes, juveniles, adults, and also to identify adults of each species of nematode found in figs. The data were recorded from 5-6 figs for each foundress category per crop, and five crops from five different trees (Table 6.2). The female fig wasps that were introduced into B-phase figs in each experimental tree came from five different crops on five different trees.

6.4.4 Data analysis

Data were analysed using the *lme4* package since the response variables were count data. Poisson distribution was used as error in the models. Over dispersion in each model was corrected by including fig identity as individual level random effect. The response variables were: (1) numbers of nematodes carried per female pollinator in each category of emergence, (2) numbers of total nematodes, juveniles, and adults in C-phase figs, and (3) numbers of adult nematodes of each species found in C-phase figs. Linear mixed effect models were applied, because the crops were nested within the trees, generating both fixed and random factors. Emergence sequence was treated as a continuous fixed effect in each analysis. Crops were included in the models as random effects. The effects were considered to be significant at P-values <0.05 and are reported together with z-values.

Table 6.2. The *Ficus racemosa* trees where figs had fig wasps introduced experimentally (the tree identification codes are the same as the ones used in previous chapters, however the crops used for the present study were different).

Tree	Crop	Circumference (cm)	Diameter (cm)	Height (m)	Location (from GPS)	
					East	North
R2	C6	136	43.3	17	95° 15’ 30.54”	05° 22’ 43.98”
R3	C7	118	37.6	17	95° 15’ 30.48”	05° 22’ 43.98”
R4	C8	135	43.0	25	95° 14’ 31.20”	05° 20’ 40.02”
R13	C8	150	27.7	30	95° 15’ 0.78”	05° 19’ 6.84”
R11	C10	82	26.1	25	95° 15’ 05.34”	05° 19’ 22.92”

6.5 Results

6.5.1 Numbers of nematodes carried by emerging female pollinators

All adult fig wasps (pollinators and non-pollinators) that were going to emerge (some fail to leave their galls) had emerged from the figs within one hour after the first wasp emerged. The wasps mostly emerged in the first ten minutes, the remaining wasps left the figs slowly. Out of the five crops, one (Crop 2) showed relatively lower number of nematodes attached per female pollinator at emergence compared to those of the other four crops (Fig. 6.1). Not all of the sampled female fig wasps had nematodes with them, but there were four figs that had nematodes in all the sampled individuals. The highest proportion of female fig wasps with nematodes was found on the first females to emerge (emergence within the first five minutes), ranging between 50 and 100 % in each fig. The second emergence period had nematodes in between 30 and 80 % of the females and the last emergence group had nematodes in between 20 and 60 % in different figs.

The mean number of nematodes carried per female ranged between 0.60-6.40 for the first five minutes of female emergence, between 0.53-4.42 for the second period, and 0.57-4.53 for the last period, across five crops (Table 6.3). The first five minutes of emergence had nematode numbers that ranged from 0-51 per female, the second period (between 5 and 10 minutes) had 0-55 nematodes per female, and last females to emerge (after 10 minutes) had 0-33 nematodes per female. Position in the emergence sequence showed a significant effect on the number of nematodes carried by female pollinators (lmer, z = -3.441, P = 0.0006). The first females to emerge carried significantly more nematodes than those that emerged later, while the second and last groups of females to emerge carried nematodes in similar numbers (Fig. 6.1).

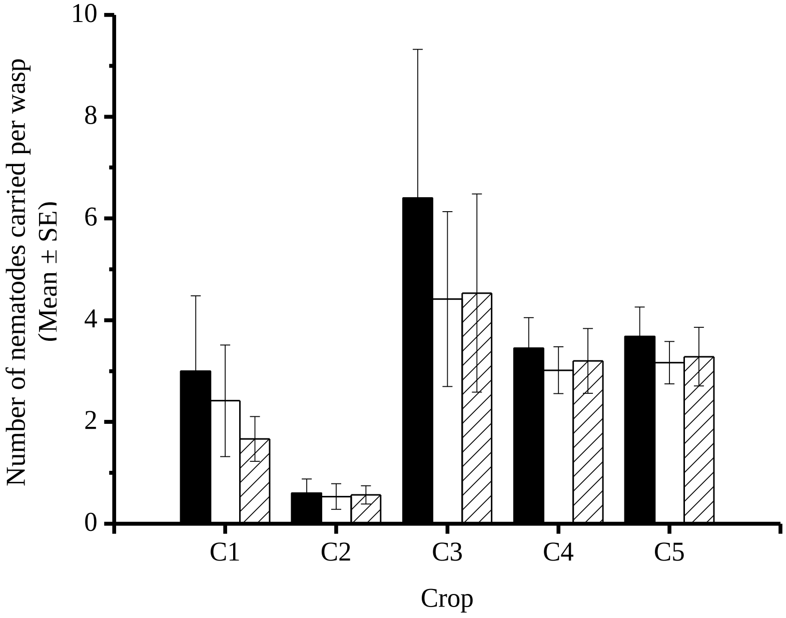


Fig. 6.1. The numbers of nematodes carried by each female pollinator *Ceratosolen fusciceps* in relation to emergence times (female pollinators that carried no nematodes were included in calculations). The emergence sequence of female pollinating wasps was divided into three phases: (1) the wasps that emerged during the first five minutes (solid bars), (2) the wasps that emerged after 5 to10 minutes from the first emergence (open bars), and (3) the wasps that emerged more than 10 minute after the first emergence (hatched bars). (N = 30 wasps per fig, 6 figs per crop).

Table 6.3. The numbers of nematodes carried by female pollinators as they emerged from their natal figs in relation to their position in the emergence sequence. N = numbers of figs sampled. N2 = number of wasps sampled in each fig. Number of wasps sampled in each crop was 180, and in total there were 900 wasps sampled across five crops observed.

Crop	N1	N2	Number of nematodes carried per emerging female pollinator in relation to their emergence times (Mean ± SE)		
			< 5 minutes	5-10 minutes	> 10 minutes
C1	6	30	3.00 ± 1.48	2.42 ± 1.10	1.67 ± 0.44
C2	6	30	0.60 ± 0.28	0.53 ± 0.25	0.57 ± 0.18
C3	6	30	6.40 ± 2.92	4.42 ± 1.72	4.53 ± 1.95
C4	6	30	3.45 ± 0.60	3.02 ± 0.46	3.20 ± 0.63
C5	6	30	3.68 ± 0.58	3.17 ± 0.42	3.28 ± 0.58

6.5.2 Numbers of nematodes in experimental figs

All the bagged B-phase figs were collected 15-17 days after introduction of the foundresses. The figs were hand-picked when they were in late C-phase, the stage with the highest number of adult and larval nematodes. Emergence sequence significantly affected the total number (adults and juveniles combined) of nematodes present in the figs that they entered (lmer, z = -2.197, P = 0.028) and the number of juveniles present (lmer, z = -2.53, P = 0.011). However, the number of adult nematodes present within the figs was statistically similar across emergence categories (lmer, z = 1.591, P = 0.112). Numbers of adult nematodes were much lower than the juvenile stages in each crop (Fig. 6.2). The total and juvenile nematodes in figs entered by foundresses that emerged in the first five minutes were higher than those of figs with foundresses that emerged later, except for Crop 8 (Fig. 6.2).

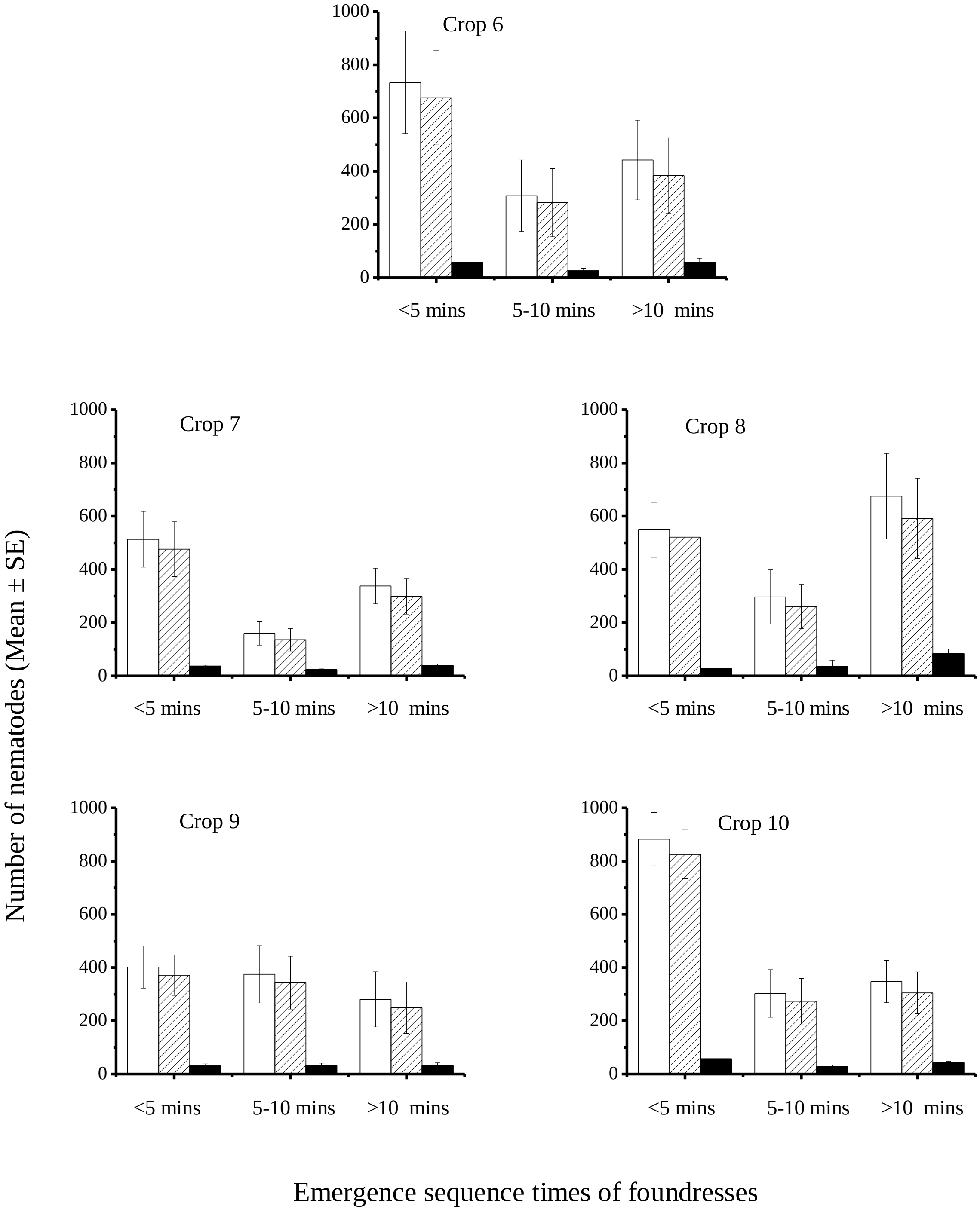


Fig 6.2. Numbers of nematodes (adult and juveniles combined) in C-phase figs where five foundresses (*Ceratosolen fusciceps*) had been introduced. The foundresses had emerged from their natal figs at different times. Open bars = total nematodes, hatched bars = juveniles, solid bars = adults. All foundresses used for each crop were obtained from the same D-phase fig from another tree. (N = 5-6 figs/each emergence category/crop).

6.5.3 Nematode species in experimental figs

Nematode species within the figs of *F. racemosa* could only be recognized when they were in their adult stage. There were eight species of nematodes found in the C-phase figs, the same as described in the previous chapter (Chapter 5) (Table 6.4). Not all species were usually found in any one fig, but only one crop (Crop 8) had figs with only one species present. Six species of nematodes was the most common occurrence in any one fig, and all eight species of nematodes were found together in one fig from Crop 8 and two figs from Crop 10. There was one fig in Crop 6 (5-10 minutes emergence group), two figs in Crop 8 (both 0-5 minutes emergence), and one fig in Crop 9 (0-5 minutes emergence) that had no adult nematodes inside. Only juvenile nematodes were found in these figs. The combinations of nematode species varied among the different emergence groups (Fig. 6.3). *Teratodiplogaster fignewmani* and *Parasitodiplogaster* sp. were more common than other species in each category of emergence and in each crop. The percentage of *Mononchoides* was also pretty high, except in Crops 8 and 9 (Fig. 6.4).

The number of each nematode species present in each fig was highly variable in each emergence category in each crop. The mean numbers of each adult nematode species are presented in Table 6.5. Position in the emergence sequence of foundresses that was introduced into B-phase figs did not affect the number each species of adult nematodes found in the figs they entered, except for *Parasitodiplogaster* sp. (Table 6.6). Interestingly, the number of *Parasitodiplogaster* sp. present in C-phase figs entered by late-emerging foundresses was higher than in figs entered by earlier-emerging foundresses (Table 6.5).

Table 6.4. Nematode species transported by pollinating fig wasps into figs of *F. racemosa*.

No	Nematodes species	Family	Biology
1.	*Teratodiplogaster fignewmani*	Diplogastridae	Bacterial-feeding (?)
2.	*Teratodiplogaster* sp.	Diplogastridae	Bacterial-feeding (?)
3.	*Parasitodiplogaster* sp.	Diplogastridae	Parasite of pollinating wasp
4.	*Mononchoides* sp.	Diplogastridae	Unknown
5.	*Schistonchus* sp 1	Aphelechoididae	Plant parasite
6.	*Schistonchus* sp. 2	Apelenchoididae	Plant parasite
7.	Umbrella-like sp. 1	Diplogastridae	Unknown
8.	Umbrella-like sp. 2	Diplogastridae	Unknown

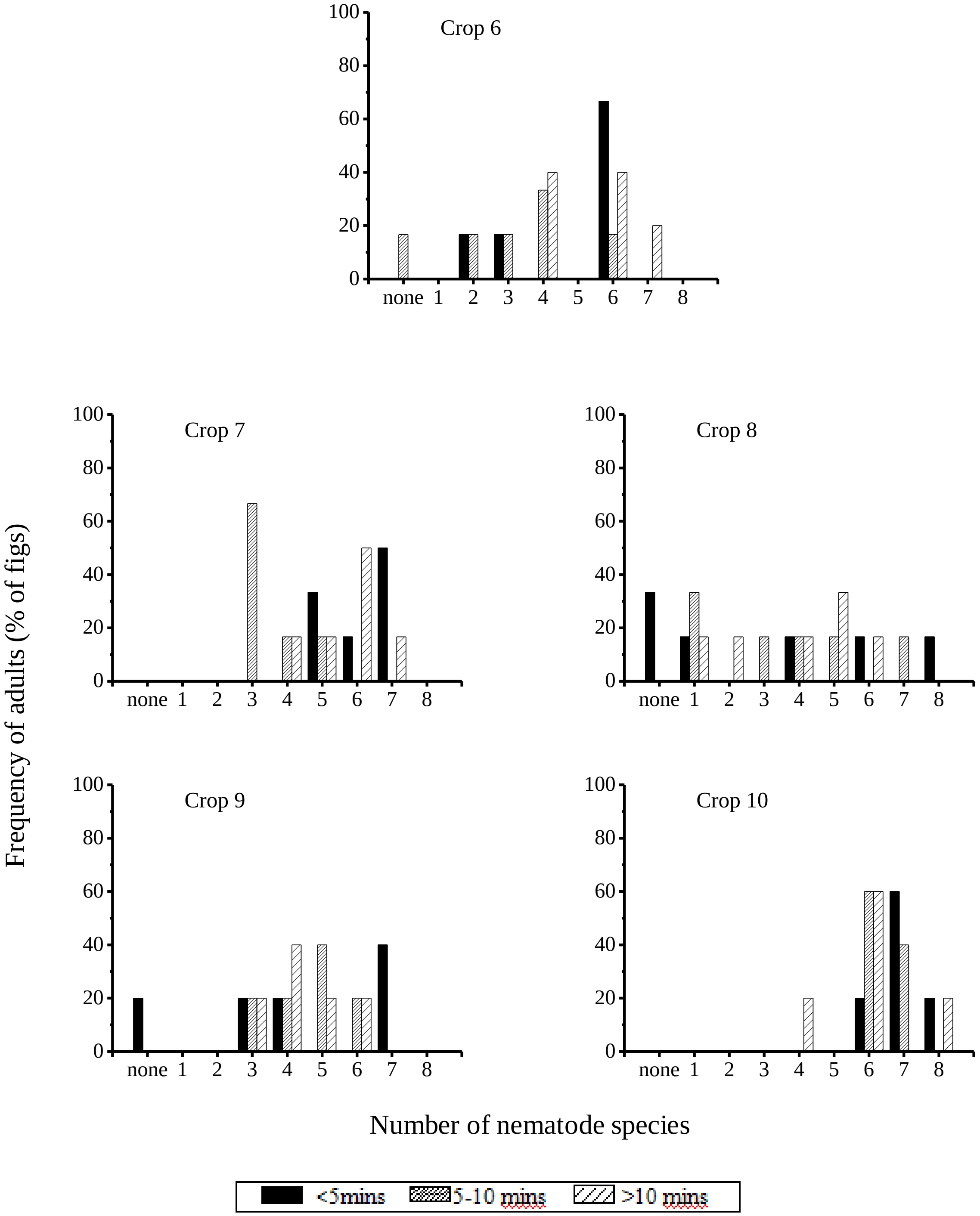


Fig. 6.3. The numbers of species of nematodes sharing C-phase figs of *Ficus racemosa* figs where five foundresses had been introduced from different groups in emergence sequences. Based on adult nematodes only. None = no adult nematodes, N = 5-6 figs/each emergence category/crop.

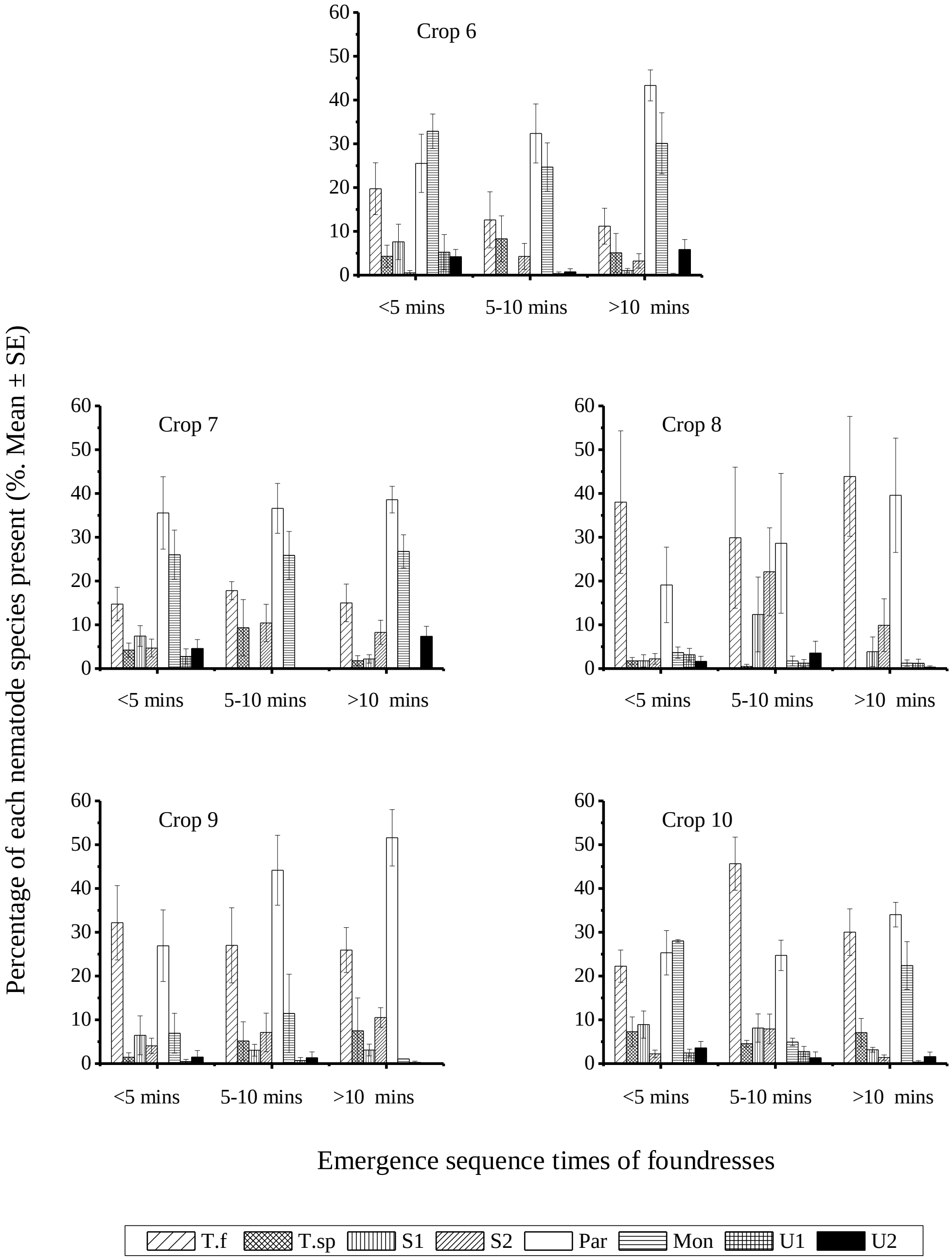


Fig. 6.4. Percentage of adult nematodes of each species in C-phase figs of *Ficus racemosa* figs where five foundresses that had emerged at different sequence times had been introduced. (T.f = *Teratodiplogaster fignewmani*, T.sp = *Teratodiplogaster* sp., S1 = *Schistonchus* sp.1, S2 = *Schistonchus* sp.2, Par = *Parasitodiplogaster*, Mon = *Mononchoides*, U1= umbrella-like sp.1, U2 = Umbrella-like sp.2). (N = 5-6 figs/each emergence category/crop).

Table 6.5. Numbers of adults of each nematode species (mean ± SE) in C-phase figs entered by females that had emerged at different times. Numbers of nematodes were counted through extraction. (T.f = *Teratodupligaster fignewmani*, T.sp = *Teratodiplogaster* sp., S1 = *Schistonchus* sp. 1, S2 = *Schistonchus* sp. 2, Par = *Parasitodiplogaster* sp., Mon = *Mononchoides* sp., U1= Umbrella like sp. 1, and U2 = Umbrella like sp.2). (Means ± SE).

Crop	Emergence (minutes)	N	T.f ± SE	T.sp ± SE	S1 ± SE	S2 ± SE	Par ± SE	Mon ± SE	U1 ± SE	U2 ± SE
C6	< 5	6	9.83 ± 4.96	5.80 ± 4.35	5.00 ± 2.73	0.33 ± 0.33	15.17 ± 4.59	18.00 ± 6.34	1.50 ± 1.12	3.67 ± 1.84
	5-10	6	3.50 ± 1.78	3.17 ± 2.61	0.00 ± 0.00	1.50 ± 1.31	9.50 ± 3.11	7.83 ± 3.31	0.17 ± 0.17	0.33 ± 0.33
	> 10	5	6.20 ± 3.22	4.80 ± 4.32	0.80 ± 0.37	2.00 ± 1.10	22.17 ± 7.44	14.80 ± 4.59	0.20 ± 0.20	2.80 ± 0.97
C7	< 5	6	5.83 ± 1.74	1.33 ± 0.49	2.83 ± 0.83	1.83 ± 0.75	11.67 ± 1.45	10.50 ± 2.68	1.17 ± 0.75	1.67 ± 0.80
	5-10	6	4.17 ± 0.60	2.17 ± 1.42	0.00 ± 0.00	2.00 ± 0.77	9.17 ± 2.12	6.33 ± 1.67	0.00 ± 0.00	0.00 ± 0.00
	> 10	6	7.00 ± 3.13	0.67 ± 0.42	0.83 ± 0.31	2.83 ± 0.83	14.50 ± 0.76	11.00 ± 2.58	0.00 ± 0.00	2.67 ± 0.71
C8	< 5	6	8.33 ± 3.51	0.67 ± 0.33	1.50 ± 1.50	1.50 ± 1.31	14.20 ± 9.99	1.33 ± 0.71	1.67 ± 1.31	0.50 ± 0.50
	5-10	6	3.83 ± 2.85	0.17 ± 0.17	13.50 ± 3.10	13.33 ± 1.21	4.00 ± 2.07	0.50 ± 0.34	0.33 ± 0.21	0.33 ± 0.21
	> 10	6	31.17 ± 3.42	0.00 ± 0.00	4.83 ± 4.44	5.67 ± 5.07	39.00 ±12.87	1.50 ± 0.96	1.33 ± 0.99	0.33 ± 0.33
C9	< 5	5	12.20 ± 3.26	0.60 ± 0.40	2.40 ± 1.69	1.60 ± 0.68	10.00 ± 2.77	2.80 ± 1.83	0.20 ± 0.20	0.60 ± 0.60
	5-10	5	7.80 ± 3.57	2.40 ± 2.16	1.00 ± 0.55	2.40 ± 1.75	12.20 ± 3.99	5.40 ± 4.42	0.20 ± 0.20	0.20 ± 0.20
	> 10	5	7.80 ± 2.27	1.20 ± 1.20	1.40 ± 0.93	4.00 ± 2.26	16.60 ± 5.84	0.40 ± 0.24	0.20 ± 0.20	0.00 ± 0.00
C10	< 5	5	13.00 ± 3.56	5.40 ± 3.46	4.80 ± 1.88	1.20 ± 0.37	13.00 ± 2.07	16.00 ± 2.88	1.60 ± 0.87	2.20 ± 0.97
	5-10	5	13.60 ± 3.09	1.20 ± 0.20	1.80 ± 0.37	2.20 ± 1.28	7.80 ± 2.22	1.40 ± 0.40	0.80 ± 0.37	0.20 ± 0.20
	>10	5	12.00 ± 1.70	3.60 ± 1.96	1.40 ± 0.40	0.60 ± 0.24	14.20 ± 1.36	10.00 ± 2.65	0.20 ± 0.20	0.80 ± 0.58

Table 6.6. The effects of foundress emergence times on the numbers of each species of nematode present in C-phase figs of *Ficus racemosa* where five foundresses were introduced. All five crops are combined. All foundresses used for each crop on each experimental tree had come from the same D-phase fig from another tree. N = 5-6 figs per category for each crop. Analysis was in R package of lme4, command lmer.

Number	Nematode species	z- values	P-values
1.	*Teratodiplogaster fignewmani*	0.88	0.38
2.	*Teratodiplogaster* sp.	-1.27	0.20
3.	*Schistonchus* sp. 1	-1.59	0.11
4.	*Schistonchus* sp. 2	1.40	0.16
5.	*Parasitodiplogaster* sp.	1.97	0.05*
6.	*Mononchoides* sp.	-1.05	0.29
7.	Umbrella-like sp. 1	-1.87	0.06
8.	Umbrella-like sp.2	-0.51	0.61

6.6 Discussion

The emergence sequence of female pollinators in this study was similar to that described by Zachariades, Schatz and Compton (2010) for an African species, with a rapid exit of pollinator females immediately after the exit hole is created by the males. Whether the first females to emergence from the figs come from the galls located toward the centre of figs or from peripheral galls toward the fig wall is still unknown. This study found that although there is a great variation between crops in nematode loads on each female pollinator, there are generally more nematodes carried on the first females to emerge. Reflecting this, the maximum mean numbers of nematodes carried per female was 6.40 for the early female emergence period, 4.42 for the next five minutes of emergence, and 4.53 for the last emergence period. When female pollinators from different times in the emergence sequence were introduced into receptive figs, populations of the new nematode generations were also higher in the figs where early-emerging females were introduced.

Ants can be a danger for pollinators because they are frequently found to capture pollinating wasps as they enter or emerge from figs (Compton and Robertson 1988; Schatz and Hossaert-McKey 2003; Schatz et al. 2008; Ranganathan, Ghara and Borges 2010). Earlier in their development, the presence of ants can be beneficial for the pollinating fig wasps because they reduce attacks by NPFW that lay eggs in younger figs (Cushman et al. 1998). The first female wasps to emerge from figs may suffer from increased predation by ants that are waiting on the fig surface during the emergence period (if the ants are not satiated by earlier-emerging fig wasps) and the rapid emergence of most females may be an adaptation to reduce ant predation (Zachariades, Schatz and Compton 2010).

The presence of predatory ants during the emergence of fig wasps from D-phase figs of *F. racemosa* is stimulated by the volatiles released by D-phase figs or by the scent of the emerging fig wasps themselves (Ranganathan and Borges 2009). The predatory ants mostly found on *F. racemosa* figs in India are *Oecophylla smaragdina* (Fabricius) and *Technomyrmex albipes* (Smith) (Ranganathan, Ghara and Borges 2010). Several other predators also attack pollinators at these times, such as dragon flies, staphylinid beetles, phorid flies, and birds (Bronstein 1988; Compton and Disney 1991)

In *Ficus sur* figs, predation on female pollinators is reduced because the ants first prey on the male pollinators that exit earlier than the females. However, when numbers of ants waiting on the fig surface were more than 15, the risk of females to be captured by ants increases (Zachariades, Schatz and Compton 2010). If the female pollinators are killed by ants, then nematodes will also die together with the females that carried them.

Female pollinating wasps are small in size and they do not feed. They also have a short life span of less than 48 hours (Ware and Compton 1994a; Dunn et al. 2008b). The success of their exit from their natal figs is crucial to the survival of both fig trees and pollinating wasps (Ware and Compton 1994a; Ware and Compton 1994b), and also for the nematodes that are carried by the females. To ensure their task is successful in finding new receptive figs, the females should leave their natal figs as quick as possible, considering their short life span. On the other hand, early emergence by females may result in a higher risk of being captured by ants and other predators, particularly when populations of predators are high. This situation does not however, seem to have been sufficient to select for avoidance of early emerging females by nematodes. Competition for females and the short time available to the nematodes for attachment are probably much more important, favouring attachment to the first female pollinators they encounter. The remaining nematodes in the figs will face a dead end, since there is no way for them to disperse to another new fig to develop and continue reproduction. The nematodes are nonetheless careful to choose female pollinators, not males or NPFW (see earlier chapters).

Up to eight species of nematodes were recorded from a single fig, though the presence of all species in one fig was very rare. Introduction of foundresses from different positions in the emergence sequence into receptive figs did not seem to affect the eventual number of adult nematodes of each species, except for *Parasitodiplogaster* sp. The number of *Parasitodiplogaster* adults was regularly found to be higher in the figs where the last foundresses to emerge were introduced. The higher number of *Parasitodiplogaster* adults suggests that higher numbers of this nematode were carried (as juveniles) by the later emerging females. *Parasitodiplogaster* are known as predatory/parasitic on female pollinators, and eat them either shortly before or after they have died in the figs (Giblin-Davis et al. 2006; Jauharlina et al. 2012). Any damage to females by this nematode is likely to occur after flights, within the figs where the foundresses lay their eggs and pollinate the female flowers. Nematode species with this lifestyle can reduce the fecundity of fig wasps (Herre 1995), so there is potential for later emerging females to suffer disproportionately in other fig pollinating wasp species on different fig trees that harbour more damaging nematodes.

Chapter 7 General Discussion

This thesis provides findings relevant to interactions between mutualists and non-mutualists species. Interactions between fig trees and their pollinating wasps have long been studied as an example on how mutualisms between species co-exist over time. Adding to this interaction is the presence of non-mutualist species (such as non-pollinating fig wasps (NPFW), mites and nematodes), which also intimately interact with the pollinating wasps and fig trees. The flightless organisms such as mite and nematodes which develop inside figs will need a ‘vehicle’ to transport them out of the fig for further development of their offspring. An attachment of such organisms to other organisms to be transferred to other suitable habitat is known as phoresy (Krishnan et al. 2010). In general, phoresy is an occurrence where certain organisms (the passengers) look for and use other organisms to disperse them out of unsuitable area for further development of the passengers or its progeny (Binns 1982; Kruitbos, Heritage and Wilson 2009). Selection of the vehicles must be done efficiently by the passengers to ensure further survival. The relationship between the passengers and their vehicles become more specific when this system involves a third organism as a shared host, as occur in the study system in this present study.

Other tritrophic interactions including annelids and ostracods transported by lizard and frogs between epiphytes (Lopez et al. 2005). Many species of mites have been known to be phoretic and dispersed by other organism into suitable habitat for feeding and further development (Barton, Weaver and Manning 2014). Flower-inhabiting mites could be dispersed by fruit bats or by hummingbirds (Tschapka and Cunningham 2004; Lopez-Orozco and Canon-Franco 2013). Flower mites also use various insects for transporting them, including butterflies, beetles, bees, and wasps (Seeman and Walter 1995). In general tritrophic interactions are often said to specific in term of the species involved (Heil 2008; Rasmann and Turlings 2008).

In this thesis I have shown how mites and nematodes as non-mutualist species choose the female pollinating wasps to transfer them between figs. Mites may disperse to another fig after being transported by female fig wasp, however nematodes exclusively rely on the female pollinators to ensure further development and reproduction of the nematodes in another suitable figs. How the mutualism between fig trees and fig wasps works in seasonal environments and survives under unfavourable seasonal weather was also investigated. A comparison of this mutualism in a temperate area and in tropical area was provided by the investigation of different species of fig trees in South Africa and in Indonesia

Seasonal changes in production of a pollinating wasp *Elisabethiella baijnathi* (the pollinator of the monoecious *Ficus burtt-davyi* figs) in Grahamstown, South Africa, were presented in Chapter 2. The third chapter focused on female *E. bajnathi* that transported mites and nematodes into receptive figs when the females enter. The fourth chapter investigated the ecology of nematodes that were transferred by a pollinating wasp *Ceratosolen solmsi marchali* into male and female figs of the dioecious *F. hispida* in Sumatra, Indonesia. Similar studies were also done with the figs of the monoecious *F. racemosa*, which are pollinated by *Ceratosolen fusciceps*. Chapter six mainly examined how the emergence sequence of females of the pollinating wasp *Ceratosolen fusciceps* affects the numbers and species of nematodes carried by female pollinators away from their natal figs. To my knowledge this is the first study to investigate the ecology of nematodes associate with fig pollinating wasps and fig trees in Indonesia.

Seasonal variation is likely to affect the reproductive success of both fig trees and their pollinating wasps, and probably also the nature of the interaction (Bronstein 1989; Wang, Yang and Yang 2005). In warmer months (summer), the crops of *F. burtt-davyi* developed in a shorter time than those in colder months (winter). Seasonal variation in temperature is known to affect the development times of figs (Zhang, Song and Yang 2006; Pereira, Teixeira and Kjellberg 2007; Jauharlina et al. 2012). In comparison to seasonal variation in Grahamstown, South Africa, seasonal changes in the Sumatran tropics were much less evident. Daily temperature and monthly rainfall had no significant effect on crop duration of *F. hispida* (dioecious species) and on *F. racemosa* (monoecious).

The female wasps of *E. baijnathi* carried mites (*Tarsonemella* sp. nr. *africanus*) and nematodes (*Parasitodiplogaster* sp.) between figs of *F. burtt-davyi* when the wasps entered the figs. Monoecious fig trees typically produce synchronized crops of figs, which forces each generation of fig wasps that emerge from their natal figs to seek another tree to find new receptive figs (Wang et al. 2008; Wang and Sun 2009). In both monoecious and dioecious fig trees it also means that short-lived flightless animals such as mites and nematodes which only reproduce inside figs need to utilize winged insects to transport them from their natal figs into new figs, usually on other trees (Chapter 3).

Many previous studies on nematodes transported into figs of dioecious fig trees in other places have focused on figs of male trees (Vovlas and Larizza 1996; Zeng, Giblin-Davis and Ye 2007), as no pollinator fig wasps can reproduce in female figs. However, Vovlas, Inserra and Greco (1992) have found nematodes in female edible figs (*F. carica*), but with much lower populations than those in caprifigs (male figs*)* in Italy. In this thesis I have shown that although fig wasps did not reproduce in female figs of *F. hispida*, nematodes transferred by female pollinators into these figs still developed and reproduced, with similar numbers to those on male figs. The new generation of nematodes has no means to be dispersed from the natal figs and eventually will die off. The nematode species found in female figs were the same as those found in male figs, comprising of *Caenorhabditis* sp. (Rhabditidae), *Schistonchus centerae* and *S. guangzhouensis* (Aphelenchoididae). The presence of rhabditid nematodes has been recorded in *F. septica* in Taiwan (Kanzaki et al. 2012b), but it is not known whether it is also *Caenorhabditis*. It is still unclear what this nematode feeds on. On the other hand, *Schistonchus* spp. are known as phytophagous nematodes that feed on flowers inside the figs. *Schistonchus* is very commonly associated with figs, of many different fig tree species. Potentially, most fig tree species could be associated with species of *Schistonchus* (Giblin-Davis et al. 1995). The figs of *F. racemosa* in Australia are known to host *S. baculum*, *S. fleckeri*, and *S. cassowaryi*. The last two species were also found in the figs of *F. hispida* in the same area (Davies et al. 2010; Davies et al. 2013). Different species of *Schistonchu*s are recorded from *F. hispida* figs in China. These are *S. guangzhou*ensis and *S. cent*erae (Zeng, Giblin-Davis and Ye 2007), the same *Schistonchus* species found in this present study. These two species of *Schistonchus* are the only nematode species found so far in *F. hispida* figs in China (Zeng, Giblin-Davis and Ye 2007).

Many other *Schistonchus* species have been recorded to associate with figs and their pollinating fig wasps in different places, such as in India, Italy, Florida, Turkey, Africa, and Australia (Reddy and Rao 1986; Vovlas, Inserra and Greco 1992; Giblin-Davis et al. 1995; Center et al. 1999; DeCrappeo and Giblin-Davis 2001; Anand 2002; Gulcu et al. 2008; Bartholomaeus et al. 2009; Bartholomaeus et al. 2012). It is suggested that host fig tree specificity may vary between the species of *Schistonchus* (Davies et al. 2013), and in

Schistonchus host switching between fig tree species of one subgenus seems to be common (Lloyd and Davies 1997; Davies et al. 2010).

There were eight species of nematodes carried by female *C. fusciceps* into figs of *F. racemosa* (Chapter 5). This is the most diverse fig nematode community recorded in one fig anywhere in the world. This is also one of few studies that looked at fig nematodes ecology, in contrast with other studies that focusses more on fig nematodes. Martin, Owen and Way (1973) recorded eight species of nematodes in figs of a native fig tree species in Zimbabwe. It was the first study described the possibility of a wide variety of tripartite relationships among African fig trees, fig wasps and nematodes. At least 20 species of nematodes were recognized from six African native fig trees. None of them was however morphologically described and no further taxonomic information on them was provided. In a recent study (Kanzaki et al. 2012a), six of the above species were described as *Schistonchus* sp., *Teratodiplogaster martini*., two *Parasitodiplogater* sp., *Mononchoides* sp., and an unknown diplogastrid genus. The presence of *Teratodiplogaster* nematodes was first recorded in *F. racemosa* figs in Australia, as *T. fignewmani* (Kanzaki et al. 2009), the same species as the one found in *F. racemosa* figs in Sumatra (Chapter 5). Different species of *Parasitodiplogaster* nematodes have also been known to exist within figs of different fig tree species elsewhere (Compton 1993; Giblin-Davis et al. 1995; Giblin-Davis et al. 2006; Kanzaki et al. 2012a; Kanzaki et al. 2013).

In the present study, the nematode community found in *F. racemosa* were more varied than those found in *F. hispida* figs. The presence of *Caenorhabditis* in *F. hispida* figs and the two undescribed diplogastrid species with unusual anatomy in *F. racemosa* figs show that the nematode communities associated with figs and their pollinating wasp are more taxonomically diverse than previously realised, and also vary in different parts of the world, even within the same species of fig tree. The development of fig wasp offspring and seeds in the same figs of the monoecious *F. racemosa* ensure a way of transportation for the new generation of nematodes out of natal figs to seek new receptive figs. This situation, where every fig can support nematode population development and transport may have stimulated more nematode species to associate with momnoecious figs. In contrast, in dioecious fig tree like *F. hispida*, the survival of nematodes is only possible if nematodes are transferred into male figs, where the fig wasp offspring are produced. About half the nematodes transferred to and developing inside female figs will have no means to transport them out of the figs, so half of the nematodes in each generation will be lost. This condition may have prevented some species of nematodes from associating with dioecious fig tree species.

In addition to nematodes, mites also have been known to be transported by female pollinating fig wasps into receptive figs of different fig tree species in Africa, Brazil, Taiwan, Australia and Thailand (Compton 1993; Pereira, Semir and Menezes Junior 2000; Walter 2000; Jauharlina et al. 2012). There were no mites found within the figs of *F. hispida* and *F. racemosa* and their pollinating wasps in Sumatra, Indonesia (Chapter 4 and Chapter 5). No mites have so far been described from dioecious fig tree species. Despite the diversity of nematodes community found in the figs of *F. racemosa*, mites did not seem to colonize this monoecious fig tree species. It belongs to subgenus Sycomorus, where the figs fill with liquid for much of their development. Possibly this prevents colonisation by mites. So far the presence of mites only reported from the figs of fig tree species belong to subgenus Urostigma (Compton 1993; Pereira, Semir and Menezes Junior 2000; Walter 2000; Jauharlina et al. 2012).

This study has shown that the number of nematodes present within the figs of both *F. hispida* and *F. racemosa* are quite high. Some species of nematodes do feed on fig flowers during the nematode development within the fig, for example *Schistonchus* spp. This feeding, however, causes no apparent impact on the development of the figs, as numbers of seeds produced are not affected. Similar results have been reported in earlier studies (Center et al. 1999; Krishnan et al. 2010). Investigations on figs of six *Ficus* species from Panama showed that each of fig trees studied contained *Schistonchus* nematodes which fed on female and/or male flowers inside the figs. Histological studies of parasitism by *Schistonchus* sp. in each fig tree species shows that there was no clear damage caused by the nematodes to the fig trees and their pollinating wasps, except for *F. laevigata*. In *F. laevigata* figs, *Schistonchus* nematodes were found to infest immature male flowers exclusively, causing apparent damage to the flowers and consequently may reduce pollen formation. Reduction of pollen potentially will decrease the reproductive success of the *F. laevigata* figs (Center et al. 1999). Reduction in pollen production caused by nematodes could also negatively affect the pollinating fig wasp, with negative consequences to the figs and to the nematode itself (Giblin-Davis et al. 1995), because pollinator larvae are often less likely to develop successfully in figs that have not been pollinated (Weiblen 2002; Herre, Jander and Machado 2008).

Despite being transported by female pollinating wasps into figs, the nematodes did not seem to affect development of the fig wasps either. The presence of *Parasitodiplogaster* sp. which is parasitic/predatory on the female figs wasps that transported the nematode did not show any detectable impact to the production of fig wasps offspring. According to Downes and Griffin (1996) many adult insects that host parasitic nematodes are still able to stay active and fly after infection. The host insects may be expecting to do so over e period of 1 to 2 days or longer when temperatures and other factors are optimal.

This study has shown that nematodes as non-mutualist have no apparent impact on the two mutualist species (fig trees and their pollinating wasps) that are being exploited by the nematodes. On the other hand, the nematodes do not offer any advantages to the two mutualist species either. The presence of nematodes in the mutualism between fig trees and their pollinating fig wasps seems to be very common. Nematodes in this case may also called as exploiters which obtain commodities and services offered to mutualist species, but they fail to reciprocate by offering advantages to the mutualist species in return (Bronstein 2001b). This non-mutualist species save the time and energy that is spent by the two partners in mutualist species (Bronstein 2001b; Orona-Tamayo and Heil 2013). Species specificity involved in the interaction between the non-mutualist and the mutualist species may act as a suitable mechanism to ensure its occurrence over time. Factors contributing to this species specificity between partners involved in multitrophic interaction, such as those that occur in phoresy, are crucial to understand evolutionary and co-evolutionary process (Thompson 2009).

Further studies

This is one of the first studies of the ecology of fig nematodes in Asia. Most previous studies have been largely taxonomic, describing the fig wasps found in different species of fig trees. This is the first attempt to investigate how the non-mutualist nematodes interact with both the fig trees and their pollinating wasps and to compare nematode populations and development in both male and female figs of dioecious species. During the course of this study, I have been in contact with two other researchers who are mostly working with taxonomy of nematodes found on fig wasps and figs of different fig tree species in several other countries. They are currently working on the further identification and description of the nematodes found in *F. hispida* and *F. racemosa*.

This study has shown that the presence of nematodes within these Asian fig scause no apparent effect to the figs and their figs wasps. However, further study needs to be done to confirm that nematodes have no effect at all to the reproductive success of figs and the pollinating wasps. This could be done by setting a controlled experiment of figs with and without nematodes introduced with fixed numbers of foundresses. This experiment is to compare the reproductive success of figs and fig wasps with and without nematode develop in the figs. I tried to set up this experiment during the course of my study, but it failed because I could not detect any foundresses that had no nematodes before introducing them into B-phase figs. Injection of a pesticide (which can also work as a nematicide) into figs was done 24 hours after foundresses were introduced, in order to kill any nematodes that might be brought into the figs. However, in later observations on the experimental figs, I found that nematodes were still alive inside the figs. Despite the unknown foundresses with and without nematodes attached, another experimental study could be done by introducing different number of foundresses into receptive figs. This treatment can be used to compare the effects of different number of foundresses introduced into figs on the reproductive success of figs and nematodes.

The effect of seasonal changes in temperature for fig tree pollination in *F. burtt-davyi* was studied. The consequences for fig pollinating wasps were recorded. However, its effects on the NPFW that interact with the mutualist species were not included. Further study needs to be done to examine how the seasonal changes will affect the structure community of the NPFW on *F. burtt-davyi* figs, which in turn may affect the mutualism between figs and the pollinating wasp. Further studies by using the fig-fig wasp-nematode system will provide a better understanding the evolution and host specificity especially the occurrence of phoresy.

References

ABDURAHIMAN, U. C. and K. J. JOSEPH 1976. Observations on the biology and behavior of *Ceratosolen marchali* Agaonidae Chalcidoidea Hymenoptera. *Entomon,* 1**,** 115-122.

AHMED, S., S. G. COMPTON, R. K. BUTLIN and P. M. GILMARTIN 2009. Wind-borne insects mediate directional pollen transfer between desert fig trees 160 kilometers apart. *Proceedings of the National Academy of Sciences of the United States of America,* 106**,** 20342-20347.

AL-BEIDH, S., D. W. DUNN, S. A. POWER and J. M. COOK 2012. Parasites and mutualism function: measuring enemy-free space in a fig-pollinator symbiosis. *Oikos,* 121**,** 1833-1839.

ALI, M. and N. CHAUDHARY 2011. *Ficus hispida* Linn.: A review of its pharmacognostic and ethnomedicinal properties. *Pharmacognosy reviews,* 5**,** 96-102.

ANAND, L. R. 2002. Studies on the association of a new nematode species *Schistonchus osmani* sp. n. (Aphlenchoidea Nickle, 1971), a wasp and fig *Ficus recemosa*. *Uttar Pradesh Journal of Zoology,* 22**,** 281-283.

ANAND, L. R. 2005. Association of a nematode *Ceratosolenus racemosa* gen. n. sp.n (Cylindrocorporidae: Rhabditida Goodey, 1939) a wasp (*Ceratosolen* sp.n.) and fig *Ficus racemosa*. *Uttar Pradesh Journal of Zoology,* 25**,** 59-62.

ANAND, L. R. 2006. *Ceratosolenus hyderabadensis* sp.n. (Cylindrocorporidae : Rhabditida, Goodey 1939) association with wasp, *Ceratosolen* sp and fig, *Ficus racemosa*. *Uttar Pradesh Journal of Zoology,* 26**,** 115-117.

ANSTETT, M. C., M. HOSSAERTMCKEY and F. KJELLBERG 1997a. Figs and fig pollinators: Evolutionary conflicts in a coevolved mutualism. *Trends in Ecology & Evolution,* 12**,** 94-99.

ANSTETT, M. C., M. HOSSAERTMCKEY and D. MCKEY 1997b. Modeling the persistence of small populations of strongly interdependent species: Figs and fig wasps. *Conservation Biology,* 11**,** 204-213.

BAI, H.-Y. and H.-H. LI 2008. Brief review of obligate pollination mutualism between Epicephala moths and Euphorbiaceae trees. *Chinese Bulletin of Entomology,* 45**,** 166-169.

BAI, L. F., D. R. YANG and S. G. COMPTON 2008. A gall midge inhabiting the figs of *Ficus benjamina* in Xishuangbanna, south-western China. *Symbiosis,* 45**,** 149-152.

BAIJNATH, H. and S. RAMCHARUN 1988. Reproductive biology and chalcid symbiosis in *Ficus burtt-davyi* Hutch. (Moraceae). *In:* P. GOLDBLATTandP. P. LOWRY (eds.) *Modern Systematics Studies in African Botany*. Lawrence, Kansas: Allen Press Inc.

BARTHOLOMAEUS, F., K. DAVIES, W. M. YE, N. KANZAKI and R. M. GIBLIN-DAVIS 2009. *Schistonchus virens* sp n. (Aphelenchoididae) and *Parasitodiplogaster australis* sp n. (Diplogastridae) from *Ficus virens* (Moraceae) in Australia. *Nematology,* 11**,** 583-601.

BARTHOLOMAEUS, F., K. A. DAVIES, W. YE and R. M. GIBLIN-DAVIS 2012. *Schistonchus* (Aphelenchoididae) from *Ficus benjamina* in Australia, with description of *S. benjamina* sp n. *Nematology,* 14**,** 1005-1013.

BARTON, P. S., H. J. WEAVER and A. D. MANNING 2014. Contrasting diversity dynamics of phoretic mites and beetles associated with vertebrate carrion. *Experimental & applied acarology,* 63**,** 1-13.

BERG, C. C. 1989. Classification and distribution of *Ficus*. *Experientia,* 45**,** 605-611.

BERG, C. C. and E. J. H. CORNER 2005. *Moraceae-Ficus. Flora Malesiana* Series I (*Seed Plants*). Volume 17, Part 2

National Herbarium of the Netherlands, Leiden.

BINNS, E. S. 1982. Phoresy as migration - some functional-aspects of phoresy in mites. *Biological Reviews of the Cambridge Philosophical Society,* 57**,** 571-620.

BRONSTEIN, J. L. 1988. Predators of fig wasps. *Biotropica,* 20**,** 215-219.

BRONSTEIN, J. L. 1989. A mutualism at the edge of its range. *Experientia,* 45**,** 622-637.

BRONSTEIN, J. L. 1991. The nonpollinating wasps fauna of *Ficus pertusa*:exploitation of a mutualism. *Oikos,* 61**,** 175-186.

BRONSTEIN, J. L. 1994. Conditional outcomes in mutualistic interactions. *Trends in Ecology & Evolution,* 9**,** 214-217.

BRONSTEIN, J. L. 2001a. The costs of mutualism. *American Zoologist,* 41**,** 825-839.

BRONSTEIN, J. L. 2001b. The exploitation of mutualisms. *Ecology Letters,* 4**,** 277-287.

BRONSTEIN, J. L. and M. HOSSAERTMCKEY 1996. Variation in reproductive success within a subtropical fig pollinator mutualism. *Journal of Biogeography,* 23**,** 433-446.

BURROWS, J. and S. BURROWS 2003. *Figs of southern & south-central Africa.,* Hatfield, Umdaus Press.

CARDONA, W., P. C. DE ULLOA and G. KATTAN 2007. Non-pollinating fig wasps associated with *Ficus andicola* (Moraceae) in the Central Andes of Colombia. *Revista Colombiana De Entomologia,* 33**,** 165-170.

CARDONA, W., G. KATTAN and P. C. DE ULLOA 2013. Non-pollinating Fig Wasps Decrease Pollinator and Seed Production in Ficus andicola (Moraceae). *Biotropica,* 45**,** 203-208.

CENTER, B. J., R. M. GIBLIN-DAVIS, E. A. HERRE and G. C. CHUNG-SCHICKLER 1999. Histological comparisons of parasitism by *Schistonchus* spp. (Nemata : Aphelenchoididae) in neotropical *Ficus* spp. *Journal of Nematology,* 31**,** 393-406.

CHEN, C. and Q. SONG 2008. Responses of the Pollinating Wasp *Ceratosolen solmsi marchali* to Odor Variation Between Two Floral Stages of *Ficus hispida*. *Journal of Chemical Ecology,* 34**,** 1536-1544.

CHEN, H.-H., D.-R. YANG, D. GU, S. G. COMPTON and Y.-Q. PENG 2013. Secondary galling: a novel feeding strategy among "non-pollinating' fig wasps from Ficus curtipes. *Ecological Entomology,* 38**,** 381-389.

CHEN, Y., S. G. COMPTON, M. LIU and X. Y. CHEN 2012. Fig trees at the northern limit of their range: the distributions of cryptic pollinators indicate multiple glacial refugia. *Molecular Ecology,* 21**,** 1687-1701.

CHOU, L.-S. and H.-M. YEH 1995. The pollination ecology of *Ficus aurantiaca* var. *parvifolia*. *Acta Zoologica Taiwanica,* 6**,** 1-12.

COMPTON, S. G. 1993. One way to be a fig. *African Entomology,* 1**,** 151-158.

COMPTON, S. G. 2002. Sailing with the wind: dispersal by small flying insects. *Dispersal Ecology***,** 113-133.

COMPTON, S. G., A. D. BALL, M. E. COLLINSON, P. HAYES, A. P. RASNITSYN and A. J. ROSS 2010. Ancient fig wasps indicate at least 34 Myr of stasis in their mutualism with fig trees. *Biology Letters,* 6**,** 838-842.

COMPTON, S. G. and R. H. L. DISNEY 1991. New species of Megaselia (Diptera, Phoridae) whose larvae live in fig syconia (Urticales, Moraceae), and adults prey on fig wasps (Hymenoptera, Agaonidae). *Journal of Natural History,* 25**,** 203-219.

COMPTON, S. G., M. D. F. ELLWOOD, A. J. DAVIS and K. WELCH 2000. The flight heights of chalcid wasps (Hymenoptera, Chalcidoidea) in a lowland bornean rain forest: Fig wasps are the high fliers. *Biotropica,* 32**,** 515-522.

COMPTON, S. G., M. D. F. ELLWOOD, R. LOW and J. WATSON 2005. Dispersal of fig wasps (Hymenoptera:Chalcidoidea) across primary and logged rainforest in Sabah (Malaysia). *Acta Soc. Zool. Bohem*, 69**,** 37-48.

COMPTON, S. G., K. GREHAN and S. VAN NOORT 2009. A fig crop pollinated by three or more species of agaonid fig wasps. *African Entomology,* 17**,** 215-222.

COMPTON, S. G. and B. A. HAWKINS 1992. Determinats of species richness in Southern African fig wasp assemblages. *Oecologia,* 91**,** 68-74.

COMPTON, S. G. and F. A. C. MCLAREN 1989. Respiratory adaptations in some male fig wasps. *Proceedings of the Koninklijke Nederlandse Akademie Van Wetenschappen Series C-Biological and Medical Sciences,* 92**,** 57-71.

COMPTON, S. G. and R. J. C. NEFDT 1990. The figs and the fig wasps of *Ficus burtt-davyi*. *Mitteilungen aus dem Institut fuer Allgemeine Botanik Hamburg,* 23**,** 441-450.

COMPTON, S. G. and H. G. ROBERTSON 1988. Complex interactions between mutualisms - ants tending Homopterans protect fig seeds and pollinators. *Ecology,* 69**,** 1302-1305.

COMPTON, S. G., S. J. ROSS and I. W. B. THORNTON 1994. Pollinator limitation of fig tree reproduction on the island of Anak-Krakatau (Indonesia). *Biotropica,* 26**,** 180-186.

COMPTON, S. G. and S. VAN NOORT 1992. Southern African fig wasps (Hymenoptera, Chalcidoidea) - resource utilization and host relationships. *Proceedings of the Koninklijke Nederlandse Akademie Van Wetenschappen-Biological Chemical Geological Physical and Medical Sciences,* 95**,** 423-435.

COMPTON, S. G., J. T. WIEBES and C. C. BERG 1996. The biology of fig trees and their associated animals. *Journal of Biogeography,* 23**,** 405-407.

COOK, J. M. and J. Y. RASPLUS 2003. Mutualists with attitude: coevolving fig wasps and figs. *Trends in Ecology & Evolution,* 18**,** 241-248.

COOK, J. M. and S. T. SEGAR 2010. Speciation in fig wasps. *Ecological Entomology,* 35**,** 54-66.

CORLETT, R. T. 1987. The phenology of *Ficus fistulosa* in Singapore. *Biotropica,* 19**,** 122-124.

CORNER, E. H. 1967. *Ficus* in the Solomon Islands and its bearing on the post-Jurassic history of Melanesia. *Philosophical Transactions of the Royal Society B,* 253**,** 23–159.

CORNER, E. J. H. 1965. *Ficus* in the Solomon Islands and its bearing on the post-Jurassic history of Melanesia. *Philosophical Transactions of the Royal Society B,* 253**,** 23–159.

CORNILLE, A., J. G. UNDERHILL, A. CRUAUD, M. HOSSAERT-MCKEY, S. D. JOHNSON, K. A. TOLLEY, F. KJELLBERG, S. VAN NOORT and M. PROFFIT 2012. Floral volatiles, pollinator sharing and diversification in the fig-wasp mutualism: insights from *Ficus natalensis*, and its two wasp pollinators (South Africa). *Proceedings of the Royal Society B-Biological Sciences,* 279**,** 1731-1739.

CROLL, N. A. and B. E. MATHEWS 1977. *Biology of Nematodes,* New York, John Wiley & Sons.

CRUAUD, A., R. JABBOUR-ZAHAB, G. GENSON, C. CRUAUD, A. COULOUX, F. KJELLBERG, S. V. NOORT and J.-Y. RASPLUS 2009. Laying the foundations for a new classification of Agaonidae (Hymenoptera: Chalcidoidea), a multilocus phylogenetic approach. *Cladistics,* 25**,** 1-29.

CUSHMAN, J. H., S. G. COMPTON, C. ZACHARIADES, A. B. WARE, R. J. C. NEFDT and V. K. RASHBROOK 1998. Geographic and taxonomic distribution of a positive interaction: ant-tended homopterans indirectly benefit figs across southern Africa. *Oecologia,* 116**,** 373-380.

DAVIES, K., F. BARTHOLOMAEUS, W. YE, N. KANZAKI and R. GIBLIN-DAVIS 2010. *Schistonchus* (Aphelenchoididae) from *Ficus* (Moraceae) in Australia, with description of *S. aculeata* sp n. *Nematology,* 12**,** 935-958.

DAVIES, K. A., F. BARTHOLOMAEUS, N. KANZAKI, W. YE and R. M. GIBLIN-DAVIS 2013. Three new species of *Schistonchus* (Aphelenchoididae) from the *Ficus* subgenus Sycomorus (Moraceae) in northern Australia. *Nematology,* 15**,** 347-362.

DECRAPPEO, N. and R. M. GIBLIN-DAVIS 2001. *Schistonchus aureus* n. sp and *S-laevigatus* n. sp (Aphelenchoididae): Associates of native Floridian *Ficus* spp. and their *Pegoscapus* pollinators (Agaonidae). *Journal of Nematology,* 33**,** 91-103.

DOWNES, M. J. and C. T. GRIFFIN 1996. Dispersal behaviour and transmission strategies of the entomopathogenic nematodes Heterorhabditis and Steinernema. *Biocontrol Science and Technology,* 6**,** 347-356.

DUFAY, M. and M. C. ANSTETT 2003. Conflicts between plants and pollinators that reproduce within inflorescences: evolutionary variations on a theme. *Oikos,* 100**,** 3-14.

DUNN, D. W., S. T. SEGAR, J. RIDLEY, R. CHAN, R. H. CROZIER, D. W. YU and J. M. COOK 2008a. A role for parasites in stabilising the fig-pollinator mutualism. *PLoS Biol,* 6**,** e59.

DUNN, D. W., D. W. YU, J. RIDLEY and J. M. COOK 2008b. Longevity, early emergence and body size in a pollinating fig wasp-implications for stability in a fig-pollinator mutualism. *Journal of Animal Ecology,* 77**,** 927-935.

EZOE, H. 2012. Evolutionary stability of one-to-many mutualisms. *Journal of Theoretical Biology,* 314**,** 138-144.

FOSTER, K. R. and T. WENSELEERS 2006. A general model for the evolution of mutualisms. *Journal of Evolutionary Biology,* 19**,** 1283-1293.

GALIL, J. 1977. Fig biology. *Endeavour,* 1**,** 52-56.

GALIL, J. and D. EISIKOWICH 1968. On the pollination ecology of *Ficus sycomorus* in East Africa. *Ecology,* 49**,** 259-269.

GALIL, J. and D. EISIKOWICH 1968b. Flowering cycles and fruit types in *Ficus sycomorus* in Israel. *New Phytologist,* 67**,** 745-758.

GALIL, J., M. ZERONI and D. BOGOSLAV 1973. Carbon dioxide and ethylene effects in coordination between pollinator *Blastophaga quadraticeps* and syconium in *Ficus religiosa*. *New Phytologist,* 72**,** 1113-&.

GHARA, M. and R. M. BORGES 2010. Comparative life-history traits in a fig wasp community: implications for community structure. *Ecological Entomology,* 35**,** 139-148.

GIBERNAU, M., M. HOSSAERTMCKEY, M. C. ANSTETT and F. KJELLBERG 1996. Consequences of protecting flowers in a fig: A one-way trip for pollinators? *Journal of Biogeography,* 23**,** 425-432.

GIBLIN-DAVIS, R. M., B. J. CENTER, H. NADEL, J. H. FRANK and W. RAMIREZ 1995. Nematodes associated with fig wasps, *Pegoscapos* spp (Agaonidae), and syconia of native Floridian figs (*Ficus* spp). *Journal of Nematology,* 27**,** 1-14.

GIBLIN-DAVIS, R. M., K. A. DAVIES, K. MORRIS and W. K. THOMAS 2003. Evolution of parasitism in insect-transmitted plant nematodes. *Journal of Nematology,* 35**,** 133-141.

GIBLIN-DAVIS, R. M., W. M. YE, N. KANZAKI, D. WILLIAMS, K. MORRIS and W. K. THOMAS 2006. Stomatal ultrastructure, molecular phylogeny, and description of *Parasitodiplogaster laevigata* n. sp (Nematoda : Diplogastridae), a parasite of fig wasps. *Journal of Nematology,* 38**,** 137-149.

GOODEY, T. 1963. *Soil and Fresh water Nematodes* Methuen & Co Ltd, London

GULCU, B., S. HAZIR, R. M. GIBLIN-DAVIS, W. M. YE, N. KANZAKI, H. MERGEN, N. KESKIN and W. K. THOMAS 2008. Molecular variability of *Schistonchus caprifici* (Nematoda: Aphelenchoididae) from *Ficus carica* in Turkey. *Nematology,* 10**,** 639-649.

HARRISON, R. D. 2003. Fig wasp dispersal and the stability of a keystone plant resource in Borneo. *Proceedings of the Royal Society of London Series B-Biological Sciences,* 270**,** S76-S79.

HARRISON, R. D. 2005. Figs and the diversity of tropical rainforests. *Bioscience,* 55**,** 1053-1064.

HARRISON, R. D. and J. Y. RASPLUS 2006. Dispersal of fig pollinators in Asian tropical rain forests. *Journal of Tropical Ecology,* 22**,** 631-639.

HARRISON, R. D. and N. YAMAMURA 2003a. A few more hypotheses for the evolution of dioecy in figs (*Ficus,* Moraceae). *Oikos,* 100**,** 628-635.

HARRISON, R. D., N. YAMAMURA and T. INOUE 2000. Phenology of a common roadside fig in Sarawak. *Ecological Research,* 15**,** 47-61.

HEIL, M. 2008. Indirect defence via tritrophic interactions. *New Phytologist,* 178**,** 41-61.

HEITHAUS, E. R., D. C. CULVER and A. J. BEATTIE 1980. MODELS OF SOME ANT-PLANT MUTUALISMS. *American Naturalist,* 116**,** 347-361.

HERATY, J. M., R. A. BURKS, A. CRUAUD, G. A. P. GIBSON, J. LILJEBLAD, J. MUNRO, J.-Y. RASPLUS, G. DELVARE, P. JANSTA, A. GUMOVSKY, J. HUBER, J. B. WOOLLEY, L. KROGMANN, S. HEYDON, A. POLASZEK, S. SCHMIDT, D. C. DARLING, M. W. GATES, J. MOTTERN, E. MURRAY, A. DAL MOLIN, S. TRIAPITSYN, H. BAUR, J. D. PINTO, S. VAN NOORT, J. GEORGE and M. YODER 2013. A phylogenetic analysis of the megadiverse Chalcidoidea (Hymenoptera). *Cladistics,* 29**,** 466-542.

HERRE, E. A. 1989. Coevolution of reproductive characteristics in 12 species of new world figs and their pollinator wasps. *Experientia,* 45**,** 637-647.

HERRE, E. A. 1993. Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science,* 259**,** 1442-1445.

HERRE, E. A. 1995. Factors affecting the evolution of virulence: Nematode parasites of fig wasps as a case study. *Parasitology,* 111**,** S179-S191.

HERRE, E. A., K. C. JANDER and C. A. MACHADO 2008. Evolutionary ecology of figs and their associates: recent progress and outstanding puzzles. *Annual Review of Ecology Evolution and Systematics,* 39**,** 439-458.

HERRE, E. A., C. A. MACHADO, E. BERMINGHAM, J. D. NASON, D. M. WINDSOR, S. S. MCCAFFERTY, W. VANHOUTEN and K. BACHMANN 1996. Molecular phylogenies of figs and their pollinator wasps. *Journal of Biogeography,* 23**,** 521-530.

HO, C.-C. 1994. A new genus and two new species of Tarsonemidae from *Ficus* spp. (Acari: Heterostigmae). *International Journal of Acarology,* 20**,** 189-197.

HU, H. Y., Z. F. JIANG, L. M. NIU, Y. G. FU, Z. Q. PENG and D. W. HUANG 2009. Different Stimuli Reduce Attraction to Pollinators in Male and Female Figs in the Dioecious Fig Ficus hispida. *Biotropica,* 41**,** 717-720.

IHAKA, R. and R. GENTLEMAN 1996. R: a language for data analysis and graphics. *Journal of Computional and Graphical Statistics,* 5**,** 299-314.

JAHAN, I. A., N. NAHAR, M. MOSIHUZZAMAN, B. ROKEYA, L. ALI, A. K. A. KHAN, T. MAKHMUR and M. I. CHOUDHARY 2009. Hypoglycaemic and antioxidant activities of *Ficus racemosa* Linn. fruits. *Natural Product Research,* 23**,** 399-408.

JANDER, K. C. and E. A. HERRE 2010. Host sanctions and pollinator cheating in the fig tree-fig wasp mutualism. *Proceedings of the Royal Society B-Biological Sciences,* 277**,** 1481-1488.

JANDER, K. C., E. A. HERRE and E. L. SIMMS 2012. Precision of host sanctions in the fig tree-fig wasp mutualism: consequences for uncooperative symbionts. *Ecology Letters,* 15**,** 1362-1369.

JANSEN-GONZALEZ, S., S. D. P. TEIXEIRA and R. A. SANTINELO PEREIRA 2012. Mutualism from the inside: coordinated development of plant and insect in an active pollinating fig wasp. *Arthropod-Plant Interactions,* 6**,** 601-609.

JANZEN, D. H. 1979a. How to be a fig. *Annual Review of Ecology and Systematics,* 10**,** 13-51.

JANZEN, D. H. 1979b. How many parents do the wasps from a fig have. *Biotropica,* 11**,** 127-129.

JAUHARLINA, J., E. E. LINDQUIST, R. J. QUINNELL, H. G. ROBERTSON and S. G. COMPTON 2012. Fig wasps as vectors of mites and nematodes. *African Entomology,* 20**,** 101-110.

JEEVANANDAM, N. and R. T. CORLETT 2013. Fig wasp dispersal in urban Singapore. *Raffles Bulletin of Zoology,* 61**,** 343-347.

JIA, X. C., J. Y. YAO, Y. Z. CHEN, J. M. COOK and R. H. CROZIER 2008. The phenology and potential for self-pollination of two Australian monoecious fig species. *Symbiosis,* 45**,** 91-96.

JOUSSELIN, E., M. HOSSAERT-MCKEY, E. A. HERRE and F. KJELLBERG 2003. Why do fig wasps actively pollinate monoecious figs? *Oecologia,* 134**,** 381-387.

JOUSSELIN, E. and F. KJELLBERG 2001. The functional implications of active and passive pollination in dioecious figs. *Ecology Letters,* 4**,** 151-158.

JOUSSELIN, E., J. Y. RASPLUS and F. KJELLBERG 2003. Convergence and coevolution in a mutualism: Evidence from a molecular phylogeny of Ficus. *Evolution,* 57**,** 1255-1269.

JOUSSELIN, E., S. VAN NOORT, V. BERRY, J. Y. RASPLUS, N. RONSTED, J. C. ERASMUS and J. M. GREEFF 2008. One fig to bind them all: Host conservatism in a fig wasp community unraveled by cospeciation analyses among pollinating and nonpollinating fig wasps. *Evolution,* 62**,** 1777-1797.

KANZAKI, N., R. M. GIBLIN-DAVIS, K. DAVIES, W. M. YE, B. J. CENTER and W. K. THOMAS 2009. *Teratodiplogaster fignewmani* gen. nov., sp nov (Nematoda: Diplogastridae) from the Syconia of *Ficus racemosa* in Australia. *Zoological Science,* 26**,** 569-578.

KANZAKI, N., R. M. GIBLIN-DAVIS, K. A. DAVIES and B. J. CENTER 2012a. *Teratodiplogaster martini* n. sp. and *Parasitodiplogaster doliostoma* n. sp. (Nematoda: Diplogastridae) from the syconia of *Ficus* species from Africa. *Nematology,* 14**,** 529-546.

KANZAKI, N., R. M. GIBLIN-DAVIS, W. YE, E. A. HERRE and B. J. CENTER 2013. Description of *Parasitodiplogaster pharmaconema* n. sp and redescription of *P-maxinema* from *Ficus maxima* Mill. (Moraceae). *Nematology,* 15**,** 957-974.

KANZAKI, N., R. TANAKA, R. M. GIBLIN-DAVIS, E. J. RAGSDALE, C. N. NGUYEN, H. F. LI and Y. C. LAN 2012b. A preliminary survey of fig-associated nematodes in the Asian subtropics. *Journal of Nematology,* 44**,** 470-470.

KERDELHUE, C. and J. Y. RASPLUS 1996. Non-pollinating Afrotropical fig wasps affect the fig-pollinator mutualism in Ficus within the subgenus Sycomorus. *Oikos,* 75**,** 3-14.

KERDELHUE, C., J. P. ROSSI and J. Y. RASPLUS 2000. Comparative community ecology studies on old world figs and fig wasps. *Ecology,* 81**,** 2832-2849.

KIONTKE, K., M. HIRONAKA and W. SUDHAUS 2002. Description of *Caenorhabditis japonica* n. sp (Nematoda : Rhabditida) associated with the burrower bug Parastrachia japonensis (Heteroptera : Cydnidae) in Japan. *Nematology,* 4**,** 933-941.

KIONTKE, K. and W. SUDHAUS 2006. Ecology of *Caenorhabditi*s species. *In: WORMBOOK* (ed.) *The C. elegans Research Community. Wormbook,* doi10.1895/wormbook.1.37.1,http://www.wormbook.org

KJELLBERG, F., J. L. BRONSTEIN, G. VAN GINKEL, J. M. GREEFF, J. C. MOORE, N. BOSSU-DUPRIEZ, M. CHEVOLOT and G. MICHALOUD 2005. Clutch size: a major sex ratio determinant in fig pollinating wasps? *Comptes Rendus Biologies,* 328**,** 471-476.

KJELLBERG, F., B. DOUMESCHE and J. L. BRONSTEIN 1988. Longevity of a fig wasp (*Blastophaga psenes*). *Proceedings of the Koninklijke Nederlandse Akademie Van Wetenschappen Series C-Biological and Medical Sciences,* 91**,** 117-122.

KJELLBERG, F., E. JOUSSELIN, J. L. BRONSTEIN, A. PATEL, J. YOKOYAMA and J. Y. RASPLUS 2001. Pollination mode in fig wasps: the predictive power of correlated traits. *Proceedings of the Royal Society of London Series B-Biological Sciences,* 268**,** 1113-1121.

KJELLBERG, F. and S. MAURICE 1989. Seasonality in the reproductive phenology of *Ficus* - its evolution and consequences. *Experientia,* 45**,** 653-660.

KRISHNAN, A., S. MURALIDHARAN, L. SHARMA and R. M. BORGES 2010. A hitchhiker's guide to a crowded syconium: how do fig nematodes find the right ride? *Functional Ecology,* 24**,** 741-749.

KRUITBOS, L. M., S. HERITAGE and M. J. WILSON 2009. Phoretic dispersal of entomopathogenic nematodes by Hylobius abietis. *Nematology,* 11**,** 419-427.

KUARAKSA, C. and S. ELLIOTT 2013. The Use of Asian Ficus Species for Restoring Tropical Forest Ecosystems. *Restoration Ecology,* 21**,** 86-95.

KUARAKSA, C., S. ELLIOTT and M. HOSSAERT-MCKEY 2012. The phenology of dioecious *Ficus* spp. tree species and its importance for forest restoration projects. *Forest Ecology and Management,* 265**,** 82-93.

LACHAISE, D. 1977. Niche separation of African *Lissocephala* within *Ficus* drosophilid community. *Oecologia,* 31**,** 201-214.

LAMBERT, K. and S. BEKAL 2002. Introduction to Plant-Parasitic Nematodes. *The Plant Health Instructor. DOI: 10.1094/PHI-I-2002-1218-01*

LEIGH, E. G. 2010. The evolution of mutualism. *Journal of Evolutionary Biology,* 23**,** 2507-2528.

LINDQUIST, E. E. 1986. The world genera of Tarsonemidae (Acari, Heterostigmata) -a morphological, phylogenetic, and systematic revision, with a reclassification of family-group taxa in the Heterostigmata. *Memoirs of the Entomological Society of Canada***,** 1-517.

LIU, C., D.-R. YANG, S. G. COMPTON and Y.-Q. PENG 2013. Larger Fig Wasps Are More Careful About Which Figs to Enter - With Good Reason. *Plos One,* 8.

LLOYD, J. and K. A. DAVIES 1997. Two new species of Schistonchus (Tylenchida: Aphelenchoididae) associated with Ficus macrophylla from Australia. *Fundamental and Applied Nematology,* 20**,** 79-86.

LOPEZ-OROZCO, N. and W. A. CANON-FRANCO 2013. Phoretic mites identified on andean hummingbirds (Trochilidae) of Caldas, Colombia. *Revista Brasileira De Parasitologia Veterinaria,* 22**,** 194-200.

LOPEZ, L. C. S., B. FILIZOLA, I. DEISS and R. I. RIOS 2005. Phoretic behaviour of bromeliad annelids (Dero) and ostracods (Elpidium) using frogs and lizards as dispersal vectors. *Hydrobiologia,* 549**,** 15-22.

LUNAU, K. 2004. Adaptive radiation and coevolution - pollination biology case studies. *Organisms Diversity & Evolution,* 4**,** 207-224.

MACHADO, C. A., E. JOUSSELIN, F. KJELLBERG, S. G. COMPTON and E. A. HERRE 2001. Phylogenetic relationships, historical biogeography and character evolution of fig-pollinating wasps. *Proceedings of the Royal Society B-Biological Sciences,* 268**,** 685-694.

MARTIN, G. C., A. M. OWEN and J. I. WAY 1973. Nematodes, figs and wasps. *Journal of Nematology,* 5**,** 77-78.

MARUSSICH, W. A. and C. A. MACHADO 2007. Host-specificity and coevolution among pollinating and nonpollinating New World fig wasps. *Molecular Ecology,* 16**,** 1925-1946.

MCKEY, D. 1989. Population Biology of figs-application for conservation. *Experientia,* 45**,** 661-673.

MURTI, K., V. LAMBOLE and M. PANCHAL 2011. Effect of *Ficus hispid*a L. on normal and dexamethasone suppressed wound healing. *Brazilian Journal of Pharmaceutical Sciences,* 47**,** 855-860.

NASON, J. D., E. A. HERRE and J. L. HAMRICK 1998. The breeding structure of a tropical keystone plant resource. *Nature,* 391**,** 685-687.

NEFDT, R. J. C. and S. G. COMPTON 1996. Regulation of seed and pollinator production in the fig fig wasp mutualism. *Journal of Animal Ecology,* 65**,** 170-182.

OKUMURA, E., Y. ISHIKAWA, R. TANAKA and T. YOSHIGA 2013. Propagation of *Caenorhabditis japonica* in the Nest of Its Carrier Insect, *Parastrachia japonensis*. *Zoological Science,* 30**,** 174-177.

ORONA-TAMAYO, D. and M. HEIL 2013. Stabilizing Mutualisms Threatened by Exploiters: New Insights from Ant-Plant Research. *Biotropica,* 45**,** 654-665.

PAARAKH, P. M. 2009. *Ficus racemosa* Linn. - An overview. *Natural Product Radiance,* 8**,** 84-90.

PATEL, A. 1996. Variation in a mutualism: Phenology and the maintenance of gynodioecy in two Indian fig species. *Journal of Ecology,* 84**,** 667-680.

PATEL, A. and M. HOSSAERT-MCKEY 2000. Components of reproductive success tn two dioecious fig species, *Ficus exasperata* and *Ficus hispida*. *Ecology,* 81**,** 2850-2866.

PATEL, A. and D. MCKEY 1998. Sexual specialization in two tropical dioecious figs. *Oecologia,* 115**,** 391-400.

PENG, Y.-Q., S. G. COMPTON and D.-R. YANG 2010. The reproductive success of *Ficus altissima* and its pollinator in a strongly seasonal environment: Xishuangbanna, Southwestern China. *Plant Ecology,* 209**,** 227-236.

PEREIRA, R. A., S. D. TEIXEIRA and F. KJELLBERG 2007. An inquiline fig wasp using seeds as a resource for small male production: a potential first step for the evolution of new feeding habits? *Biological Journal of the Linnean Society,* 92**,** 9-17.

PEREIRA, R. A. S. and A. P. DO PRADO 2005. Non-pollinating wasps distort the sex ratio of pollinating fig wasps. *Oikos,* 110**,** 613-619.

PEREIRA, R. A. S., J. SEMIR and A. D. O. MENEZES JUNIOR 2000. Pollination and other biotic interactions in figs of *Ficus eximia* Schott (Moraceae). *Revista Brasileira de Botanica,* 23**,** 217-224.

POINAR, G. O. 1979. *Parasitodiplogater sycophilon* gen. n. sp. n (Diplogasteridae, Nematoda), a parasite of *Elisabethiella stuckenbergi* Grandi(Agaonidae, Hymenoptera) in Rhodesia. *Proceedings of the Koninklijke Nederlandse Akademie Van Wetenschappen Series C-Biological and Medical Sciences,* 82**,** 375-381.

POINAR, G. O. J. and E. A. HERRE 1991. Speciation and adaptive radiation in the fig wasp nematode *Parasitodiplogaster* Diplogasteridae Rhabditida in Panama. *Revue de Nematologie,* 14**,** 361-374.

PROFFIT, M., B. SCHATZ, R. M. BORGES and M. HOSSAERT-MCKEY 2007. Chemical mediation and niche partitioning in non-pollinating fig-wasp communities. *Journal of Animal Ecology,* 76**,** 296-303.

RANGANATHAN, Y. and R. M. BORGES 2009. Predatory and trophobiont-tending ants respond differently to fig and fig wasp volatiles. *Animal Behaviour,* 77**,** 1539-1545.

RANGANATHAN, Y., M. GHARA and R. M. BORGES 2010. Temporal associations in fig-wasp-ant interactions: diel and phenological patterns. *Entomologia Experimentalis Et Applicata,* 137**,** 50-61.

RASMANN, S. and T. C. J. TURLINGS 2008. First insights into specificity of belowground tritrophic interactions. *Oikos,* 117**,** 362-369.

REDDY, Y. N. and P. N. RAO 1986. *Schistonchus racemosa* new species, a nematode parasite of wasp *Ceratolen* sp associated with the fig *Ficus racemosa*. *Indian Journal of Nematology,* 16**,** 135-137.

RONSTED, N., G. D. WEIBLEN, J. M. COOK, N. SALAMIN, C. A. MACHADO and V. SAVOLAINEN 2005. 60 million years of co-divergence in the fig-wasp symbiosis. *Proceedings of the Royal Society B-Biological Sciences,* 272**,** 2593-2599.

SACHS, J. L. and E. L. SIMMS 2006. Pathways to mutualism breakdown. *Trends in Ecology & Evolution,* 21**,** 585-592.

SANITJAN, S. and J. CHEN 2009. Habitat and fig characteristics influence the bird assemblage and network properties of fig trees from Xishuangbanna, South-West China. *Journal of Tropical Ecology,* 25**,** 161-170.

SCHATZ, B. and M. HOSSAERT-MCKEY 2003. Interactions of the ant *Crematogaster scutellaris* with the fig/fig wasp mutualism. *Ecological Entomology,* 28**,** 359-368.

SCHATZ, B., F. KIELLBERG, S. NYAWA and M. HOSSAERT-MCKEY 2008. Fig wasps: A staple food for ants on *Ficus*. *Biotropica,* 40**,** 190-195.

SEEMAN, O. D. and D. E. WALTER 1995. Life-history of *Afrocypholaelaps africana* (Evans) (Acari, Ameroseiidae), a mite inhabiting mangrove flowers and phoretic on honeybees. *Journal of the Australian Entomological Society,* 34**,** 45-50.

SEGAR, S. T. and J. M. COOK 2012. The dominant exploiters of the fig/pollinator mutualism vary across continents, but their costs fall consistently on the male reproductive function of figs. *Ecological Entomology,* 37**,** 342-349.

SHANAHAN, M., S. SO, S. G. COMPTON and R. CORLETT 2001. Fig-eating by vertebrate frugivores: a global review. *Biological Reviews,* 76**,** 529-572.

SIRISHA, N., M. SREENIVASULU, S. K. and C. M. CHETTY 2010. Antioxidant properties of Ficus species-a review. *International Journal of PharmTech Research,* 2**,** 2174-2182.

SMITH, C. M. and J. L. BRONSTEIN 1996. Site variation in reproductive synchrony in three neotropical figs. *Journal of Biogeography,* 23**,** 477-486.

SPENCER, H., G. WEIBLEN and B. FLICK 1996. Phenology of Ficus variegata in a seasonal wet tropical forest at Cape Tribulation, Australia. *Journal of Biogeography,* 23**,** 467-475.

SRIWATI, R., S. TAKEMOTO and K. FUTAI 2006. Seasonal changes in the nematode fauna in pine trees killed by the pinewood nematode, *Bursaphelenchus xylophilus*. *Japanese Journal of Nematology,* 36**,** 87-100.

SULEMAN, N., S. RAJA and S. G. COMPTON 2012. Only pollinator fig wasps have males that collaborate to release their females from figs of an Asian fig tree. *Biology Letters,* 8**,** 344-346.

SULEMAN, N., S. RAJA and S. G. COMPTON 2013. Parasitism of a pollinator fig wasp: mortalities are higher in figs with more pollinators, but are not related to local densities of figs. *Ecological Entomology,* 38**,** 478-484.

TARACHAI, Y., S. G. COMPTON and C. TRISONTHI 2008. The benefits of pollination for a fig wasp. *Symbiosis,* 45**,** 29-32.

THOMPSON, J. N. 1994. *The Coevolutionary Process,* Chicago The University of Chicago Press.

THOMPSON, J. N. 2009. The coevolving web of life. *American Naturalist,* 173**,** 125-140.

THOMPSON, J. N., C. SCHWIND, P. R. GUIMARAES, JR. and M. FRIBERG 2013. Diversification through multitrait evolution in a coevolving interaction. *Proceedings* *of the National Academy of Sciences of the United States of America,* 110**,** 11487-11492.

THORNTON, I. W. B., S. G. COMPTON and C. N. WILSON 1996. The role of animals in the colonization of the Krakatau Islands by fig trees (*Ficus* species). *Journal of Biogeography,* 23**,** 577-592.

TSCHAPKA, M. and S. A. CUNNINGHAM 2004. Flower mites of *Calyptrogyne ghiesbreghtiana* (Arecaceae): evidence for dispersal using pollinating bats. *Biotropica,* 36**,** 377-381.

VALDEYRON, G. and D. G. LLOYD 1979. Sex-differences and flowering phenology in the common fig, *Ficus carica* L. *Evolution*, 33**,** 673-685.

VAN NOORT, S. 1994. Systematics of the sycoecine fig wasps (Agaonidae, Chalcidoidea, Hymenoptera). 5. (*Philocaenus*, concluded-generic key-checklist). *Proceedings of the Koninklijke Nederlandse Akademie Van Wetenschappen-Biological Chemical Geological Physical and Medical Sciences*, 97**,** 341-375.

VAN NOORT, S. and S. G. COMPTON 1996. Convergent evolution of agaonine and sycoecine (Agaonidae, Chalcidoidea) head shape in response to the constraints of host fig morphology. *Journal of Biogeography,* 23**,** 415-424.

VAN NOORT, S., A. B. WARE and S. G. COMPTON 1989. Pollinator-specific volatile attractants released from the figs of *Ficus burtt-davyi*. *South African Journal of Science,* 85**,** 323-324.

VERKERKE, W. 1989. Structure and function of the fig. *Experientia,* 45**,** 612-622.

VOVLAS, N., R. N. INSERRA and N. GRECO 1992. *Schistonchus caprifici* parasitizing caprifig (*Ficus carica sylvestris*) florets and the relationship with its fig wasp (*Blastophaga psenes*) vector. *Nematologica*, 38**,** 215-226.

VOVLAS, N. and A. LARIZZA 1996. Relationship of *Schistonchus caprifici* (Aphelenchoididae) with fig inflorescences, the fig pollinator *Blastophaga psenes*, and its cleptoparasite *Philotrypesis caricae*. *Fundamental and Applied Nematology,* 19**,** 443-448.

VOVLAS, N., A. TROCCOLI, S. VAN NOORT and E. VAN DEN BERG 1998. *Schistonchus africanus* n. sp (Aphelenchida : Aphelenchoididae) associated with

Ficus thonningii (Moraceae) and its pollinator wasp *Elisabethiella stuckenbergi* (Chalcidoidea : Agaonidae). *Journal of Nematology,* 30**,** 404-410.

WALTER, D. E. 2000. First record of a fig mite from the Australian Region: *Paratarsonemella giblindavisi* sp n. (Acari : Tarsonemidae). *Australian Journal of Entomology,* 39**,** 229-232.

WANG, B., J. H. XIAO, S. N. BIAN, L. M. NIU, R. W. MURPHY and D. W. HUANG 2013a. Evolution and expression plasticity of opsin genes in a fig pollinator, *Ceratosolen solmsi*. *Plos One,* 8.

WANG, H., J. RIDLEY, D. W. DUNN, R. WANG, J. M. COOK and D. W. YU 2013b. Biased oviposition and biased survival together help resolve a fig-wasp conflict. *Oikos,* 122**,** 533-540.

WANG, R. W., L. SHI, S. M. AI and Q. ZHENG 2008. Trade-off between reciprocal mutualists: local resource availability-oriented interaction in fig/fig wasp mutualism. *Journal of Animal Ecology,* 77**,** 616-623.

WANG, R. W. and B. F. SUN 2009. Seasonal change in the structure of fig-wasp community and its implication for conservation. *Symbiosis,* 47**,** 77-83.

WANG, R. W., C. Y. YANG, G. F. ZHAO and J. X. YANG 2005. Fragmentation effects on diversity of wasp community and its impact on fig/fig wasp interaction in *Ficus racemosa* L. *Journal of Integrative Plant Biology,* 47**,** 20-26.

WANG, R. W., J. X. YANG and D. R. YANG 2005. Seasonal changes in the trade-off among fig-supported wasps and viable seeds in figs and their evolutionary implications. *Journal of Integrative Plant Biology,* 47**,** 144-152.

WANG, R. W. and Q. ZHENG 2008. Structure of a fig wasp community: Temporal segregation of oviposition and larval diets. *Symbiosis,* 45**,** 113-116.

WARE, A. B. and S. G. COMPTON 1992. Breakdown of pollinator specificity in an African fig tree. *Biotropica,* 24**,** 544-549.

WARE, A. B. and S. G. COMPTON 1994. Responses of fig wasps to host-plant volatile cues. *Journal of Chemical Ecology,* 20**,** 785-802.

WARE, A. B. and S. G. COMPTON 1994a. Dispersal of adult female fig wasps.1. Arrival and departures. *Entomologia Experimentalis Et Applicata,* 73**,** 221-229.

WARE, A. B. and S. G. COMPTON 1994b. Dispersal of adult female fig wasps. 2. Movements between trees. *Entomologia Experimentalis Et Applicata,* 73**,** 231-238.

WARE, A. B., P. T. KAYE, S. G. COMPTON and S. VANNOORT 1993. Fig volatiles-their role in attracting pollinators and maintaining pollinator specificity. *Plant Systematics and Evolution,* 186**,** 147-156.

WEIBLEN, G. D. 2002. How to be a fig wasp. *Annual Review of Entomology,* 47**,** 299-330.

WEIBLEN, G. D. 2004. Correlated evolution in fig pollination. *Systematic Biology,* 53**,** 128-139.

WEIBLEN, G. D. and G. L. BUSH 2002. Speciation in fig pollinators and parasites. *Molecular Ecology,* 11**,** 1573-1578.

WEIBLEN, G. D., D. W. YU and S. A. WEST 2001. Pollination and parasitism in functionally dioecious figs. *Proceedings of the Royal Society B-Biological Sciences,* 268**,** 651-659.

WEST, S. A. and E. A. HERRE 1994. The ecology of the new-world fig-parasitizing wasps *Idarnes* and impliactions for the evolution of the fig-pollinator mutualism. *Proceedings of the Royal Society of London Series B-Biological Sciences,* 258**,** 67-72.

WEST, S. A., E. A. HERRE, D. M. WINDSOR and P. R. S. GREEN 1996. The ecology and evolution of the New World non-pollinating fig wasp communities. *Journal of Biogeography,* 23**,** 447-458.

WIEBES, J. T. 1979. Co-evolution of figs and their insect pollinators. *Annual Review of Ecology and Systematics,* 10**,** 1-12.

WIEBES, J. T. 1981. The phylogeny of the Agaonidae (Hymenoptera,Chalcidoidea). *Netherlands Journal of Zoology,* 32**,** 395-411.

WU, T., D. W. DUNN, H. Y. HU, L. M. NIU, J. H. XIAO, X. L. PAN, G. FENG, Y. G. FU and D. W. HUANG 2013. The occurrence of fig wasps in the fruits of female gynodioecious fig trees. *Acta Oecologica-International Journal of Ecology,* 46**,** 33-38.

YANG, D.-R., R.-W. WANG, Q.-S. SONG, G.-M. ZHANG and T.-Z. ZHAO 2000. Rule of seasonal changes of the *Ceratosolen* sp. in the tropical rainforest of Xishuangbanna, China. *Forest Research,* 13**,** 477-484.

YANG, D. R., Y. Q. PENG, Q. S. SONG, G. M. ZHANG, R. W. WANG, T. Z. ZHAO and Q. Y. WANG 2002. Pollination biology of *Ficus hispida* in the tropical rainforests of Xishuangbanna, China. *Acta Botanica Sinica,* 44**,** 519-526.

YEO, C. K. and H. T. W. TAN 2009. Variation in reproductive output of *Ficus superba* despite aseasonal reproduction. *Plant Ecology,* 205**,** 235-248.

YU, D. W., J. RIDLEY, E. JOUSSELIN, E. A. HERRE, S. G. COMPTON, J. M. COOK, J. C. MOORE and G. D. WEIBLEN 2004. Oviposition strategies, host coercion and the stable exploitation of figs by wasps. *Proceedings of the Royal Society B-Biological Sciences,* 271**,** 1185-1195.

YU, H. and S. G. COMPTON 2012. Moving your sons to safety: galls Containing male fig wasps expand into the centre of figs, away from enemies. *Plos One,* 7.

ZACHARIADES, C., B. SCHATZ and S. G. COMPTON 2010. Wasp emergence from the figs of *Ficus sur*: characteristics and predation by ants. *Tropical Zoology,* 23**,** 121-138.

ZENG, Y., W. YE, R. M. GIBLIN-DAVIS, C. LI, Z. DU and C. ZHAO 2010. *Schistonchus hirtus* n. sp (Nematoda: Aphelenchoididae), an associate of *Ficus hirta* in China. *Nematology,* 12**,** 543-556.

ZENG, Y., W. YE, R. M. GIBLIN-DAVIS, C. LI, S. ZHANG and Z. DU 2011a. Description of *Schistonchus microcarpus* n. sp (Nematoda: Aphelenchoididae), an associate of *Ficus microcarpa* in China. *Nematology,* 13**,** 221-233.

ZENG, Y. S., R. M. GIBLIN-DAVIS and W. M. YE 2007. Two new species of *Schistonchus* (Nematoda : Aphelenchoididae) associated with *Ficus hispida* in China. *Nematology,* 9**,** 169-187.

ZENG, Y. S., W. M. YE, R. M. GIBLIN-DAVIS, C. H. LI, S. N. ZHANG and Z. J. DU 2011b. Description of Schistonchus microcarpus n. sp (Nematoda:

Aphelenchoididae), an associate of Ficus microcarpa in China. *Nematology*, 13**,** 221-233.

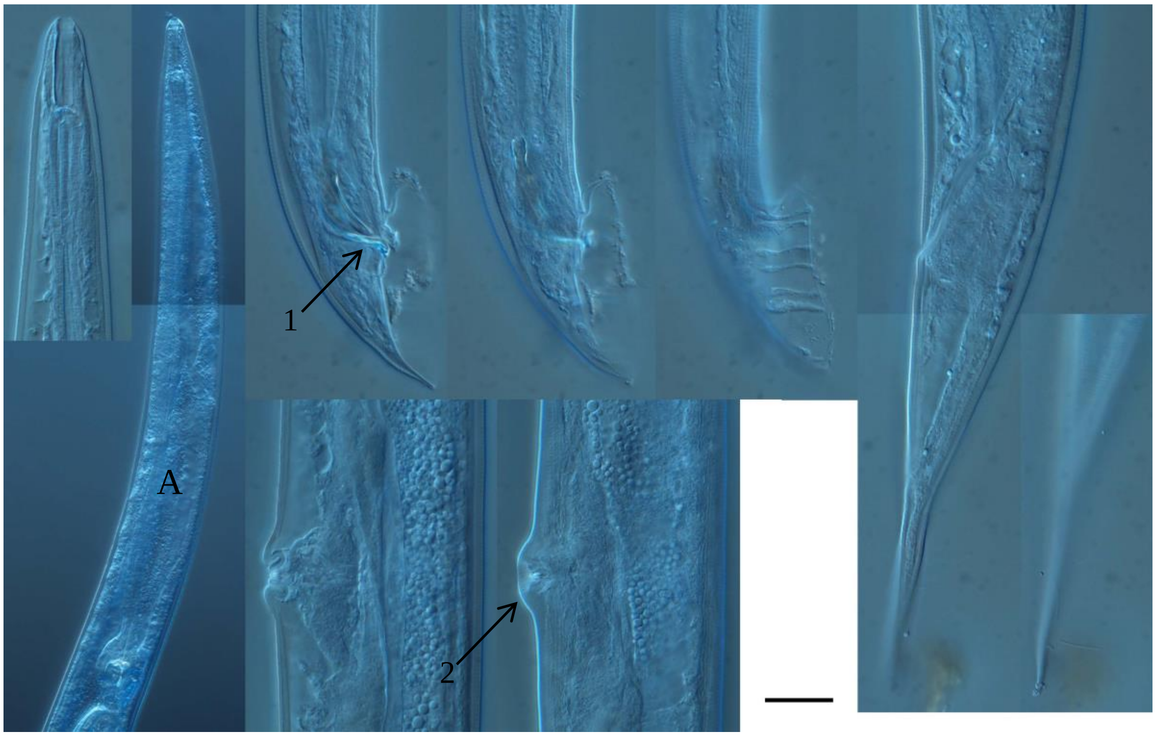
ZHANG, G. M., Q. S. SONG and D. R. YANG 2006. Phenology of *Ficus racemosa* in Xishuangbanna, Southwest China. *Biotropica,* 38**,** 334-341.

ZHANG, Y., D. R. YANG, Y. Q. PENG and S. G. COMPTON 2012. Costs of inflorescence longevity for an Asian fig tree and its pollinator. *Evolutionary Ecology,* 26**,** 513-527.

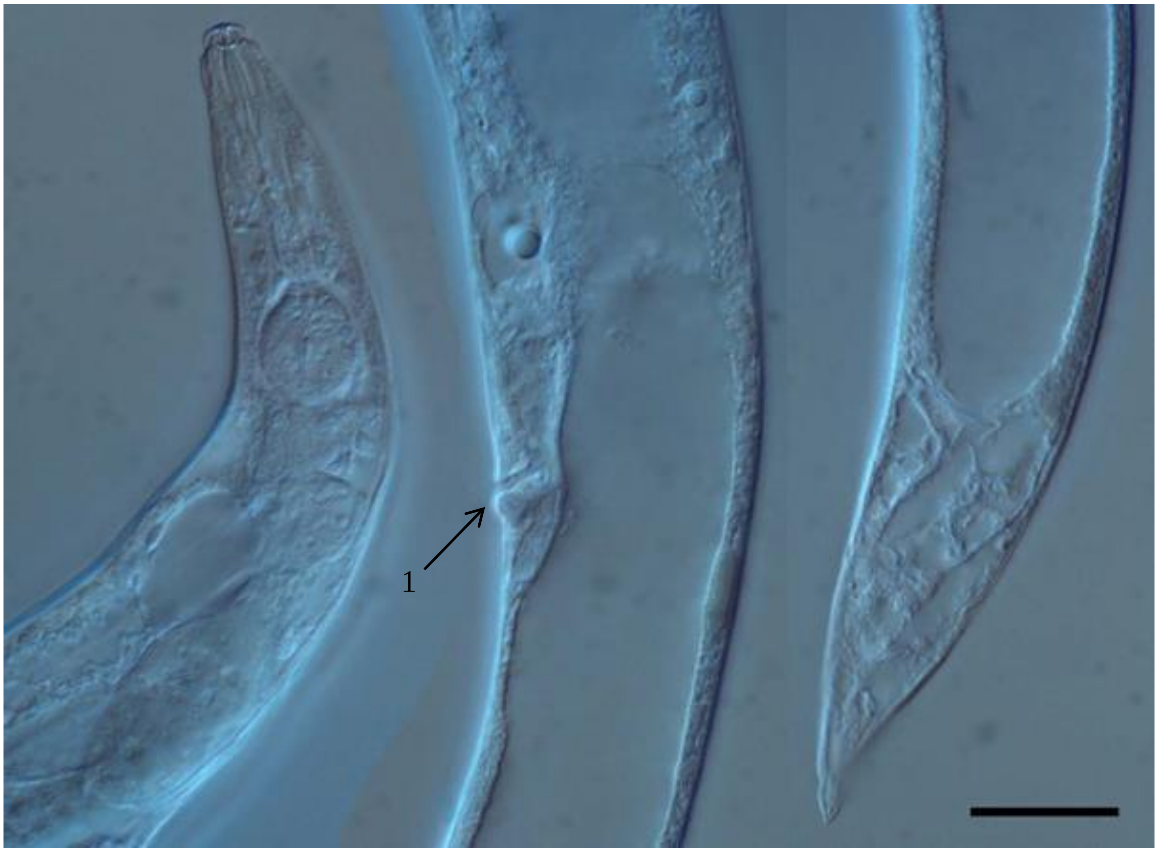
Appendices

Nematode pictures

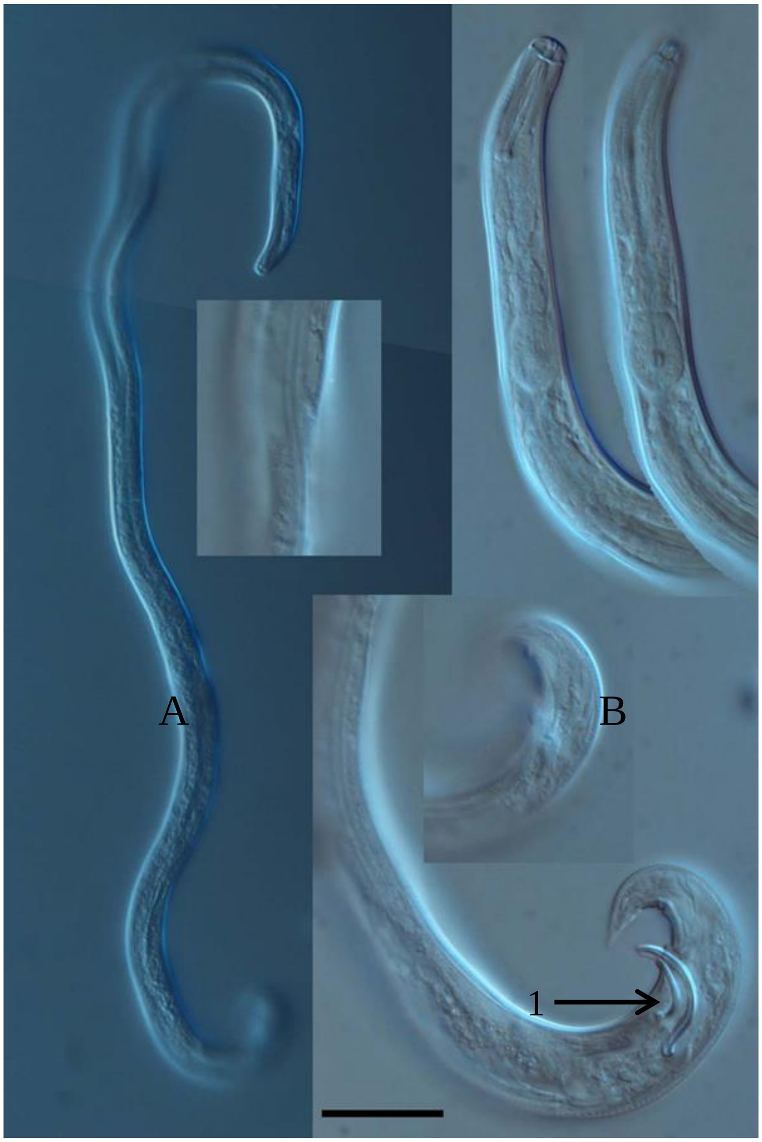
All nematode pictures were provided by Dr. Natsumi Kanzaki (taken in Forestry and Forest Products Research Institute, Tsukuba, Japan). All pictures are of adult nematodes.



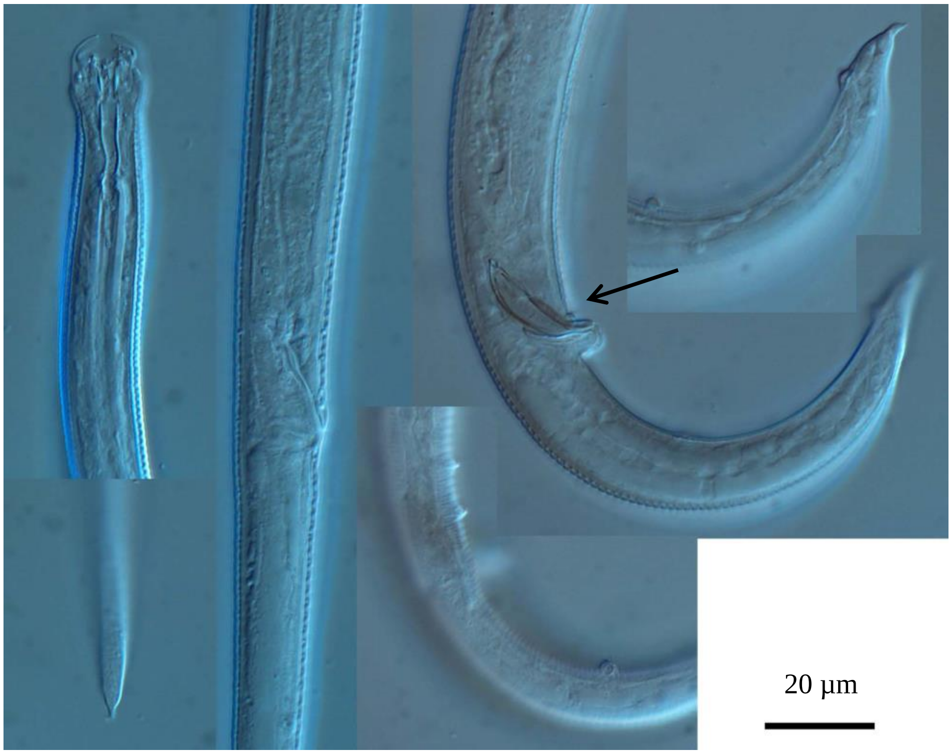
Appendix 1. *Caenorhabditis* adult males (upper) showing the spicule (1) close to the tail and females (lower) showing the vulva (2) located around the middle part of the nematode. Bar represents 50 µm for image A, and 20 µm for the rest of the images.



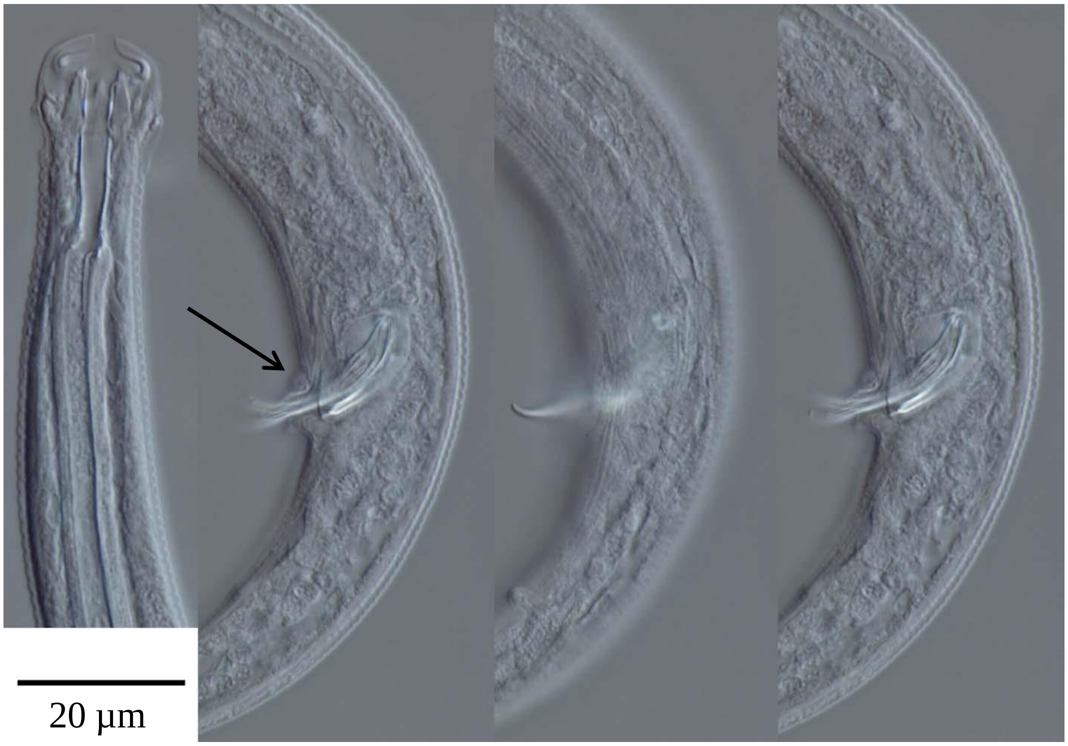
Appendix 2. Female adults of *Schistonchus centerae*. 1 = vulva. Bar represents 20 µm.



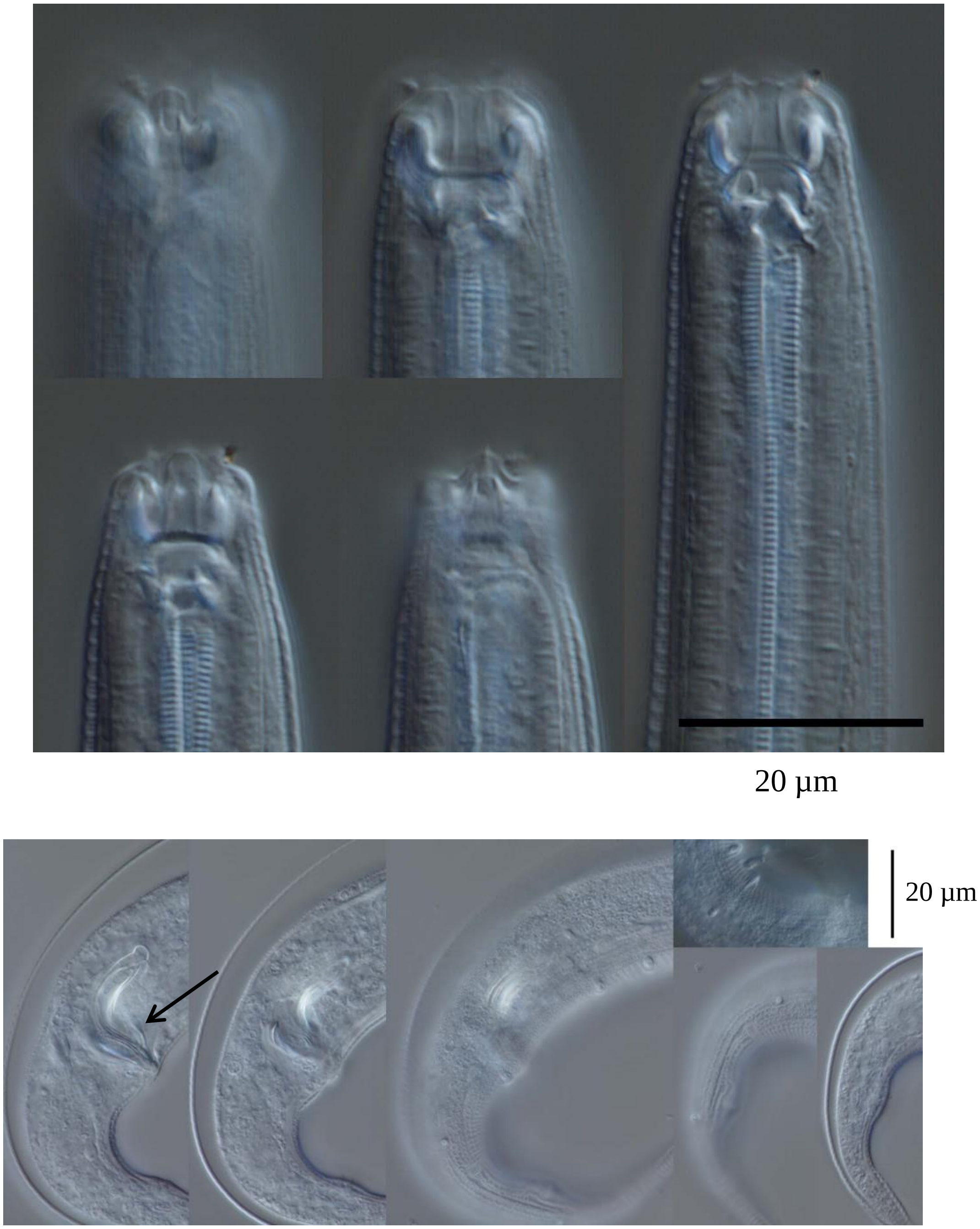
Appendix 3. Adult males of *Schistonchus guangzhouensis*.. The bar represents 50 µm for section A (darker background), and 20 µm for section B.



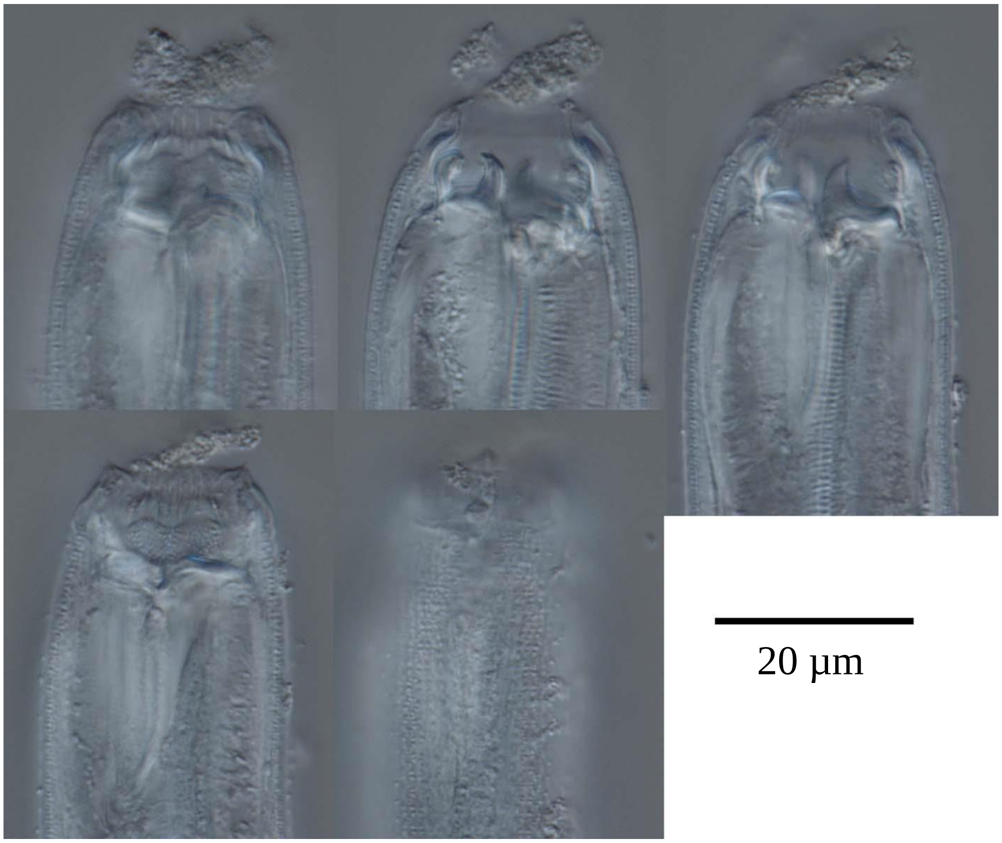
Appendix 4. *Teratodiplogaster fignewmani* (male). Arrow sign is showing the spicule (male genitalia) of the nematode.



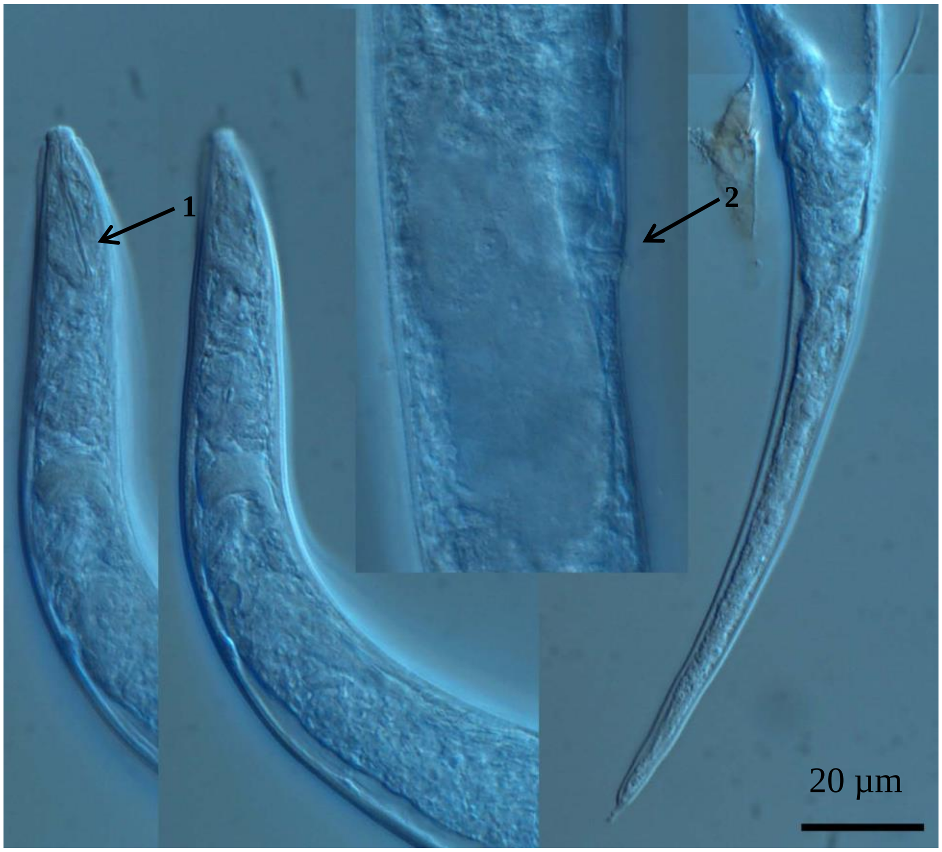
Appendix 5. *Teratodiplogaster* sp. (male). Arrow sign is showing the spicule (male genitalia) of the nematode.



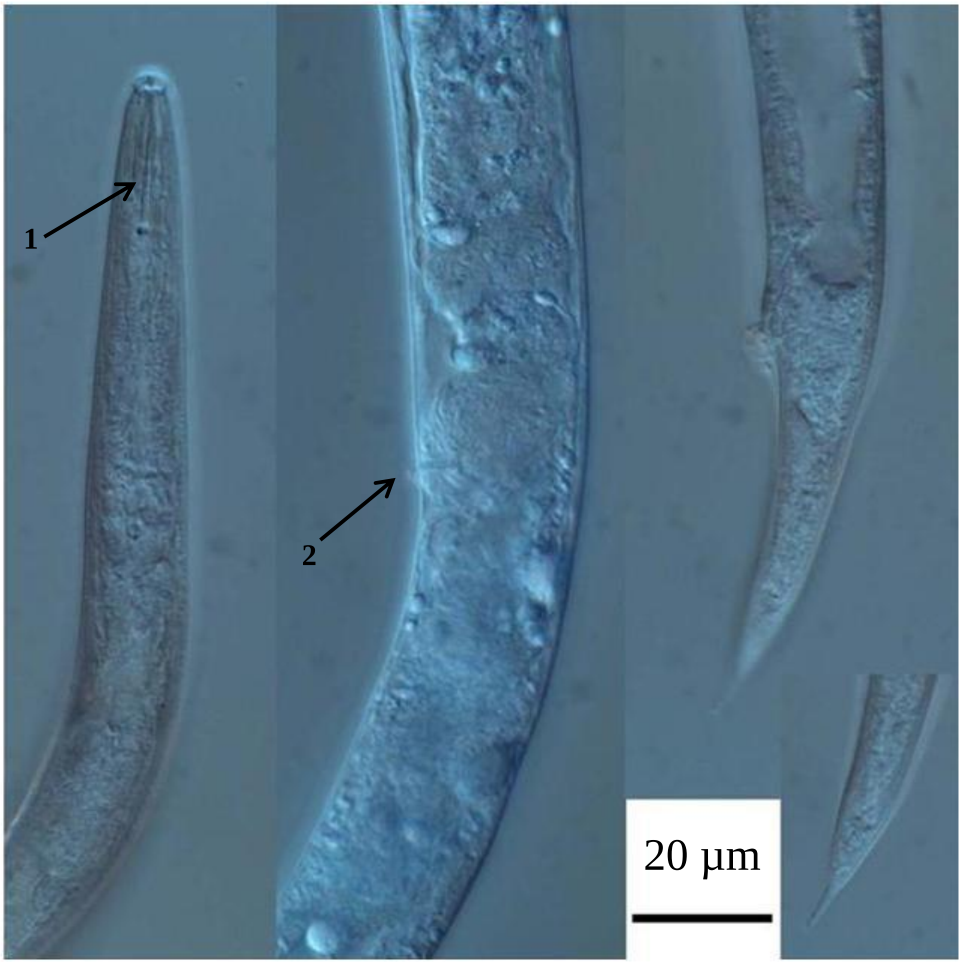
Appendix 6. *Parasitodiplogaster* sp. male showing stomatal structure (top), and spicule (arrow sign, bottom).



Appendix 7. *Mononchoides* sp. male showing stomatal structure.

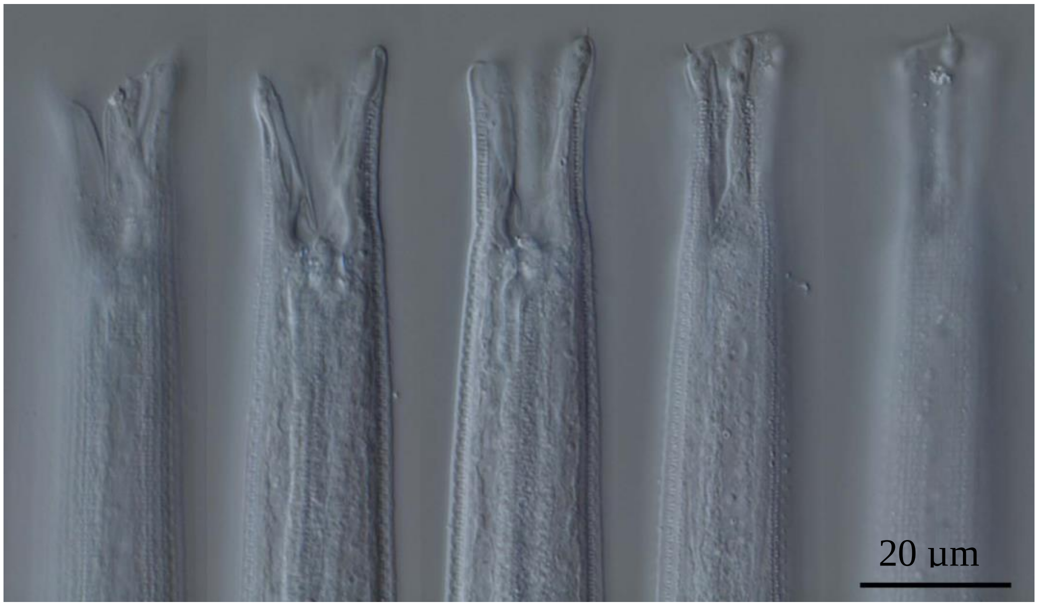


Appendix 8. *Schistonchus* sp1. female showing stylet (1) and vulva (female genitalia)(2).



Appendix 9. *Schistonchus* sp2. female showing stylet (1) and vulva (female genitalia)(2).

Appendix 10. Umbrella-like sp. 1 female showing stomatal structure.



Appendix 11. Umbrella-like sp. 2 female showing stomatal structure.