Fig Pollinating Wasp Transfers Nematodes into Figs of *Ficus racemosa* in Sumatra, Indonesia

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Abstract. The fruits (figs) of fig trees (*Ficus* spp, known as 'bak ara' in Aceh), are the source of food for many species of faunas in the forest, including birds, monkeys, orangutans, etc. Pollination within the figs totally depends on female fig wasps that belong to family Agaonidae. Fig trees and their pollinating wasps rely on each other to survive. Female fig wasps are known to transport nematodes into receptive figs when the wasps enter the figs to lay eggs. An investigation on the nematodes carried by female pollinating wasps *Ceratosolen fusciceps* Mayr into figs of *Ficus racemosa* was conducted in Sumatra, Indonesia. The figs on the trees were regularly sampled to determine the presence of nematodes and infer their ecology. The Baermann funnel method was employed to extract the nematodes from the figs. 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. The highest nematode populations were routinely found in D-phase figs, when the new generations of wasps were about to emerge. 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.
Key words: Agaonidae, mutualism, non-mutualism, *Diplogastridae, Apelenchoidae*

Introduction

Fig trees are often considered as a 'keystone' group in tropical forests. Their often all-year-round production of fig fruits potentially maintain frugivore populations, particularly, at times of the year when other fruits are not easily available (Shanahan et al., 2001; Harrison, 2005). *Ficus racemosa* L., known as the cluster fig, is a monoecious fig tree that is distributed from India to Australia and grows commonly in Southeast Asia . *Ficus racemosa* is a large-sized tree species, reaching 25-40 m in height and 60-120 cm in trunk diameter (at breast height). It grows in evergreen forests, moist localities and the banks of streams in deciduous forests up to an elevation of 1800 m. The tree usually grows in groups of five to ten. The tree is often cultivated for shade and its edible fruits (Zhang et al., 2006).

The tree has unisexual male and female flowers inside its figs that mature asynchronously, which prevents self-pollination. 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. The developmental stages of figs are starting from the A-phase (pre-receptive), then B (receptive, when the wasps enter), follwed by the subsequent C-phase in which the wasps and seeds develop. The mated female wasps leave the figs, loaded with pollen in the D-phase or 'emission' phase. Upon the wasps' departure, the figs then ripen in E- phase. At this phase, the figs become soft and ready to be eaten by vertebrates or eventually abort (Galil and Eisikowich, 1968; Shanahan *et a*l., 2001)

Pollination within the figs of *F. racemosa* depends on its pollinating wasp *Ceratosolen fusciceps* Mayr. The wasps depends on the fig to lay eggs and complete their life cycle. The number of pollinating wasps that enter a receptive fig of *F. racemosa* is between five and 30, but sometimes it can reach more than 70 (Wang and Sun, 2009).

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 (Kanzaki *et al*., 2012; Davies *et al*., 2013; Davies *et al*., 2015) . High numbers of nematodes, up to 50,000, has been recorded from one fig. The nematodes associate with the unique habitat within the figs where they develop and feed. 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).

This study was conducted to investigate the nematode species associated with figs of *F. racemosa* and their relationship with the fig tree and its pollinating wasp in Sumatra, Indonesia.

Materials and Methods

Routine sampling. The study sites of the fig trees were located in the northern part of Sumatra Island in Aceh Province, Indonesia. The study was undertaken for 11 months in 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. 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 (Data obtained from Blang Bintang Station, the closest Meteorological Station under the Indonesian Meteorological and Geophysical Agency).

Each sampled tree was marked with black paint at the base and its location was recorded using a GPS. All fig samples were analysed in the Plant Disease Laboratory, Agrotechnology Department, Faculty of Agriculture, Syiah Kuala University, Banda Aceh, Indonesia. There were 20 *F. racemosa* trees that were marked, some of which were used for weekly routine sampling, and others were used for additional data collection. For routine sampling, ten figs were sampled haphazardly from each crop, from A-phase through to E-phase. For each fig, the developmental stage and the colour, were recorded. On opening of the fig, the number of foundresses inside the figs was counted (B and C phases) and they were then placed on glass slides in 40% sugar water. Any nematodes that emerged from the foundresess were observed and counted.

Fig extraction. The contents of each fig were extracted individually. The figs (including liquid if any) were placed in extraction funnels. The Baermann funnel method used to extract the nematodes from the figs was adapted from Sriwati *et al*. (2006). Numbers of nematodes per fig were calculated based on the observation on the fig extracts.

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 et al., 2007).

Impact of nematodes on pollinating wasps and seeds. Six to Seven early D-phase figs were brought to the laboratory to investigate 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. 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 or seeds were recorded for each fig. These data were collected from five crops came from five different trees.

Data analysis. Fig wasps and the nematodes biology were described based on the observations on the fig samples. Impact of nematodes presence on the fig wasps and seeds were analysed using R programme (Ihaka and Gentleman, 1996) version 2.14.12. Number of wasps and number of seeds were treated as continuous data because their means were high. They were analysed using a linear mixed effect models using *lme* package. Assumptions were checked by looking at the normality of residuals. Linear mixed effect models were applied, because the crops were nested within the trees, generating both fixed and random factors. 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).

Results and Discussion

The communities of nematodes recorded in *F. racemosa* figs consisted eight species, all transferred by the pollinating wasp *Ceratosolen fusciceps* into figs of *F. racemosa* (Table 1). 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). 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 1. Identification of Nematode species transferred 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

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 (Davies et al., 2013). *Mononchoides* species have never been described before from *F. racemosa* figs, even though its presence has been noticed elsewhere recently. 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.

The nematodes were transferred into figs as juveniles, except for the *Schistonchus* spp., which were occasionally transferred as adults. Nematodes found on B-phase figs were presumed to be the ones being transferred into the figs along with the foundresses. 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) across all D-phase figs extracted from the four crops (Fig. 1.). They mostly consisted of nematode larvae from new generations. 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.

At early D phase, 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). 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. Most females of non-pollinating wasps do not enter another fig after they emerged from their natal fig. They will lay eggs from the outer surface of figs 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.

The high numbers of nematodes inside D-phase figs increased the chances that nematodes would be picked up by each female pollinator. 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.

In onoecious 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 on early D-phase figs showed that the number of nematodes did not significantly affect the number of galls containing female pollinator offspring (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) (Table 2).

These results showed that there was no evidence to believe the presence of nematodes within the figs caused any harm to the female pollinators, 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. 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.

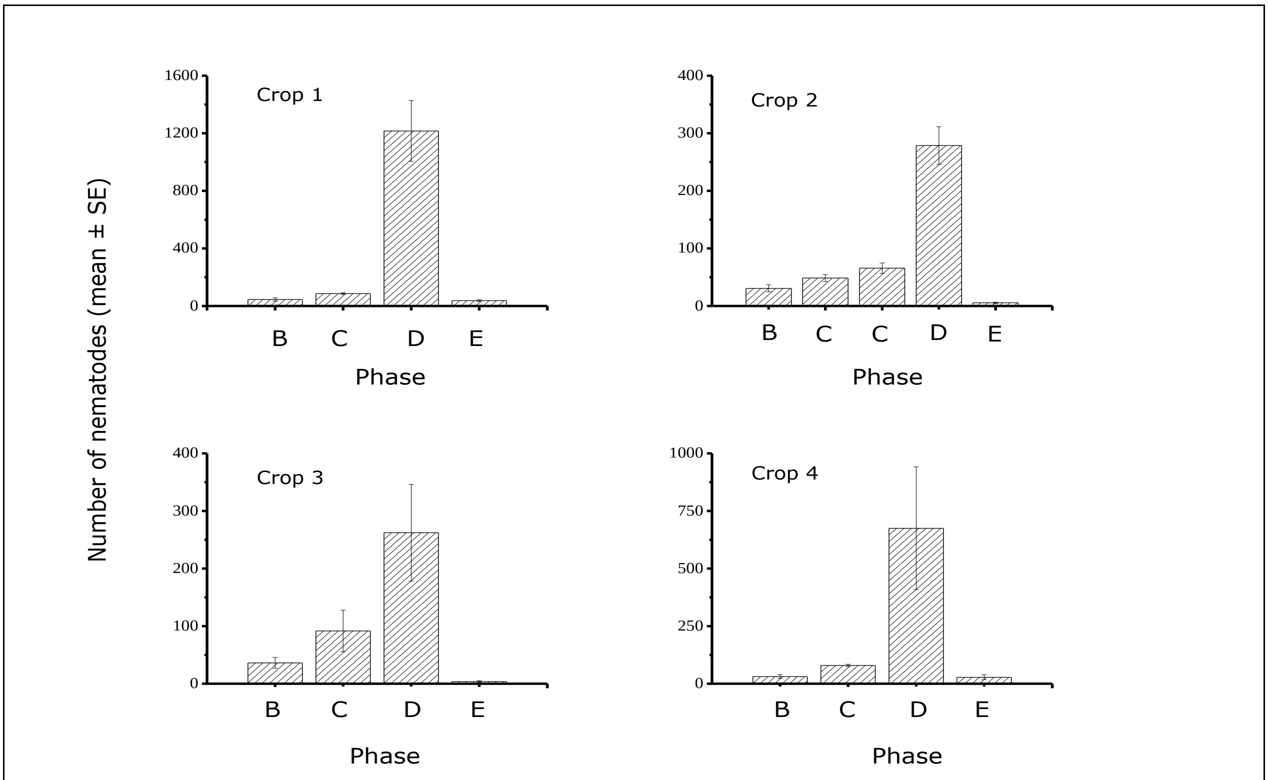

Figure 1. Number of nematode per fig during the development of *F. racemosa* figs. Numbers were obtained from extraction of whole figs (note the varying scale of the Y-axis).

Conclusions

There were eight species of nematodes recorded, all transferred by the pollinating wasp *Ceratosolen fusciceps* into figs of *F. racemosa.* Despite the high comminities nematodes found, their development within a fig has no clear negative impact on the female pollinating wasps and on the seeds.

Table 2. The effect of nematodes number on seeds and galls containing adult female pollinators in early D-phase figs of *F. racemosa* (N = Number of figs sampled).

Crop	**N**	**Numbers per fig (Mean ± SE)**		
		Nematodes	**Seeds**	**Female pollinators**
Crop 5.	6	313.2 ± 52.1	323.5 ± 29.0	438.5 ± 46.2
Crop 6.	6	308.8 ± 64.7	357.2 ± 60.9	560.7 ± 39.6
Crop 7.	7	966.0 ± 312.6	400.7 ± 50.2	561.7 ± 44.7
Crop 8.	6	429.8 ± 105.7	406.7 ± 167.4	483.3 ± 191.9
Crop 9.	6	2644.7 ± 847.8	287.7 ± 9.2	446.8 ± 43.7

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