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POLLINATING FIG WASP AND SEED PRODUCTIONS OF *Ficus deltoidea* var. *angustifolia* AT SELECTED OIL PALM PLANTATIONS IN PENINSULAR MALAYSIA

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ABSTRACT

The relationship between *Ficus deltoidea* (family Moraceae) and pollinator fig wasp (family Agaonidae) are extremely specific in which each partner depends on the other for their reproductive success. There are about seven described varieties of *Ficus deltoidea* can be found in peninsular Malaysia. *Ficus deltoidea* is a dioecious species that are primarily epiphytes. They are unharmful for their host tree where one of the host tree is oil palm tree. In this study, epiphytic *Ficus deltoidea* var. *angustifolia* from oil palm plantations in Banting, Dengkil, Changkat Lobak and Batu Pahat, Malayaia were studied to determine the flower variation and reproductive output (fig wasp and seeds) by the figs of different individual plants and locations. A total of ten matured figs from each male and female tree from all locations were collected and later dissected to

count the number of flowers, galls, female and male fig wasp for male tree while the number of seed and female flowers were counted from female tree. Data were analysed by using one-way ANOVA. Overall results showed that different individual plants were significantly varied in their floral numbers and reproductive output (pollinators and seeds) (P < 0.05) for both and male figs except for seed productions at Changkat Lobak (P = 0.067). Whilst there were significant different (P < 0.01) in the production of pollinators and seeds by the figs among locations. The reproductive output (pollinators and seeds) were greatly influenced by number of flowers and number of fig wasp visitations per fig.

Keywords: Ficus deltoidea, fig wasp, fig, epiphytes

ABSTRAK

Hubungan antara pokok Mas Cotek (family Moraceae) dan penyengat ara (family Agaonidae) adalah sangat specific dimana mereka bergantung antara satu sama lain bagi kejayaan reproduktif masing-masing. Terdapat tujuh varieti pokok Mas Cotek di Semenanjung Malaysia yang telah dikenal pasti. Pokok Mas Cotek adalah spesies epifit diesius yang tidak merosakkan pokok hos nya, salah satu pokok hos pokok Mas Cotek adalah pokok kelapa sawit. Dalam kajian ini, pokok Mas cotek epifit (Ficus deltoidea var. angustifolia) daripada ladang kelapa sawit di Banting, Dengkil, Changkat Lobak dan Batu Pahat telah dikaji bagi menentukan variasi bunga dan hasil reproduktif (penyengat ara dan biji benih) bagi buah ara daripada individu pokok yang berbeza serta dari tempat yang berbeza. Sebanyak 10 buah ara yang telah matang dikutip daripada pokok jantan dan pokok betina dari semua kawasan kajian dan kemudian bilangan bunga, hempedu, penyengat ara jantan dan betina bagi pokok jantan manakala bilangan biji benih dan bunga betina telah dikira bagi pokok betina. Data yang diperolehi dianalisis

menggunakan kaedah ANOVA satu hala. Secara kesuluruhan, individu pokok Mas Cotek yang berbeza menunjukkan perbezaan yang ketara dari segi bilangan bunga dan hasil reproduktif mereka (penyengat ara dan biji benih) (P <0.05) bagi kedua-dua pokok jantan dan betina kecuali penghasilan biji benih di Changkat Lobak (P= 0.067). Manakala terdapat perbezaan yang ketara (P < 0.01) bagi penghasilan penyengat ara dan biji benih dari lading yang berbeza. Hasil reproduktif (pendebunga dan biji benih) adalah dipengaruhi oleh bilangan bunga dan kedatangan penyengat ara bagi setiap buah ara.

Kata kunci: pokok mas cotek, penyengat ara, buah ara, epifit

INTRODUCTION

Ficus deltoidea (family Moraceae) and their fig wasp (family Agaonidae) are partners in obligate mutualism where the loss of one species has detrimental effect on the other (Janzen 1979, Herre et al. 2008). Ficus need the pollinators for the pollination process while the pollinators require the figs as their brood site. There are approximately 750 Ficus species worldwide with only 300 fig wasp species have been described so far (Osborne 2012). Roughly half of the Ficus species are functionally dioecious (Ronsted et al. 2008) including Ficus deltoidea in which the female trees produce only seed-bearing fruit while the male tree produces only pollen and pollen-carrying wasp progeny (Dumont et al. 2014). Ficus deltoidea or mistletoe fig is native of Peninsular Malaysia and widely distributed throughout Southeast Asia. Ficus deltoidea also known locally as Mas Cotek because of the presence of golden spots on the upper surface of the leaves. This species is also acknowledged for its medicinal value as it contains pharmacological properties (Hamidun Bunawan et al. 2014). According to Fatihah et al. (2014) there are seven described varieties of Ficus deltoidea in

Peninsular Malaysia namely var. *deltoidea* Corner, var. *angustifolia* (Miq.) Corner, var. *trengganuensis* Corner, var.

*bilobat*a Corner, var. *intermedia* Corner, var. *kunstleri* (King) Corner, and var. *motleyana* (Miq.). Morphological studies of these varieties portray a high variability among the varieties including the number of flowers. *Ficus deltoidea* complex is said to be truly epiphytes (Rosnah et al. 2015) whilst most of the *Ficus* species are strangler or hemiepiphytes with one of the host is oil palm trees. The massive transformation from forest to oil palm plantation in Malaysia has offered this complex to colonise them and probably extend their ranges. Oil palm agricuture has been heavily criticized as it generally causes the loss of biodiversity (Azhar et al. 2015) contrary figs are consider as keystone mutualist of tropical forest, thus the presence of figs as an epiphytic plant for oil palm will minimize the negative impact of oil palm cultivation.

Traditionally each species of fig tree is pollinated by one fig wasp species however some exceptions have been reported in which more than one species of pollinator can occur in single host fig tree (Rasplus 1996, Michaloud et al. 1985). There are two types of fig wasp pollinator which are active and passive pollinator (Kjelberg et al. 2001). The active pollinator will show a pollination behaviour in which it will collect the pollen and put it inside their thoracic pollen pockets then transfer the pollen grain to stigmas by using their front legs (Jander and Herre. 2010). While for the passive pollinator, it shows no specialized behaviour as it will be covered by the pollen that simply stick to the wasp body (Bain et al. 2015). In order to oviposit their eggs, foundress female fig wasp enter the figs through a bract-line tunnel known as ostiole which temporarily open during the receptive phase of the figs. For male tree, once inside the female fig wasp oviposit their egg down the style of female flowers

where the larvae developed in and feed on galled ovaries and form a single male or female progeny from each gall. After few \weeks, wingless male fig wasp will emerge first and mating with female fig wasp by chewing a hole on galled ovules containing female fig wasp and insert their telescopic gasters.

Later, fully developed female fig wasp collect pollen from anthers and escape from the figs to seek out other receptive figs and start the cycle anew while the male fig wasp will die inside the fig cavity (West et al. 1996). Whereas for female foundress that entered female trees, they are unable to oviposit their eggs as the style length of the female flower for female fig is way longer than their ovipositor, thus they only pollinate the flower which will eventually developed into seeds (Harrison 2003, Anstett et al. 1997). The aim of this paper is to determine the variation of Ficus deltoidea var. angustifolia in their number of flowers between individual plants as well as plant from different locations. We also examined the production of pollinators and seeds between individual plants and between plants from different locality. Having these data or information is very useful for measuring the population of fig wasp in a disturbed environment as a diversity indication which result from transformation from forest to agricultural forest such as oil palm.

METHODOLOGY

This research was conducted at 4 different oil palm plantations located at Banting (2°50.094'N, 101°35.074'E), Dengkil (2°51.125'N, 101°39.424'E), Changkat Lobak (5°07.070'N, 100°39.445'E) and Batu Pahat (1°57.693'N, 102°48.580'E). All *Ficus deltoidea* var. *angustifolia* studied were grown directly from wild that act as epiphyte in oil palm trees with synchronized figs development. The phases of figs development

were classified according to Harrison (2005) namely phase A, B, C, D and E. Phase A is the immature fig with tightly closed ostiolar bracts, followed by B phase which is the receptive phase for foundress female fig wasp to enter the fig through

ostiole. Phase C is the longest phase in which the wasps developed on male trees and seed developed on female trees. Phase D is when the female progeny emerged from the fig which only occur in male tree, thus female trees lack this phase. Lastly the fig will developed to E phase where it forms a fruit structure. The unpollinated figs will be aborted later as it developed into O phase.

From June 2016 to July 2017, the characters of figs from wild epiphytic Ficus deltoidea var. angustifolia at different location were studied. The study population at Banting consisted of 28 male trees and 24 female trees, Dengkil consisted of 50 male trees and 48 female trees, Changkat Lobak consisted of 14 males and 13 female trees and Batu Pahat consisted of 34 male trees and 11 female trees. Ten trees for each sex from each oil palm plantation were labeled and observed during study period. When the figs reached mature phase (Phase D) ten figs from each male and female tree was collected at random from all plantations. Figs collected from male trees were left in tightly closed mesh-topped containers for 24 hours to let the fig wasp emerge naturally. The following day after the wasp had emerged from figs, the figs were put inside vials with 75% alcohol to preserved. Meanwhile figs collected from female trees were preserved the same way right after collected from study sites.

Later, the samples of figs from both male and female plants were brought to the laboratory and dissected under stereo microscope for counting process. For male figs, the individual figs were cut open into 2 or 4 parts and the numbers of male flowers, female flowers, galls where the wasps had emerged or still contained un-exited wasps were counted. Plus, the number of female flowers, and male flowers were also counted and recorded. Similarly, for female figs the numbers of seeds and total female flowers were counted and recorded as well. Data for the number of flowers produced in each fig, fig wasps and seeds production by fig wasps between individual fig trees and from four different oil plantations were analysed by using oneway ANOVA and if result is significant the means were compared by Turkey's and P < 0.05. Analysis were on IBM SPSS statistic software version 20.

RESULT AND DISCUSSION

There was a highly significant variation in the numbers of male flowers produced by different male plants for each location (P < 0.01) (Table 1). Different plants also showed significant variation in the number of female flowers produced with means numbers varying from 67 to 309 by fig plants at Banting and Dengkil (Table 1). The male plants reproductive output in term of pollinators produced varied significantly with the lowest is plants from Banting while the highest is from Changkat Lobak with 3 and 211 respectively.

In contrast, the number of flowers of female figs produced by different plants from each location showed significant value P (<0.01) (Table 2). Whereas the reproductive output (number of seeds per fig) of figs at Banting, Dengkil and Changkat Lobak showed highly significant differences between female plants (P < 0.01). However, the production of seeds by different female plants from Changkat Lobak was not significantly varied (P = 0.067).

Fig content	Location	Mean±SE	F	sig		
a) Male Flower						
	Banting	28.73±0.377	6.277	0.000		
	Dengkil	28.54 ± 0.480	33.484	0.000		
	Changkat Lobak	29.32±0.319	3.281	0.002		
	Batu Pahat	29.32±0.395	27.868	0.000		
b) Female Flowe	er					
	Banting	147.32±3.617	25.401	0.000		
	Dengkil	175.19 ± 4.750	36.582	0.000		
	Changkat Lobak	157.00±2.550	7.450	0.000		
	Batu Pahat	165.6±1.857	3.396	0.001		
c) Pollinators						
	Banting	65.79±3.546	4.655	0.000		
	Dengkil	85.90±4.002	4.390	0.000		
	Changkat Lobak	86.00±4.039	2.781	0.006		

Table 1. Variation in male fig among Ficus deltoidea var.angustifolia from different locations

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Fig content	Locations	Mean±SE	F	sig
a) Female Flower				
	Banting	5.48 ± 0.063	7.239	0.000
	Dengkil	4.86±0.077	5.602	0.000
	Changkat Lobak	4.80±0.060	1.688	0.103
	Batu Pahat	4.51±0.080	3.261	0.002
b) Seed				
	Banting	4.93±0.112	4.230	0.000
	Dengkil	4.36±0.101	3.300	0.001
	Changkat Lobak	3.82±0.125	1.868	0.067
	Batu Pahat	3.77±0.129	3.310	0.002

Table 2. Variation in female fig among Ficus deltoidea var.angustifolia from different locations.

The number of male and female flowers per male fig among different oil palm plantations showed no significant difference for male flowers (P = 0.923) and female flowers (P =0.189). Meanwhile for female flowers, they were significantly varied (P < 0.001). Similarly, the number of pollinators produced by *Ficus deltoidea* var. *angustifolia* from different location was also highly significant different (P = 0.008) (Fig.1). The highest production of pollinators was at Changkat Lobak with the mean pollinators are 86 which was significantly different compared to mean for the lowest pollinators recorded from Batu Pahat with 61.01. However, the different production of pollinators from Changkat Lobak and Dengkil as well as from Banting and Batu Pahat has only slight differences. The P value for seeds production from different locations was significant (P < 0.01) (Fig.2), indicating that the number of seed produced are varied among locations. The highest and lowest production of seed recorded was from Banting (4.93 ± 0.112) and Batu Pahat (3.77 ± 0.129) respectively.



Figure 1. Mean pollinators production of *Ficus deltoidea* var. *angustifolia* from different locations



Figure 2. Mean seed productions of *Ficus deltoidea* var. *angustifolia* from different locations

Different individual plants of of Ficus deltoidea var. angustifolia have different numbers of flowers they produced. In order to stabilized selection, morphology of flowers within species tend to be constant however in term of quantitative variation there are often vary among conspecific (Galen 1999, Herrera 2005). Female flowers inside each fig are the likely major determinants of fig wasp progeny and seed productions. According to Nazia et al. (2013) the number of flowers may vary between figs, individual plants as well as geographically isolated site. Pornwiwan et al. (2016) stated that the flowering phenologies for several dioecious Ficus species are related with abiotic climatic factors such as water and light. This is corresponding with results obtained in this study where there is significant variation in the number of flowers of Ficus deltoidea var. angustifolia collected from different individual plants and plants from different location for female figs.

From this study, figs sample were collected from 4 different oil palm plantations that has different seasonal variation in environmental factor, management system, soil types as well as oil palm trees ages which may result in variation of flowers produced from different locations. Yasmin (2012) suggested that the production of flowers, pollen and nectar can be influenced by soil quality which result in changes of visitation patterns by pollinator. However, the results obtained was contrary for the numbers of flowers in male figs collected from different locations as they are not significantly different for both male and female flowers. This might be because the number of flowers inside male figs are extremely high compared to female figs, so that it will only significant when the differences among places is high. Meanwhile for female figs only a slight different could result in significant value. The other factors that may cause this variation is the cross pollination among different plants and the genetic mutation of the fig itself. Other than genetic variation, the locations of figs in the branch also can cause different number of flowers produced as individual leaves can be the major source of carbohydrates (Herre 1989) for figs nearer to the leaves.

Fig trees and their pollinating fig wasp are known as a highly specific plant-insect mutualism with high level of behavioural and morphological co-adaptation (Liu et al. 2013). Based on our finding, the production of fig wasp progeny and seeds from *Ficus deltoidea* var. *angustifolia* was significantly varied among individual plants and plantations which demonstrate different patterns of visitation of pollinating fig wasp. There are various factors determine the number of fig wasp progeny and seeds productions. One of it is the number of eggs fig wasp carried when emerge from their natal figs. Foundresses which carried more eggs will utilized more female flowers to oviposit their eggs. Peng et al. (2014) claimed that the number of eggs carried by fig wasp correlates with its body size which means larger fig wasp carried more eggs. In this study, the size for pollinators varied from 1.0 to 1.19 mm in body length measurement for pollinator from different figs which explained why there are differences in numbers of fig wasp production by each figs.

Fig wasp favor male figs as it provides nutrition and shelter for the fig wasp larvae to complete their development (Susheela et al. 2016). The production of fig wasp offspring can only happen if the female wasp enters figs on the male tree (Herre et al. 2008). Tarachai (2008) had claimed female flowers for male figs, even if they do receive the pollen from fig wasp they are physiologically not capable to produce seeds. Fig wasp is generally unable to distinguished between male and female figs because of the inter-sexual mimicry by plants as their mechanism to maintain this mutual relationship. Thus, in order to avoid discrimination by their pollinating fig wasps both receptive male and female figs released sufficiently confusable volatiles through the ostiole (Grison-pige et al. 2001). This is corresponding with the result obtained as there are numerous seed productions recorded from this study. If polen-bearing adult fig wasp had entered female fig, they cannot reproduce as they are unable to oviposit due to the style lengths being longer than their ovipositors (Nefdt and Compton 1996) thus once inside female fig wasp can only pollinated the flowers inside. They can re-emerge from the old figs, but their movement is limited to the nearby figs of the same plant only and they have no chance to subsequently reaching male plant. This is because they might have detached their wings and part of their antennae when entering the first fig and because of their short adult life span (Ahmed et al. 2009, Jevanandam et al. 2013). According to Shazia et al. (2008) male and female figs has similar frequency

and timing of emergence of foundress from figs, however due to the higher rates in pollination compared to oviposition there are generation of numerous seeds in female figs.

The average number foundresses entering fig wasp could result in variation of fig wasp offspring and seed productions. It is not easy for the fig wasp to get inside the fig as there are chemical and physical barrier produced by the plant to ensure that only the right pollinator can enter the fig so that the hybridization between Ficus species can be prevented. The specific volatiles emit by figs during receptive phase was believed to be the main mechanism in maintaining pollinator specificity in fig and fig wasp relationship. This floral volatile signature act as a chemical barrier in which only the right pollinator can have recognized the chemical cue by their olfactory receptors (Ware and Compton 1992, Zacharuk 1985). Meanwhile, ostiolar diameter of fig and style length of female flowers play role as a physical barrier in the prevention of entry by different pollinators. The head size and the length of ovipositor of the pollinator need to be adapted to that particular Ficus species ostiolar morphology and female flower style length to gain entry the fig and oviposit their eggs (Noort et al. 1996). The fig that has been pollinated will be less attractive a few hours after entered by pollinators depending on Ficus species, while the unpollinated figs will remain receptive for much longer (Nazia 2011). As fig wasp of Ficus deltoidea var. angustifolia has short adult lifespan which is less than 24 hours. They do not eat during adult stage and only spend their time to seek for receptive figs and oviposit their eggs for their next generation due to the time pressures. The best strategy for fig wasp to oviposit their eggs in the limited time is to simply enter the first fig they encounter as the chances to find another receptive fig before they die is very low (Patel et al. 1995, Moore et al. 2003). The entry of foundresses inside the fig also

affected by the density of fig wasp population in areas surrounding the fig trees. Fig wasp is known as a weak flyer; thus it is difficult to reach tree located far from the their natal fig tree. In other word, fig plants that are surrounded by male fig plants has higher possibilities to have multiple foundresses in one fig as only male figs can produced the fig wasp progeny. Consequently, the multiple foundress that entered the figs will utilized as many flowers they can to oviposit their eggs.

CONCLUSION

In conclusion, variation in figs floral is influenced by several factors operating at different levels included genetic variation as well as environmental factors. This is proven by the result obtained from this research in which different individual plants as well as plants from different locations were significantly varied in their floral numbers. On the other hand, the production of fig wasps and seeds are greatly affected by the number of flowers available to be oviposit and the number of fig wasp's visitations in each fig. Thus, further work need to be conducted to understand the behaviour and interactions between these extremely species-specific figs and fig wasps' relationship

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