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Insect Pollination of *Aquilaria crassna* (Thymelaeaceae): Effect of Moths for the Fruit Setting in Thailand

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The diversity and abundance of insect visitors to flowers of *Aquilaria crassna* were investigated in a natural forest in Khao Yai National Park and a plantation in Trat Province, Thailand. The behavior and pollinating effectiveness of the major insect visitors were also examined. Data were collected from 2006 to 2008 during the flowering season from March to May. A total of 103 insect species were found, representing 34 families from 4 orders. The highest number of species belonged to the order Lepidoptera (61 species), followed by Hymenoptera (26), and Coleoptera and Diptera (8). The efficiency of moth visitors was measured by the mean number of pollen grains found on the visitor's proboscis. *Endotricha* species (Pyralidae, Lepidoptera) were the most abundant and frequent visitors in both areas, and had pollen deposits of 16.29±5.36 (n=9) grains per proboscis. Fruit setting following different pollination treatments was not significantly different in the natural forest and the plantation, and no significant difference was found between hand pollination and open pollination. However, there was a significant difference in fruit setting between hand pollination and closed pollination, indicating that pollen transfer from anthers to stigma by vectors was required for fruit setting of *A. crassna* flowers.

Keywords: insect pollinators, moths, *Aquilaria crassna*, pollination, fruit setting

INTRODUCTION

Successful fruit setting after pollination is important for most flowering plants. The effectiveness of animals as pollinators depends on the reproductive system of the plant (Proctor *et al.*, 1996; Kato *et al.*, 2003; Okamoto *et al.*, 2008) and the availability of the associated pollinators (Richards, 1996; Grazoul *et al.*, 1998). Insects are major pollinators of angiosperms (Proctor *et al.*, 1996), and this includes some moth species (Proctor *et al.*, 1996; Kato *et al.*, 2003; Sugiura and Yamazaki, 2005; Makholela and Manning, 2006; Okamoto *et al.*, 2008). Insect visitors vary with regard to pollination efficiency and in their preferences for floral enticements (Lau *et al.*, 2004).

Resinous wood, mainly from the tree genus *Aquilaria* (Family Thymelaeaceae), is an important raw material for producing perfume and incense in the Middle East and Asia. *Aquilaria crassna* Pierre ex Lec. (Kritsana/ Agar Wood/ Eagle Wood) is one of the most important commercial resinous heartwood tree species. The tree is native to Cambodia, Laos, Thailand, and Vietnam (Nghia, 1998; Phengklai and Khamsai, 1985). The resinous wood of this species found in natural forests is generally considered to be of high quality and fetches a high price, due to its effective immune response to fungal attack (Nghia, 1998; Khangsap and Watcharinrath, 2005). In recent years, the demand for this resinous wood has increased so that the species is critically endangered, as defined by the criteria of the IUCN (International Union for Conservation of Nature) red list, and is listed in Appendix Ⅱ by the Convention on International Trade in Endangered Species (CITES) of Wild Fauna and Flora (Nghia, 1998). Therefore, without a proper management and protection system, *A. crassna* may become extinct in the near future.

To address this issue, *Aquilaria* plantations have been expanding rapidly in Southeast Asia (Tran *et al.*, 2003; Donovan and Puri, 2004; Khangsap and Watcharinrath, 2005). The production of high quality seeds is a key issue in the establishment of the plantation system. Low germination rate of seeds is a major problem because seed viability declines rapidly, and the proportion of viable seeds falls to 25% in the third week (Khangsap and Watcharinrath, 2005). Tissue culture is potentially an alternative way of propagating *A. crassna* but this technique requires substantial capital investment and is still in development. Thus, seed production plantations are still the most practical way to increase the quality and quantity of seed available for farmers. For effective production of large quantities of high quality seed, it is important to understand the factors affecting the pollination processes. Some research has focused on reproduction in the genus *Aquilaria* (Soehartono and Newton, 2001) but information on *A. crassna* is limited. Most members of the Thymelaeaceae family are presumed to be entomophilous (Whitehead *et al.*, 1987)

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but the diversity and roles of the pollinators are not clear, and it is not known if there are differences between natural forests and plantations in terms of the abundance and efficiency of pollinators.

This study investigates and compares the role of pollinators during the *A. crassna* flowering season in canopies of natural forest and plantation stands. The diversity and abundance of flower visitors, the behavior of moth visitors to *A. crassna* flowers, and their effectiveness in pollination and fruit setting were examined.

MATERIALS AND METHODS

Study site

The study was conducted at two locations in Thailand, a natural forest at Kao Yai National Park and private plantation at Khaosaming District. Kao Yai National Park (14° 26´ N, 101° 22´ E), the second largest national park in Thailand covering 216,000 ha in Nakhon Ratchasima, Saraburi, Prachinburi and Nakhon Nayok Provinces. This area holds diverse and significant habitat in tropical rain forest ecosystems, containing hill evergreen forest, dry evergreen forest, dry deciduous forest, mixed deciduous forest, secondary forests and grassland (TDRI, 1995). The elevation is about 600–700 m and the average annual rainfall is 1,152.8 mm. The average annual temperature (from 1995 to 2005) is 26.09 ˚C. According to the official record, the temperatures were observed to be the highest at 33.85 ˚C and the lowest at 17.15 ˚C in April and January, respectively (Sangtabtim, 2008). Kao Yai National Park has a wide variety of generally endangered fauna and flora, which also included *A. crassna* for the conservation in Thailand. In 2005 the park was designated to be an UNESCO World Heritage Site under the name Dong Phaya Yen–Khao Yai Forest Complex (United Nations, 1992–2007; DNP, 2005).

The private plantation at Khaosaming District (12° 21´ N, 102° 29´ E) is located in Trat Province, eastern part of Thailand at about 15–20 m elevation, covering 12.8 ha. The average annual rainfall was 1,753.0 mm. The climate of average annual was 28.15 ˚C. The highest of temperature was officially observed in May at 33.40 ˚C, while the lowest was in December at 22.22 ˚C (Thaieasterncluster, 2008). This plantation contains 15 year old *A. crassna* trees growing at 2 m×4 m spacing.

Measures of visitor species diversity

A. crassna flowers are small (12.1×4.9 mm width and length) and hermaphroditic, blooming at the canopy of the tree (Fig. 1). To clarify the species diversity of flower visitors, we observed and sampled on six trees of *A. crassna* canopies in each habitat which was randomly selected based on accessibility, flowering performance and isolation. Scaffolding was erected to a height of 4 to 8 m around each tree.

To investigate abundance of each visitor and species richness, the insects were collected with air–flight malaise traps, which are effective in collecting Hymenoptera and Diptera (Grazoul, 1997). The flower visitors were collected every week during the flowering period. In addition, the visitor collection was supplemented by sweep net, which is rapidly swept back and forth through the canopy during night time (18:00 to 06:00) and day time (06:00 to 18:00). The number of flower visitors and their behaviors were recorded for a 20 minute period each hour. A number of individual and species, observed in ten inflorescences (n=10), were recorded in the first 10 minutes while the visitor behaviors including mean time of visit (second/flower) and frequency of flower visits (number of visitors and anthesis on flowers) were then recorded in the second 10 minutes. The flowers began to open around 16:00 and most flowers opened by 18:00

Fig. 1. *Aquilaria crassna* trees and collecting sites; A) flowers, B) fruits, C) a scaffolding to observe visitors at natural forest and D) an air–flight malaise trap (arrow) to catch visitors at plantation on flowering *A. crassna* canopies.

normally. The peak blooming period of *A. crassna* is dry season. The observation was performed during March to May of three years (2006–2008). Time and areas of trapped and swept flower visitors were labeled and recorded. The visitors' species were identified at the laboratory of forest entomology, Faculty of Forestry, Kasetsart University, Thailand and Institute of Tropical Agriculture, Kyushu University, Japan.

Estimation of nectar volumes and sugar concentrations

The volume and sugar concentration of nectar in a flower can affected a number and behavior of visitors (Heinrich and Raven, 1972; Proctor *et al.*, 1996; Tasen *et al.*, 2000–2002) to pollen transfer at the flower (Galen and Plowright, 1985; Thomson, 1986). To investigate the volume and quantity of nectar production by individual flower, nectar volumes and sugar concentrations were directly measured at the study site in the evening or period of each hour (16:00 to 24:00) after inflorescence starts blooming. Nectar volumes were measured by inserting a 2 μL capillary tube down to the base of each flower (n=90 for sugar of nectar concentration, n=54 for nectar volume). Because of a small amount of produced nectar, several flowers were pooled for measuring fresh nectar concentration by using a hand–held refractometer (N.O.W. Tokyo, Japan). The measurement was conducted during 27 to 30 March, in 2007 and 2008.

Efficiency of flower visitors

To investigate the potential pollination effectiveness of insect visits on the flower, direct measures were conducted by counting pollen loads on visitors to 10–15 inflorescences of each flowering trees. Firstly, unopened flowers in each inflorescence were covered with a flower bags. The bags were then opened at 18:00, which is start point of blooming. The major insect visitors were collected to examine pollen deposit on the part of body after visiting the flowers. A number of pollen grains deposited on the part of specimen were counted using a light dissecting stereomicroscope. The higher the number of pollen represents, the higher possibility of pollination resulted in higher efficiency of fruit setting. The efficiency and importance of visitors for pollination were indicated by number of pollen grain deposited on the part of the body after visiting the flowers (Moeller, 2005), which the pollen deposited or transferred by the visitor, is shown to result in fertilization of the ovule (Cox and Knox, 1988).

Pollination experiments

To detect the fruit setting in different conditions, pollination experiments were conducted in natural forest and plantation stands, which were the same sites as in the observation of flower visitors. The treatments included; (1) closed–pollination; flowers were covered by bags, (2) hand–pollination; bagged flowers were hand–pollinated with the pollen from a tree of more than 50 m distant (3) open–pollination; flowers were not covered and left open to allow the pollinators to visit freely and (4) moth–pollination; flowers were bagged and the bags were removed at 18:00 and the flowers were tagged immediately after the moth visits. Each treatment was done on six branches of individual tree.

In the hand–pollination treatment, the undehisced stamens were removed and the flowers were bagged before flower opened between 16:00 to 17:00 on the day time. The flowers were hand–pollinated by touching the stigma with fresh–pollen from newly opened flowers. Each bag was removed for hand–pollinations from receptive flowers between 18:00 to 21:00.

The flowers of each treatment were distinguished by tagging with colored thread. All of the un–pollinated flowers were withered and fallen down during the first week (Sangtabtim, 2008). Fruit setting was recorded at two weeks after starting the pollination experiments and was calculated based on the proportion of total flowers treated.

Data analyses

Means and standard error of the means were calculated for all the measurements. The comparative means by analysis of variance (ANOVA) between natural forest and plantation stands. Duncan New Multiple Range Test at P<0.05 was used to compare the means and determine the significance of differences between variables.

RESULTS

Diversity of flower visitors

A total of 103 species were collected, belonging to 34 families from four insect orders; Lepidoptera, 61 species; Hymenoptera, 26 species; Diptera, 8 species; Coleoptera, 8 species (Table 1). 86 species from 33 families were found in the natural forest site, while 38 species from 15 families were found in the plantation site, and 21 species from 13 families were common to the natural forest and plantation sites.

Shannon's index of species diversity H' (Ludwig and Reynolds, 1988) was higher in the natural forest than in the plantation sites (3.61 and 3.24 respectively). The most frequent species in the forest was a pyralid moth (*Xanthomelaena schematia*), while *Endotricha* spp. was the most common species found in both the natural forest and plantation stands. Margalef's index of species abundance (Magurran, 2004) was higher in the natural forest than in the plantation (32.23 and 19.42, respectively). However, Pielou's index of species evenness (Ludwig and Reynolds, 1988) was slightly higher in the plantation than in the natural forest. This indicates that the distribution of insect visitors to both sites was not differed for variable of evenness index in floral species (Table 2).

The proportion of each species on the flowers was analyzed by Whittaker's measure of β diversity or differentiation diversity (Whittaker, 1977). ANOVA confirmed that species richness in the natural forest was higher than in the plantation (F=6.267, d.f.=1, 69, P= 0.015). The Morisita–Horn similarity index (Wolda, 1981) for the two sites was 0.36, a moderate to low value.

Behavior and foraging periods

There were two peaks in the visiting times of insects (Fig. 2). The first peak was 20:00 to 22:00 and the second was 10:00 to 12:00. The number of insect visitors was lowest during 04:00 to 06:00. This pattern was the same in the natural forest and plantation plots.

At night time, foraging behavior of nocturnal visitors was related to the sugar concentration and quantity of nectar secreted by the flowers. The nectar volume and sugar concentration were 0.06±0.02 μL per flower and 12.54±1.22%, respectively. The nectar volume was highest from 18:00 to 20:00, and the sugar concentration increased until 19:00 and then leveled off. This implies that 18:00 to 20:00 is the best time for visitors to obtain nectar from *A. crassna* flowers in terms of the quality and quantity. Indeed, the first peak of visitors almost coincides with this period, and the decrease in number of visitors paralleled the decrease in nectar volume. While the sugar concentration is not related to visitation frequency in Fig. 3 because it still remain high after 21:00.

The number of visits during the day peaked from 10:00 to 12:00. The highest number of species belonged to the order Hymenoptera, followed by Lepidoptera (butterflies), Coleoptera (beetles) and Diptera (flies). The five diurnal species contributing most flower visits were *Chrysomya* sp. (Calliphoridae, Diptera; 11.96% all of diurnal visits), *Bactocera correcta* (Tephritidae, Diptera; 11.57%), *Lasioglossum* sp. (Halictidae, Hymenoptera; 10.20%), *Apis florea* (Apidae, Hymenoptera; 5.69%)

Table 1. Number of species and percentage of individual of insects visiting *A. crassna* flowers in natural and plantation stands (N=Night time visitors, D=Day time visitors)

Order/Family	Number of species: Natural forest	Number of species: Plantation	Total of species	% individual of visits
Lepidoptera				
Noctuidae	10(N)	5(N)	11	3.87
Arctiidae	5(N/D)	6(N/D)	10	4.08
Pyralidae	10(N)	7(N)	14	32.68
Lasiocampidae	1(N)	–	1	0.14
Thyrididae	1(N)	–	1	0.42
Geometridae	–	1(N)	1	0.49
Nymphalidae	9(D)	4(D)	12	3.59
Papilionidae	1(D)	–	1	0.07
Pieridae	2(D)	2(D)	3	0.14
Lyceanidae	6(D)	1(D)	6	0.56
Hesperiidae	1(D)	–	1	
Hymenoptera				2.39
Apidae	6(D)	4(D)	7	5.56
Braconidae	3(D)	–	3	3.59
Chalcididae	1(D)	–	1	0.63
Formicidae	5(D)	–	5	1.41
Halictidae	2(D)	1(D)	2	4.30
Megachilidae	2(D)	–	2	0.70
Scoliidae	1(D)	–	1	0.28
Sphecidae	1(D)	–	1	0.14
Crabronidae	3(D)	–	3	0.35
Vespidae	1(D)	–	1	0.56
Diptera				
Culicidae	1(D)	1(D)	1	9.08
Muscidae	1(D)	1(D)	1	6.41
Tachinidae	1(D)	–	1	0.56
Tephritidae	1(D)	1(D)	1	4.15
Asilidae	1(D)	–	1	0.49
Calliphoridae	2(D)	2(D)	2	5.42
Syrphidae	1(D)	–	1	0.56
Coleoptera				
Buprestidae	2(D)	–	2	0.28
Cerambycidae	1(N/D)	1(D)	2	0.21
Chrysomelidae	1(D)	–	1	0.56
Coccinellidae	1(D)	–	1	0.14
Elateridae	1(D)	–	1	0.07
Scarabaeidae	1(D)	1(D)	1	0.56
Total	86	38	103	

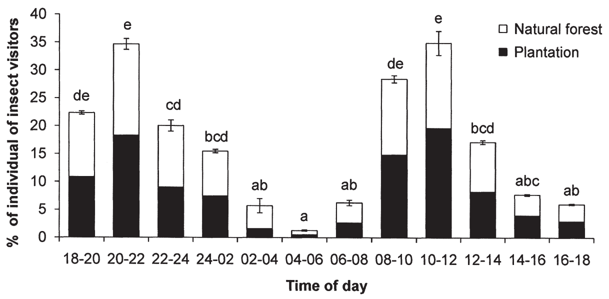

Fig. 2. Numbers of insect visitors in different flowering periods in the natural forest and the plantation. Vertical bars show the standard error of the mean for each variable. The same letter indicates the means are not significantly different at $P<0.05$ as determined by Duncan's New Multiple Range Test.

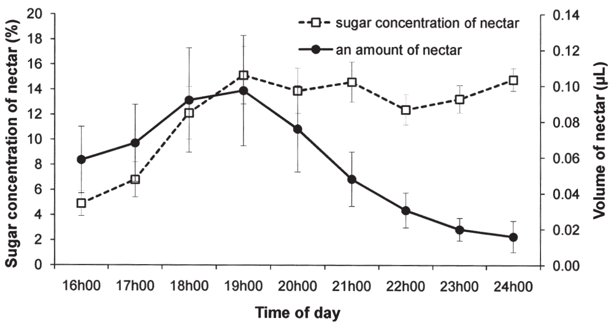

Fig. 3. Relationship between sugar concentration of nectar (%) and the volume of nectar (μL) in *A. crassna* flowers in each observation period from 16:00 to 24:00 h. Vertical bars show the standard error of the mean for each variable.

Table 2. Number of individuals and species diversity indices of insect visitors collected from A. crassna flowers in natural and plantation stands

Habitat	Number of individuals	Diversity indices			
		H' (Shannon's index)	Evenness	Richness	β diversity
Natural forest	1039	3.61	0.52	32.23	7.76
Plantation	377	3.24	0.55	19.42	4.26

and *Catopsilia pomona* (Pieridae, Lepidoptera; 5.29%). The diurnal visitors can potentially support or assist pollination of *A. crassna* flowers not pollinated by nocturnal visitors. The present study indicates that the diurnal insect groups were mostly present before noon in both the natural forest and plantation.

The mean duration of visits in the natural forest and plantation were not significantly different (F=1.04, d.f.=1, 11, P=0.461), but the foraging behaviors of the nocturnal and the diurnal pollinators were different (F=44.03, d.f.=1, 11, P=0.000). The mean duration of visits of the nocturnal visitors or moths was 25.36±1.78 second per flower (n=46) while the mean duration of visits of the Hymenopteran (wild bees, wasps and ants) diurnal visitors was 10.39±0.70 second per flower (n=40). Mean visit durations of Lepidoptera (butterflies), Coleoptera (beetles) and Diptera (flies) were 10.94±0.17 (n=110), 11.28±0.59 (n=3) and 11.93±2.66 (n=22) second per flower respectively. The staying time on a flower is significantly longer for the nocturnal visitors. The quality and quantity of nectar of *A. crassna* flowers were not measured in the day time, but it is certain that the volume of nectar is distinctly lower than at night, which may be why the diurnal visitors stay on flowers for a relatively short time.

Efficiency of flower visitors

Examination of the pollination efficiency of insect visitors mainly focused on the nocturnal visitors, since the highest number pollen grains were found on the proboscis of this group (Fig. 4). The highest number of pollen grain deposited on the proboscis per individual was found in the noctuid moth (*Parallelia rigidistria*) (25.57±8.01, n=7).

The second and third highest numbers of pollen grains were found on the proboscis of the pyralid moths *Salma* sp. (18.17±10.85, n=6) and *Sameodes cancellalis* (17.67±2.60, n=3), However, the lowest number of pollen grains found per proboscis was recorded in *Endotricha* species (16.29±5.36, n=9), the most frequent and abundant species in both the natural forest and plantation (Table 3). *Xanthomelaena schematias* spent the longest time foraging and the mean duration of visits was 30.64±4.59 second per flower (n=11), and the lowest number of pollen grains per proboscis (3.86±1.30, n=17), were found on this species.

Pollination experiments

Fruit setting in *A. crassna* was not significantly different (F=3.96, d.f.=3, P=0.109) in the natural forest and plantation. However, there was a significant difference between treatments (F=37.97, d.f.=3, P=0.002). Fruit setting in the closed pollination treatment (0.53%) was significantly lower than in the hand pollination treatment (3.62%). Fruit setting in the hand pollination and open pollination treatments was not significantly different. This indicates that fruit set do not restricted by pollen lacking and pollen transfer from anthers to stigma by vectors is required for fruit setting in *A. crassna*. Fruit setting by moth pollination was higher than in the closed–pollination treatment, but the difference was not statistically significant (F=1.99, d.f.=3, P=0.117).

Flower visitors in the open pollination treatment were not directly observed, but the treatment allows for the influence of insect visitors in pollination. Fruit setting in moth–pollination was not significantly lower than in open–pollination in both the natural forest and plantation. In the natural forest, fruit setting of moth pollinated trees was 1.09% compared to 2.23% in open pollinated trees. In the plantation, fruit setting in moth pollinated trees was 1.33% compared to 2.78% in open pollinated trees (Fig. 5).

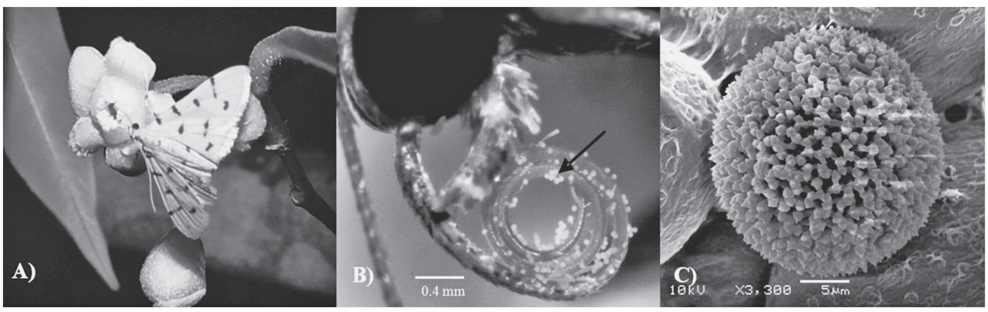

Fig. 4. Moth visitor and pollen grain of *A. crassna* flower; A) *Endotricha* species visiting on flower, B) pollen grains deposited on proboscis of a pyralid moth (arrows) and C) scanning electron micrographs (SEM) of pollen grain.

Table 3. Mean (±SE) time of visit and mean (±SE) number of pollen grains on proboscis of ten moth visitors during 18:00 to 22:00 found in the natural forest and the plantation

Family/ Species	Mean time of visit (second/flower)	n	Mean number of pollen grains on moth proboscis	n
Pyralidae				
1. *Xanthomelaena schematias*	30.64±4.59	11	3.86±1.30	17
2. *Salma* sp.	27.25±3.12	4	18.17±10.85	6
3. *Sameodes cancellalis*	26.14±5.46	7	17.67±2.60	3
4. *Endotrich*a spp.	29.75±5.20	8	16.29±5.36	9
5. *Crypsiptya* sp.	26.33±4.07	6	5.80±2.24	5
6. *Gadessa nilusalis*	24.22±4.24	9	8.25±3.87	4
Noctuidae				
7. *Parallelia rigidistria*	20.59±4.49	7	25.57±8.01	7
Arctiidae				
8. *Ceryx imaon*	21.75±4.99	4	9.67±4.91	3
9. *Cyana* spp.	20.60±5.59	5	11.25±1.80	4
Geometridae				
10. *Cleora alienaria*	21.67±7.43	6	10.67±2.19	3

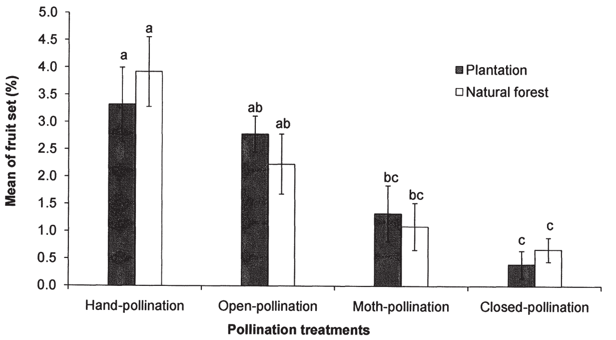

Fig. 5. Percentage of mean *A. crassna* fruit setting in hand pollination, open pollination, closed pollination, and moth pollination treatments. Means of each variable with the same letter are not significantly different at $P<0.05$ as determined by Duncan's New Multiple Range Test. Vertical bars represent standard error.

DISCUSSION

These observations showed that the number visitors of *A. crassna* flowers were about hundred species of nocturnal and diurnal insects. In comparison, only 20 species, half of which were nocturnal, visited *Aquilaria* flowers at plantation plots in Bogor, Indonesia (Soehartono and Newton, 2001). The insect visitors at the *A. crassna* study sites at Khao Yai National Park and the plantation at Trat Province are far more diverse.

Species diversity was analyzed by Shannon's index of species diversity and the species abundance was finding out by Margalef's index. The results showed that the visitor diversity in the natural forest was higher than in the plantation sites. Chey *et al.* (1997) found similar a species diversity of moths in plantations and natural forest, while Tangmitcharoen *et al.* (2006) reported higher diversity and abundance of insects in teak (*Tectona grandis* L.f.) canopies in natural forest than in plantations during the flowering season.

The results suggest that the quality and quantity of nectar secreted have important effects on insect foraging behavior. The same relationship between nectar and frequency of visitors has been reported in other flowers (Alekseyeva and Bureyko, 2000; Cawoy *et al.*, 2008), and nectar properties are among the parameters affecting pollination and fruit setting (e.g. Cresswell, 1999; Shafir *et al.*, 2003; Leiss *et al.*, 2004; Kudo and Harder, 2005). The stigma of *A. crassna* flowers is receptive to pollen for at least the first 12 hours of flowering, and the most receptive period is from 18:00 to 21:00 (Sangtabtim, 2008). If insect visitors contribute to pollination of *A. crassna* flowers, there are distinct and different opportunities for pollination in night time and in day time. Therefore, we divided the insect visitors into two groups: nocturnal visitors, and diurnal visitors. Nocturnal visitors are mainly moths, and they are highly efficient pollinators of *A. crassna* in the early flowering period (night time) in both the natural forest and plantation sites. Moths are also important pollinators in *Aquilaria* spp. in Indonesia (Soehartono and Newton, 2001) and in *Struthiola ciliate* (Thymelaeaceae) in southern Africa (Makholela and Manning, 2006).

In this study, moths seem to be the most important visitors, especially noctuids and pyralids. The moth may be also transferring of pollen from anthers to stigma by the proboscis. However, Okamoto *et al.* (2008) stated that moths carried lower number of pollen grains but still moths are important pollinator because their visitation behavior is suitable for preventing geitonogamous pollination. So this quotation is inadequate. Pollen grains can be mostly observed on the tip of the proboscis (Sugiura and Yamazaki, 2005; Makholela and Manning, 2006), and the proboscis of moths is usually used for feeding on nectar and for carrying pollen to flowers (Proctor *et al.*, 1996; Sugiura and Yamazaki, 2005). The moth visitors play a role in moving pollen to and from flowers within and among plants, thereby influencing the reproductive success of the plants (Talavera *et al*., 2001).

Results of the pollination experiments indicated that the *A. crassna* flowers for fruit succession are required for transferring of pollen to stigma by some vectors. Fruit setting was limited in moth pollinated flowers, which was associated with fewer flowers being left open to other insect visits. Visitor efficiency can be defined as the amount of pollen deposited on a stigma relative to the vector pollen load (Inouye *et al*., 1994), and the number of pollen grains on the proboscis of the moths may not be sufficient to disperse deposits on the *A. crassna* stigma.

Diurnal visitors (including wild bees, butterflies, wasps, flies and beetles) are potentially effective agents for *A. crassna* pollination and successful reproduction. The plants may be visited by multiple types of pollinators which transfer pollen between flowers with varying efficiency (Fishbein and Venable, 1996), and therefore, the insect visitors are necessary for *A. crassna* pollination.

In conclusions, the visitors to *A. crassna* flowers at the study sites include a diverse range of insects, and their visits are concentrated in particular periods of the night and morning. Moths are the major insect visitors, and play an important role in the pollination of *A. crassna*. The diversity and abundance of insect pollinators depends on several factors, including geographic and climatic conditions. Result of hand–pollination suggest that maximum fruit set of *A. crassna* is 3–4% in this field. So, great increase of fruit set would not be expected if pollination status is favor. Thus, the chances of pollination can be improved by pollinators, leading to an increase in fruit setting of *A. crassna*, which is a typically low fruit setting hermaphroditic plant species (Stephenson, 1981; Sutherland, 1986; Guitian, 1993). Therefore, to conservation and artificial supplementation of efficient pollinators could be an alternative way to increase fruit production in *A. crassna* seed plantations.

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