Songklanakarin J. Sci. Technol.
31 (3), 255-259, May - Jun. 2009

Songklanakarin Journal
of Science and Technology
http://rdo.psu.ac.th/sjst

Original Article

Fruit set as affected by pollinators of teak (*Tectona grandis* L. f.) at two tree spacings in a seed orchard

Suwan Tangmitcharoen[1]*, Wattanachai Tasen[2], John.N. Owens[3] and Jutitep Bhodthipuks[1]

[1] *Forest Research and Development Bureau, Royal Forest Department, Chatuchak, Bangkok, 10900 Thailand.*

[2] *Faculty of Forestry, Kasetsart University, Chatuchak, Bangkok, 10900 Thailand*

[3] *Centre for Forest Biology, University of Victoria, Victoria, B.C., Canada V8W 3N5 and Timber West 1450 Mt. Newton Cross Roads, Saanichton, B.C., Canada V0S 1M0*

Received 14 February 2008; Accepted 4 May 2009

Abstract

Fruit set was determined at 2, 3 and 4 wks after pollination (WAP) in a teak seed orchard having 12x12 and 6x6 m spacings. Generally, the percentages of fruit set at 2 WAP were much higher than at 3 and 4 WAP. Repeated measures analysis indicated there was a significant difference in percent fruit set among different WAP for both spacings. *Trigona collina* (stingless bee) commonly visited teak flowers, whereas *Ceratina* sp. (small carpenter bee) had fewer visits. At 4 WAP, the percentage of fruit set at both spacings for *T. collina* pollinated flowers was the highest, followed in decreasing order by open-pollinated, cross-pollinated, *Ceratina*-pollinated, and self-pollinated flowers. There was no significant difference in the percent fruit set between the two spacings and among trees for all pollination treatments. A significant difference was detected only for the percentge of fruit set among pollination treatments. Percent fruit set for self- and *Ceratina* sp. pollinations differed significantly from cross-, open-, and *T. collina*-pollinations. Only 3% of self-pollinated and 5% of *Ceratina*-pollinated flowers set fruit, whereas 9% of cross-pollinated flowers, 8% of open-pollinated flowers, and 13% of *T. collina*-pollinated flowers set fruit. This result supports other studies that there is a relationship between size of pollinators and their behaviors. Our results suggested that *T. collina*, larger size, contribute to increased cross-pollinations resulting in higher fruit set.

Key words: Ceratina, fruit set, pollinator, Trigona collina, Tectona grandis

1. Introduction

Earlier studies in Nigeria and India showed that insect pollinators play crucial roles in pollination and fruit set of teak (*Tectona grandis* L. f.) (Egenti, 1981; Mathew *et al.*, 1987). In Thailand, several studies (Hedegart, 1976; Tangmitcharoen and Owens, 1997a; Tangmitchareon *et al.*, 2006 a;b) have indicated that the lack of efficient pollinators was the main cause of low fruit production. Tangmitcharoen and Owens (1997a) reported that 78% of flowers from open pollinations in conventional plantations were pollinated and *Ceratina* sp. (a carpenter bee in the Anthophoridae) was an important pollinator. They indicated that these pollinators contributed to a high amount of selfing because the insects tended to forage on flowers within an inflorescence and on the same tree. Recent studies (Tangmitcharoen *et al.* 2006a; b), compared the main pollinators in a wild stand and in a seed orchard in northern Thailand and reported that the most frequent flower visits were by *Trigona collina* (a stingless bee in the Apidae), accounting for 67% of teak flowers that

*Corresponding author.
Email address: suwan@forest.go.th, suwantang@hotmail.com

were pollinated. In a comparison of seed orchard versus wild trees, they also suggested that the amount of cross-pollinations may be related to the close proximity of trees. They found that pollinators on wild trees, having a more scattered distribution, were more likely to move short distances and visit flowers within the same inflorescence. There have been no studies, as is reported here, on how spacing within a seed orchard, affects pollinations by *Trigona collina* and *Ceratina* sp.

2. Materials and Methods

2.1 Study sites and plant materials

Three 30-year-old teak trees growing at 6x6 m spacing and three trees growing at 12x12 m spacing were selected in the Maegar Clonal Seed Orchard (CSO) in the Phayao Province, Northern Thailand (19^o 10' N and 99^o 55' E). The six trees were selected based on flowering performance and accessibility and about 100 m away from one to another. They were the same age and size and had diameters at breast height (DBH) of about 30 cm. Scaffolding was erected to a height of 8-12 m adjacent to each tree.

2.2 Pollination experiments

The effect of the different pollinators on fruit set used both control pollinations and observations of pollinations by the pollinators, *Trigona collina* and *Ceratina* sp. during the peak of the flowering season, in August, 2000. Pollinations were made during the receptive period of teak flowers (Tangmitcharoen and Owens, 1997a) on a minimum of five inflorescences per tree. At pollination, each flower was tagged with colored thread and one of the following pollination treatments was carried out: (1) flowers were not emasculated, then hand self-pollinated and bagged (self-pollination); (2) flowers were emasculated, then hand cross-pollinated and bagged (cross-pollination); and, (3) flowers were not emasculated and left unbagged for open-pollinations.

For the emasculated treatments, the undehisced stamens were removed from the newly opened flowers between 0700 and 0800 h on the day of receptivity. Cellophane bags were then placed over the group of open flowers to exclude all potential pollinators. Each bag was removed from the receptive flowers for about 15 min. while hand pollinations were made. The pollen was applied by tapping an anther, held with forceps, over the stigma of a receptive flower between 1100 and 1200 h. Pollen donor trees were chosen from a different clone. For self-pollinations, pollen from the same tree was used and applied as described above. Flowers pollinated in the different ways were tagged with different colored thread. The bags were removed the day after all of the open flowers in the bags were polllinated. A total of 6,439 flowers were hand-controll pollinated, 2,804 from trees at 6x6 m spacing and 3,635 from 12x12 m spacing (Table 1).

2.3 Pollination by *Trigona collina* and *Ceratina* sp.

Before receptivity, pollination bags were placed over a group of flowers (7-15 flowers per inflorescence) to exclude all potential pollinators. Each bag was then removed, for a short time (less than 10 min.) to allow pollination by these insects. After a single visit by either *Trigona collina* or

Table 1. Percentage of fruit set at 2, 3 and 4 weeks after pollination (WAP) using various pollinations on the six trees in each of the 12x12 m and the 6x6 m spacings in a clonal seed orchard. Numbers in brackets represent standard errors.

Pollination treatment	Percentage of fruit set						Total number of pollinated flowers	
	2 WAP		3 WAP		4 WAP			
	spacing		spacing		spacing		spacing	
	12x12m	6x6m	12x12m	6x6m	12x12m	6x6m	12x12m	6x6m
Self	15.34 (2.34)	10.51 (2.12)	6.35 (3.35)	5.64 (2.39)	3.17 (2.5)	3.42 (1.04)	910	735
Cross	24.11 (2.15)	22.02 (3.78)	14.57 (1.43)	15.58 (3.82)	9.11 (0.84)	8.57 (2.23)	1800	1369
Open	20.55 (2.13)	19.5 (1.89)	15.03 (2.05)	11.06 (2.32)	10.0 (1.07)	6.5 (1.26)	925	700
T. collina	20.4 (1.9)	21.42 (1.91)	13.67 (2.03)	17.07 (1.34)	13.10 (1.56)	12.29 (0.93)	125	347
Ceratina sp.	15.3 (6.17)	15.88 (4.12)	7.14 (7.14)	2.94 (2.94)	7.15 (7.15)	2.94 (2.94)	25	27
Total							3785	3178

Ceratina sp., the flower was tagged with colored thread. A total of 524 flowers were pollinated by both pollinators, 374 from trees at 6x6 m, 150 from 12x12 m spacing (Table 1).

2.4 Fruit set

To determine fruit set following pollinations, the variously pollinated flowers were observed and monitored at 2, 3 and 4 wks after pollinations (WAP). Fruit set as affected by pollinators was compared with fruit set of control-pollinated flowers.

2.5 Method of analysis

Repeated measures analyses were conducted on the effect of time (three levels: 2, 3 and 4 WAP) to determine precentage of the fruit set at each tree spacing. Analysis of variance was used to determine if significant differences existed in the percentages of fruits set at 4 WAP after pollination among the different pollinators and control pollinations. Untransformed means and standard errors (±) were reported throughout. The Duncan's multiple range test ($P<0.05$) was used to compare means for a significant difference among the variables. Data, in percentages, were arcsine square root transformed (Sokal and Rohlf, 1981).

3. Results

3.1 Fruit set at 2, 3 and 4 WAP using 12x12 m and 6x6 m tree spacing and different pollinations

A total of 6963 flowers were pollinated, 3785 from trees at 12x12 m spacing and 3178 from 6x6 m spacing. Generally, the percentages of fruit set at 2 WAP were much higher than that at 3 and 4 WAP for every pollination treatment, i.e., a higher fruit abortion occurred from 2 to 3 WAP than from 3 to 4 WAP (Table 1). Repeated measures analysis indicated that there was a significant difference in percent fruit set among different weeks for both the 12x12 m (F = 25.17, df = 2, $P<0.01$) and the 6x6 m (F = 46.30, df = 2, $P<0.01$) spacings. *T. collina* commonly visited teak flowers, whereas *Ceratina* sp. made fewer visits.

Two weeks after hand cross-pollinations, flowers from both the 12x12 m and the 6x6 m spacings showed the highest percentage of fruit set (24.1 and 22.0%, respectively), followed by open-pollination (20.6 and 19.5%, respectively), and pollinations by *T. collina* (20.4 and 21.4%, respectively). The percentages of fruit set from self-pollinated flowers were 15.4 and 10.5%, and those pollinated by *Ceratina* sp. were 15.3 and 15.9% for the 12x12 m and 6 x 6 m spacings, respectively. The percentages of fruit set at 3 WAP for self- and *Ceratina*-pollinated flowers decreased in both 12x12 m and 6x6 m spacings (Table 1). The percentage of fruit set at 4 WAP for *T. collina*-pollinated flowers was highest in both 12x12 m and 6x6 m spacings (13.1 and 12.3%), followed by open-pollinated (10.0 and 6.5%), cross-pollinated (9.1 and 8.6%), *Ceratina* sp.-pollinated (7.1 and 2.9%), and self-pollinated flowers (3.2 and 3.4%, respectively).

Table 2. Statistics for fruit set 4 wks after pollination (WAP) of six trees in the 12x12 m and in the 6x6m spacings in a clonal seed orchard

	Source		
	df	F	P-value
Pollination treatment	21	4.063	0.044
Spacing	1	2.714	0.109
Tree	2	1.117	0.484

3.2 Fruit set at 4 WAP using combined spacings

At 4 WAP, a significant difference was detected only for the percentage of fruit set among pollination treatments. There was no significant difference between the two spacings or among trees for any pollination treatment (Table 2).

The percentage of fruit set for self-pollinations and *Ceratina* sp.-pollinations differed significantly ($P<0.05$) from cross-, open-, and *T. collina*-pollinations. There was only 3.3% set fruit for self-pollinated flowers and 5.0% for *Ceratina* sp.-pollinated flowers, whereas 8.8% of cross-pollinated flowers, 8.3 % of open-pollinated flowers, and 12.7% of *T. collina*-pollinated flowers set fruit (Table 3).

4. Discussion

Eynard and Galetto (2002) suggested that successful fruit production depends on pollinators, their activities and the quality of the pollen transferred by the pollinators. Based on a study of fruit production in an endemic South American tree, *Geoffroea decorticans*, they found that although diverse native pollinators were observed, *Apis mellifera* (honey bees) accounted for 60% of the visits. The honey bees, however, caused mainly self-pollinations. In teak, Tangmitcharoen *et al.* (2006b) suggested that low fruit set of teak, especially in the seed orchard, is mainly related to pollinators and their foraging behavior. They also suggested that there is a relationship between size of pollinators and their foraging behaviors. Our results support their conclusion and indicate that the larger size of *T. collina* contributes to increased cross-pollinations resulting in higher fruit set than the smaller pollinators (*Ceratina* sp.).

Regarding controlled pollinations, our results indicate that there were no significant differences in the percentage of fruit set between open-pollinations among trees in the two spacings. All other pollinations were control-pollinations of some sort in which spacing was not a factor.

Ceratina sp. was reported to be the major pollinator of a tropical *Acacia* Hybrid (*A. mangium* x *A. auriculiformis*) (Sornsathapornkul and Owens, 1998) and teak (Tangmitcharoen and Owens, 1997a) at a plantation in central Thai-

Table 3. Comparison of means of fruit set 4 wks after pollination (WAP) with various types of pollinations on six trees in a clonal seed orchard. Means of each variable followed by the same superscript are not significantly different (P<0.05) as determined by the Duncan's multiple range test

Type of pollination	Fruit set	SE	Number of pollinated flowers
Self	3.32[b]	1.04	1645
Cross	8.84[a]	1.07	3169
Open	8.25[a]	1.08	1625
T. collina	12.69[a]	0.83	472
Ceratina sp.	5.04[b]	3.38	52
(Total)			6963

land. Both studies indicated that *Ceratina* sp. effected primarily self-pollinations because this insects mostly visited flowers within the same inflorescence or within the same tree. A recent study on behavior of pollinators in a clonal seed orchard reported similar findings (Tangmitcharoen *et al.* 2006b) and they suggested that the manner by which pollinators approach flowers affects the transfer of pollen to the stigma. The small (<5 mm) bees, such as *Ceratina* sp., mostly approached the flowers from the side, thus they may not be effective pollinators because their bodies usually do not contact the anthers or stigma and less pollen is transferred to other flowers. Our present results on fruit set, as affected by *Ceratina* sp., are consistent with these earlier observations. We found that fruit set at 4 WAP by self-pollinations and *Ceratina* sp.-pollinations were similar but less than those pollinated by *T. collina*, indicating that in the CSO, *Ceratina* sp. contributed to increased self-pollinations.

Trigona collina was reported as the main pollinator of Durain (*Durio zibethinus*) at an orchard in Chantaburi, Thailand (Boongird, 1992). In the teak CSO, *T. collina* recently was reported as the most important pollinator (Tangmitcharoen *et al.*, 2006b). They also found that the large (>5 mm) bees, typically landed on top of the corolla and flew (rather than walked) between flowers. The species visited a high number of flowers per minute and a low percentage of visits within the same inflorescence. This behavior increased the chance of cross-pollinations. Our present results are consistent with these earlier results. We found that fruit-set from open-pollinations, hand cross-pollinations, and *T. collina*-pollinations were similarly high. This indicated that *T. collina* promoted cross-pollinations in the CSO.

Tangmitcharoen *et al.* (2006a; b) suggested that aggregations of large numbers of flowers in many inflorescences on a tree effect the foraging movement of pollinators. Based on observation in wild and CSO trees, they found that pollinators in the CSO, where there were large aggregations of flowers in many large inflorescences on a tree, tended to move longer-distances thus increasing cross-pollinations. Our present findings support this observation. We found that, compared to *Ceratina* sp., the main pollinator, *T. collina*, contributed to cross-pollinations in both the 6x6 m and 12x 12 m spacings.

The size of pollinators may influence the number of flowers pollinated and eventual fruit set. Based on behavior of the major insect pollinators of teak in the CSO (including *T. collina* and *Ceratina* sp.), Tangmitcharoen *et al.* (2006b) suggested that the larger pollinators had a higher chance of transferring pollen between trees, thus increasing the chance of cross-pollinations. In Nigeria, Egenti (1981) reported that teak seed production was very high (0.63 to 6.56 kg per tree), much higher than in Thailand (0.02 to 0.78 kg per tree) (Pianhanurak, 1995). This has been attributed to the presence of seven pollinators in Nigeria (Egenti, 1981), six of which belonged to Nymphalidae (Lepidoptera), Vespidae (Hymenoptera), Megachilidae (Hymenoptera), and Apidae (Hymenoptera). All of these were relatively large insects. In Japan, Wang *et al.* (1998) also reported that more effective pollinations resulted from larger pollinators such as *Xylocopa circumvolan* (a carpenter bee) rather than *A. cerana* Japonica (eastern hive bee). Our results are consistent with these earlier observations. We found that the larger pollinators, such as *T. collina*, contributed to higher fruit set in teak than the smaller pollinators, such as *Ceratina* sp.

Generally, an increase in pollen load resulting from supplementary pollinations leads to an increase in fruit set (Sutherland and Delph, 1984; Sutherland, 1987). *Apis cerana* has been reported to increase pollinations of various cultivated crops and fruit trees in Sri Lanka and Bangladesh (Robinson, 1988; Hannan, 2000). In Thailand, Wonsiri *et al.* (2000) reported that this large native bee has been used mainly for honey production with coconut (*Cocos* spp.) and rubber (*Hevea brasiliensis*) trees. A recent study (Tangmitcharoen *et al.*, 2006b) suggested that apiculture of *A. cerana* should be tested to determine if this insect would increase cross-pollinations in teak seed orchards in Thailand. They suggested that this bee may be a more effective pollinator than the current main pollinator, *T. collina*. Our present results support this suggestion.

Acknowledgements

This work was supported by TRF/BIOTEC Special Program for Biodiversity Research and Training grant BRT 140008, Thailand. Mr. P. Pianhanurak, station chief at Maegar Seed Orchard, Phayao for providing the field facility. We are grateful to Ms. M. Takeaw who assisted with data collection during field work.

References

Boongird, S. 1992. Biological studies of stingless bee, *Trigona laeviceps* Smith and its role in pollination of Durian, *Durio zibethinus* L. Cultivar Chanee. Ph.D. Thesis, Department of Entomology, Kasetsart University, Thailand.

Egenti, L.C. 1981. Aspect of pollination ecology of teak (*Tectona grandis* L. f.) in Nigeria : pollinators and fruit production. *In* : Krugman, S.L., Katsuta, M. (Eds.), Proceedings of the Symposium on Flowering Physiology, IUFRO XVII World Congress, Kyoto, Japan, 27-31.

Eynard, C. and Galetto, L. 2002. Pollination ecology of *Geoffroea decorticans* (Fabaceae) in central Argentine dry forest. Journal of Arid Environments 51, 79-88.

Hannan, M.A. 2000. Apiculture in Bangladesh. Observer magazine 22, 3-5. Bangladesh.

Hedegart, T. 1976. Breeding systems, variation and genetic improvement of teak (*Tectona grandis* L. f.). *In* Burley, J., Styles, B.T. (Eds.), Tropical Trees, Variation, Breeding and Conservation. pp. 109-124. Linnean Society Symposium Series 2: Academic Press, New York.

Mathew, G., Koshy, M.P. and Mohanadas, M. 1987. Preliminary studies on insect visitors to teak (*Tectona grandis* Linn. f.) inflorescence in Kerala, India. Indian Forester 113, 61-64.

Pianhanurak, P. 1995. Analysis of teak seed orchards in Thailand. M.Sc. Thesis, Kasetsart University, Bangkok, Thailand. (in Thai).

Robinson, W.S. 1988. Beekeeping with *Apis cerana* in Sri Lanka. Bee World 69(3), 125-130.

Sokal, R.R. and Rohlf, F.J. 1981. Biometry. W. H. Freeman, New York.

Sornsathapornkul, P. and Owens, J. N. 1998. Pollination biology in a Tropical *Acacia* Hybrid (*A. mangium* Wild. × *A. auriculiformis* A. Cunn. ex Benth). Annals of Botany 81, 631-645.

Sutherland, S. 1987. Why hermaphroditic plants produce many more flowers than fruit: experiment tests with *Agave mckelveyana*. Evolution 41, 750-759.

Sutherland, S. and Delph, L.F. 1984. On the importance of male fitness in plants: patterns of fruit-set. Ecology 65, 1093-1104.

Tangmitcharoen, S. and Owens, J.N. 1997a. Floral biology, pollination, pistil receptivity, and pollen-tube growth of teak (*Tectona grandis* Linn. f.). Annals of Botany 79, 227-241.

Tangmitcharoen, S. and Owens, J.N. 1997b. Pollen viability and pollen-tube growth following controlled pollination and their relation to low fruit production in teak (*Tectona grandis* Linn. f.). Annals of Botany 80, 401-410.

Tangmitcharoen, S., Takaso, T., Siripatanadilox, S., Tasen, W. and Owens, J.N. 2006a. Behavior of major insect pollinators of teak (*Tectona grandis* L. f.): A comparison of clonal seed orchard versus wild trees. Forest Ecology and Management 222, 67-74.

Tangmitcharoen, S., Takaso, T., Siripatanadilox, S., Tasen, W. and Owens, J.N. 2006b. Insect biodiversity in flowering teak (*Tectona grandis* L.f.) canopies: Comparison of wild and plantation stands. Forest Ecology and Management 222, 99-107.

Wang G, Yamasue, Y., Itoh, K. and Kusanagi, T., 1998. Outcrossing rates as affected by pollinators and the heterozygote advantage of *Monochoria korsakowii*. Aquatic Botany 62, 135-143.

Wonsiri S, Chanchao, C., Deowanish, S., Aemprapa, S., Chaiyawong, T., Petersen, S. and Leepitakrat, S. 2000. Honey bee diversity and bee keeping in Thailand. Bee World 81(1), 20-29.