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Pollen Viability and Pollen-tube Growth Following Controlled Pollination and their Relation to Low Fruit Production in Teak (*Tectona grandis* Linn. f.)

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Pollen released at 1100 h has the highest viability (92·2%) but is no longer viable 3 d (84 h) after anthesis. *In vitro* pollen-tube growth is fast (140 μm h^{-1}) and increases significantly within the first 8 h. *In vivo* pollen tubes also grow quickly and reach the base of the style within 2 h after pollination and enter the micropyle 8 h after pollination. There is no significant difference between self- and cross-pollination in either the rate and the number of pollen tubes in the pistil and the number of ovules penetrated by a pollen tube. Teak has late-acting gametophytic self-incompatibility; the majority of pollen tubes grow through the style but some do not continue to grow from the style towards the embryo sacs. Pollen-tube abnormalities include swollen, reversed, forked and tapered tips and irregular and spiralling tubes. These are most prevalent in self-pollination (20·4%). The index of self-incompatibility of 0·17 and low fruit set following self-pollination (2·49%) indicates that teak is mostly self-incompatible. Drastic fruit abortion occurs within the first week following controlled pollination. Within 14 d, fruit size and fruit set from cross-pollination is generally much greater than from self-pollination.

Key words: *Tectona grandis*, pollen viability, pollen-tube growth, pollination, controlled pollinations, incompatibility.

INTRODUCTION

Like many hermaphroditic species (Stephenson, 1981; Willson and Burley, 1983; Sutherland, 1987), teak (*Tectona grandis* Linn. f.) produces low fruit set due to self-incompatibility (SI) (Hedegart, 1973; Tangmitcharoen and Owens, 1997). This is also true for the two other members of the Verbenaceae; *Vitex cooperi* and *Gmelina arborea* (Bolstad and Bawa, 1982; Bawa, Perry and Beach, 1985). In teak, it was suggested that insect pollinators foraged mostly on the same tree resulting in a high level of selfing which led to lower fruit set and production of self-fruits with low germination rates (Hedegart, 1973).

There have been several studies of the reproductive biology and pollination of teak (Cameron, 1968; Bryndum and Hedegart, 1969; Hedegart, 1973; Egenti, 1974; Kedarnath, 1974; Tangmitcharoen and Owens, 1997). Bryndum and Hedegart (1969) described the procedure for controlled pollination using small bags to isolate single flowers and large bags to isolate an entire inflorescence. Hand cross-pollination was reported to increase the fertilization percentage by 6 to 60% (average 20%) compared to 0·4 to 5·1% (average 1·3%) from open-pollination. However, there has been no report on the pattern of pollen-tube growth or quantification of pollen tubes within the pistil following hand-pollinations and their role in fruit production of teak.

To ensure the success of hand-pollinations, it is important to determine pollen viability prior to pollen application. In general, pollen viability indicates the ability to deliver the sperm cells to the embryo sac after pollination (Shivanna, Linskens and Cresti, 1991). In species having gametophytic self-incompatibility (GSI), pollen tends to be binucleate and germinate readily *in vitro* (Brewbaker and Majumder, 1961; Brewbaker, 1967). Teak pollen is reported to be binucleate (Siripatanadilox, 1974) but speed or ease of germination are not known. Mulcahy and Mulcahy (1983) also suggested that pollen tubes from binucleate pollen tend to be inhibited in the style in GSI.

In angiosperms, the rate of *in vitro* pollen-tube growth varies from 60–20 000 μm h^{-1}. This is at least ten times faster than gymnosperm pollen (Hoekstra, 1983). Brewbaker and Majumder (1961) proposed that *in vitro* growth of binucleate pollen tubes is approximately 10% of *in vivo* pollen-tube growth due to limited reserve food in the pollen. However, Bryndum and Hedegart (1969) reported that teak *in vitro* pollen-tube growth rate was high, reaching a maximum of 9·3 mm in 24 h.

Self-incompatibility may occur in the stigma, style or ovary (Seavey and Bawa, 1986) but inhibition in the style is thought to be more characteristic of GSI (de Nettancourt, 1977). In open-pollination of teak (Tangmitcharoen and Owens, 1997) pollen-tube inhibition from GSI occurred anywhere along the pistil, but was mostly in the lower portion of the ovary. Incompatible pollen-tube arrest within the ovary, described as late-acting SI, has now been reported to occur primarily in woody species (Seavey and Bawa, 1986; Sage, Bertin and Williams, 1994).

This study reports on pollen viability and longevity, the patterns and the rates of *in vitro* and *in vivo* pollen-tube growth following controlled pollinations, various abnormalities observed *in vivo* pollen-tube growth, rate and form

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of SI, reproductive success, fruit set and rate of fruit abortion of teak.

MATERIALS AND METHODS

Plant material and study site

Three 40-year-old plantation teak trees growing at Muaklek, Saraburi, Thailand, (14° 40′ N, 101° 17′ E) were used. Two or three trees were used for each part of the study. Scaffolding was erected to a height of 8 m adjacent to each tree. Flowering occurs during the rainy season from June to August and the most abundant flower production occurs in July and August The study began in July 1994.

Pollen germination and pollen-tube growth

Preliminary studies indicated that media containing only sucrose may not result in optimum pollen germination. Therefore, Brewbaker's solution (Brewbaker and Kwack, 1963) was used for this study. To determine pollen germination, mature pollen was extracted at 12 different times (0, 2, 4, 6, 8, 10, 12, 24, 36, 48, 72, 84 h) after flower opening (0700 h). For each collection, two flowers per inflorescence (four inflorescences per tree) were collected from each of the three trees.

The fresh pollen from each flower was then placed in a petri dish containing 10% Brewbaker's solution with 10% sucrose for 4 h. A drop of this mixture was placed on a microscope slide and covered with a cover slip. Using a light microscope, approx. 200 pollen grains per sample were observed to determine the percentage and the time of pollen germination, i.e. when the pollen tubes were equal or greater in length than pollen diameter. To determine *in vitro* pollen-tube growth, mature pollen collected at 1300 h was placed on a drop of the same solution and covered with a coverslip. Using an eyepiece gradicule, 90 germinated grains from the three trees were measured for pollen-tube length at 1, 2, 4, 8, 12 and 24 h after sowing.

Pollination experiments

In a previous study (Tangmitcharoen and Owens, 1997), pollination success was greater in flowers which opened early and during the peak flowering season than in those which opened at the end of the flowering season. Therefore, hand pollinations were generally performed on those flowers which opened at the beginning or middle of the inflorescence flowering period. At pollination, each flower was tagged with coloured thread and one of the following pollination treatments was carried out: (1) flowers not emasculated, self-pollinated, and bagged; (2) flowers emasculated, cross-pollinated, and bagged; and (3) flowers not emasculated and unbagged (open-pollination).

In the emasculated treatments, the undehisced stamens were removed from the newly opened flowers between 0600 and 0700 h on the day of receptivity. Cellophane bags were then placed over the group of open flowers (approx. ten flowers per inflorescence) to exclude all potential pollinators. Each bag was removed from the receptive flowers for about 15 min for hand pollinations. The pollen was obtained from previously bagged flowers and was applied by tapping the anther, held with forceps, over the stigma of a receptive flower between 1100 and 1200 h. Pollen donors were chosen from trees located nearby (5 m) and further (500 m) away. For self-pollinations, pollen from the same tree was used and applied as described above.

Since approx. 1 to 3% of the flowers in a given inflorescence open each day (Tangmitcharoen and Owens, 1997), hand pollinations were carried out on successive days for each inflorescence. The bags were removed the day after all of the open flowers in the bag were pollinated to prevent inflorescence damage by heavy rains and strong winds against the bags.

Microscopy

Specimens used for transmission electron microscopy (TEM) were fixed in 2·5% glutaraldehyde with 0·075 M PO_4 buffer (pH 7·2) for 2 h at room temperature, rinsed in 0·075 M PO_4 buffer, then postfixed in 1% osmium tetroxide for 1 h. Specimens were dehydrated in an ethanol series, infiltrated with Spurr's resin (Spurr, 1969) and cured for 18 h at 60 °C. Semithin sections (1 μm) for light microscopy (LM) were stained with Richardson's stain (Richardson, Jarrett and Finke, 1960). Ultrathin sections were placed on uncoated 200-mesh copper grids, stained with 2% aqueous uranyl acetate and 0·2% lead citrate (Reynolds, 1963) and viewed with a JOEL JEM 1200 EX electron microscope.

Pollen-tube growth and self-incompatibility

To determine *in vivo* pollen-tube growth, 15 flowers from each of five inflorescences from each of two trees were collected at 1, 2, 4, 6, 8, 12, 24 and 48 h following pollination. The pistils were sliced longitudinally to remove the hairy lateral surfaces of the ovary then fixed in ethanol-acetic acid (3:1 v/v) for 24 h. After rinsing with water, they were cleared in 8N NaOH at room temperature for 2–4 d, or until most of the tissues became transparent. They were then rinsed in water and stained with 0·1% decolorized aniline blue in 0·1 K_3PO_4 modified from Dumas and Knox (1983) and Kenrick and Knox (1985). The number and lengths of pollen tubes in the pistils were examined in approx. 350 flowers using epi-fluorescence microscopy.

Fruit set and fruit size

To determine fruit set and fruit size following controlled pollination, the variously pollinated flowers were observed and monitored every day for 1 month. The index of self-incompatibility (ISI) was determined by dividing the percentage of fruit set from self-pollination by the percentage of fruit set from cross-pollination (Zapata and Arroyo, 1978).

Seed efficiency and reproductive success from open-pollination

Seed efficiency (SE) was calculated as the number of filled seeds, divided by seed potential, multiplied by 100. To

determine filled seed per fruit, 450 fruits were collected and examined using the cutting tests and x-ray at 25 kv for 60 s. Reproductive success is the product of the fruit to flower ratio (Fr/Fl) and the seed to ovule ratio (S/O) (Weins *et al.*, 1987). Ten inflorescences from each of the three trees were counted to obtain the total number of flowers. From the same inflorescences, the number of flowers that developed into mature fruits were counted.

Statistical analysis

Means ± s.e. were calculated for all measurements. Arcsine transformations were applied to percentage of pollen germination, the percentage of pollinated flowers, percentage of fruit abortion, and distribution of number of seeds per fruit prior to analysis of variance (ANOVA). The among tree variation in *in vitro* pollen germination, rate of *in vitro* and *in vivo* pollen-tube growth, and rate of fruit abortion from controlled pollinations were assessed by ANOVA. The Duncan new multiple range test at $P < 0{\cdot}05$ was used to compare the means for a significant difference among the variables.

RESULTS

In vitro *pollen germination and pollen-tube growth*

Pollen released at 1100 h (4 h after anthesis) had the highest viability (92·2%). Pollen viability gradually decreased after 1100 h and 3 d (84 h) after flower opening, pollen was no longer viable (Fig. 1). There was no significant difference in pollen germination among trees. The peak receptive period for teak flowers was 1100 to 1300 h as reported in an earlier study (Tangmitcharoen and Owens, 1997).

Pollen germination began within 1 h of being placed in Brewbaker's solution. Pollen-tube growth was fast and increased significantly each hour from 0 to 8 h. Within the first 2 h, the average rate of tube growth was about 160 μm h^{-1}. During the 3 to 4 h period it was 280 μm h^{-1}. Overall, the rate of tube growth within the first 8 h varied from 60 to 280 μm h^{-1} with an average of 140 μm h^{-1} (Fig. 2). After 8 h, there was no significant increase in pollen-tube growth. The rate of pollen-tube growth varied among trees ($P < 0{\cdot}001$).

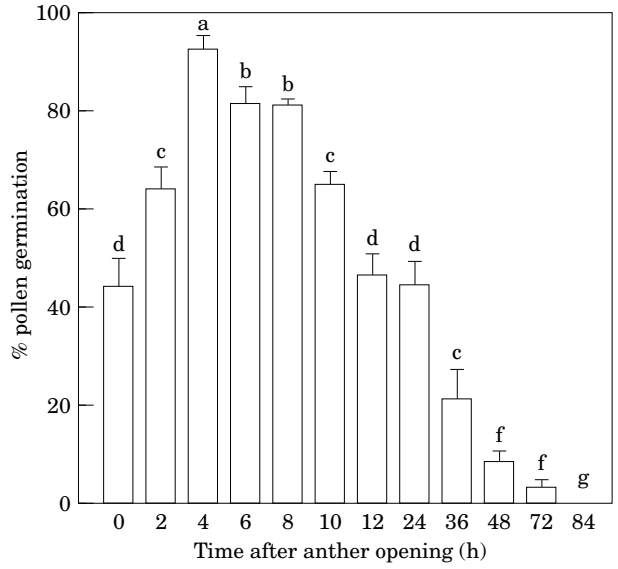

FIG. 1. *In vitro* pollen germination of teak pollen at various times after anther opening ($n = 144$). Vertical bars represent s.e. Means of each variable with the same letter are not significantly different at $P < 0{\cdot}05$ as determined by the Duncan new multiple range test.

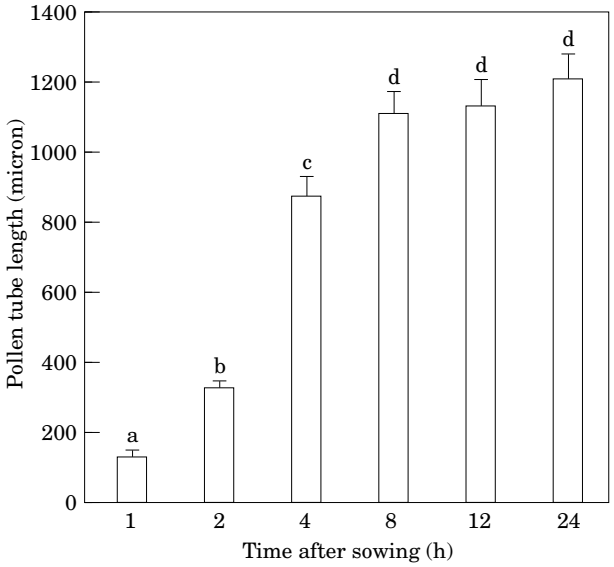

FIG. 2. *In vitro* pollen-tube growth of teak up to 24 h after sowing. Vertical bars represent s.e. Means of each variable with the same letter are not significantly different at $P < 0{\cdot}05$ as determined by the Duncan new multiple range test.

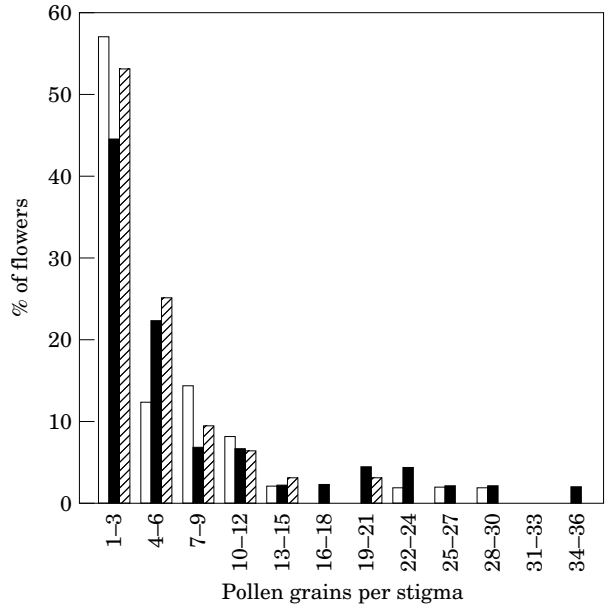

FIG. 3. Percentage of flowers having different numbers of pollen grains on the stigma of control-pollinated teak flowers; self (□), $n = 49$; open (■), $n = 54$; cross (▨), $n = 29$.

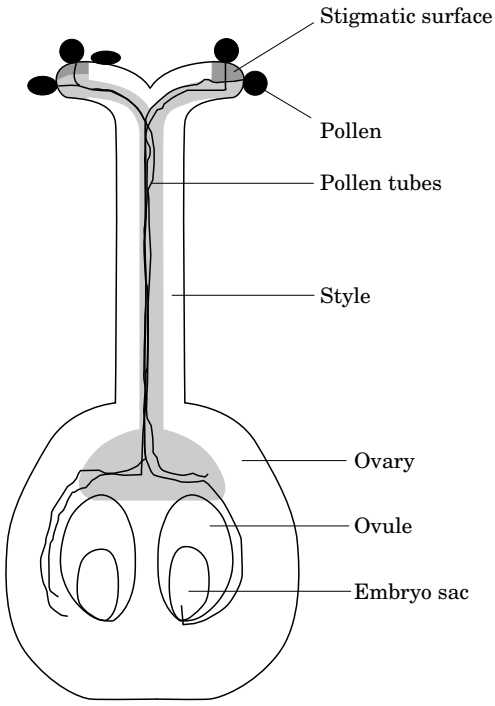

FIG. 4. Diagram of teak flower showing the pathway of pollen tubes through the stigma and hollow stylar transmitting tissue (shaded area) to the embryo sacs.

In vivo *pollen-tube growth following controlled pollination*

Of the 225 flowers observed following control self-, open- and cross-pollinations, 30 to 50% (average 35% ± 4·16) had pollen adhering to the stigma. The number of pollen grains on the stigma varied from 1 to 38 with an average of 6·2 ± 0·63 ($n = 130$), but most commonly there were only 1 to 3 grains (Fig. 3). The percentage of pollen adhering to the stigma did not differ significantly between the three types of pollination.

The pathway of pollen-tube growth to the embryo sacs is shown in Fig. 4. In all three types of pollination, a few pollen grains did not germinate on the stigma (Table 2). Pollen germinated within 1 h of being placed on a receptive stigma. Generally, self-, open-(natural), and cross-pollen tubes grew at the same rate and there were similar numbers of pollen tubes growing through the pistil for the first 24 h. The average number of pollen tubes in the style varied from 5 to 8. The majority of pollen tubes grew through the style (Fig. 6) but some did not continue to grow from the style towards the embryo sacs (Fig. 7).

Pollen-tube growth was fast during the first 4 h. Four h after pollination, pollen tubes of 24% of self-, 29% of open- and 8% of cross-pollinated flowers had reached an ovule and about 7% of pollen tubes from open-pollinated plants had reached a micropyle (Fig. 5A). The first pollen tubes were observed to reach the embryo sacs 8 h after pollination (Fig. 5B). Pollen-tube growth was slow between the 8 to 12 h and the 20 to 24 h observation periods. At the 20 to 24 h observation period, 70 to 80% of pollen tubes from the three types of pollination had reached the ovules, 40 to 60% of pollen tubes had reached the micropyle, but very few (< 5%) of pollen tubes from self- and open-pollinated flowers and about 20% from cross-pollinated plants had reached the embryo sacs (Fig. 5C). The number of pollen tubes at different positions within the pistil at 20 to 24 h following controlled pollination is shown in Table 1.

Of the 84 flowers observed 8 to 24 h after pollination, the number of flowers in which pollen tubes entered the micropyle did not differ significantly among the three types of pollination. The average number of pollen tubes in the various portions of the pistil 20 to 24 h after pollination is shown in Table 1. The number of pollen tubes entering the micropyle per flower varied from 0 to 3 but was not significantly different among the three types of pollination (Table 1). In general, only one micropyle per flower was entered by a pollen tube even though four micropyles exist per flower. Occasionally two, very rarely three and never four micropyles per flower were observed to contain pollen tubes. Observation of the pistil at 28, 32, 36 and 48 h after pollination indicated that there was no increase in the number of pollen tubes entering the micropyles after 24 h.

Abnormalities of pollen-tube growth

In the 132 pistils observed during the 24 h following controlled pollination, most pollen tubes were straight with thin pollen-tube walls and tapered tips. Pollen-tube inhibition on the stigma occasionally occurred in all types of pollination (Fig. 8). Several abnormalities occurred in pollen-tube growth at various positions within the pistil (Table 2). Abnormalities included reversing tubes (Fig. 9),

TABLE 1. *Comparison of mean numbers (± s.e.) of pollen tubes entering the stigma, at 25, 50, 75 and 100% of the style length, and in the ovule, micropyle and embryo sac at 20–24 h following pollinations*

Distance through pistil	Types of pollination			ANOVA		Average
	Self-	Open-	Cross-	*F* values	*P*	
25% of style	7·8 ± 2·0	6·6 ± 2·5	5·2 ± 1·1	0·45	0·64 NS	6·7
50% of style	7·7 ± 2·0	6·4 ± 2·5	4·9 ± 1·1	0·52	0·60 NS	6·6
75% of style	7·3 ± 2·0	6·4 ± 2·5	4·9 ± 1·1	0·50	0·62 NS	6·4
100% of style	7·3 ± 2·0	6·2 ± 2·5	4·9 ± 1·1	0·39	0·68 NS	6·3
Ovule	6·6 ± 1·9	6·0 ± 2·4	4·6 ± 1·2	0·28	0·76 NS	5·8
Micropyle	3·9 ± 1·3	4·3 ± 2·0	2·0 ± 0·63	0·70	0·50 NS	3·5
Embryo sac	0·2 ± 0·09	0·5 ± 0·22	0·5 ± 0·14	1·28	0·29 NS	0·3

NS, non significant.

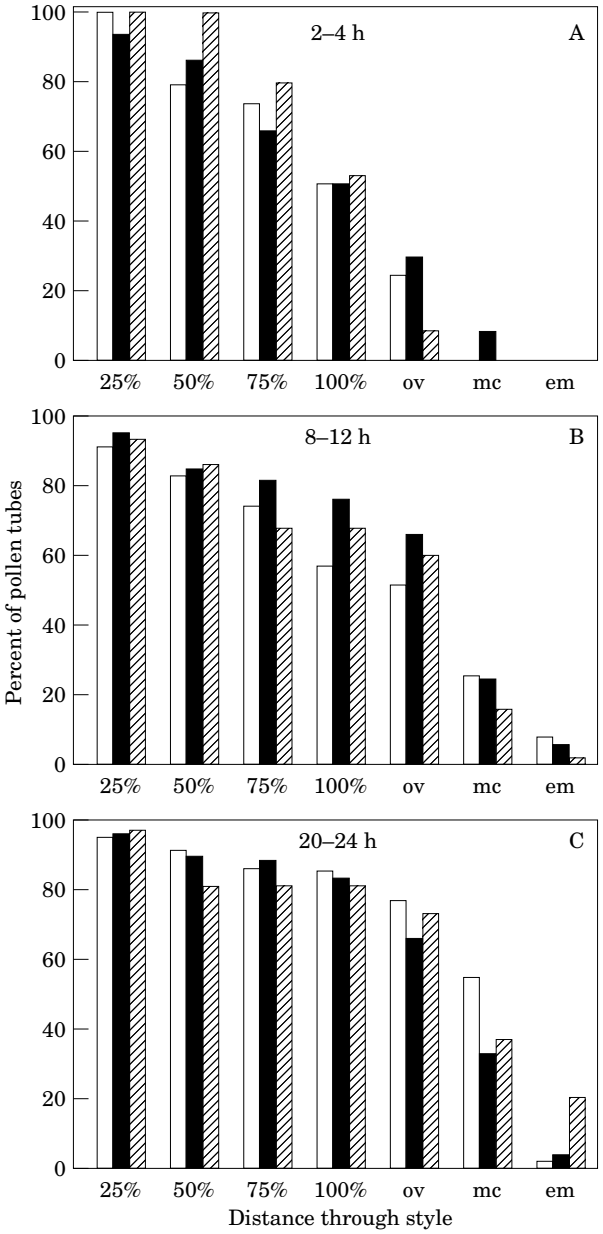

FIG. 5. Percentage of self- (□), open- (natural) (■), and cross- (▨) pollen tubes at different positions in the teak pistil after pollination. A, At 2–4 h, self- ($n = 14$); open- ($n = 14$); cross- ($n = 4$). B, At 8–12 h, self- ($n = 14$); open- ($n = 31$); cross- ($n = 11$). C, At 20–24 h, self- ($n = 17$); open- ($n = 45$); cross- ($n = 9$). 25, 50, 75 and 100% refer to percent of the style length; ov, ovule; mc, micropyle; em, embryo sac.

irregular or spiralling tubes (Fig. 10), and an increase of callose deposits resulting in swelling of the tube tip (Figs 11 and 12). Abnormalities were most prevalent in the self-pollinated flowers (20·4%) (Table 2). Swelling of the tube tip was most common, accounting for 41·7% of total abnormal tubes.

Fruit set and rate of fruit abortion following controlled pollination

The index of self incompatibility (ISI), determined by dividing the percentage fruit set from self-pollination (2·49) by the percentage fruit set from cross-pollination (14·54) was 0·17, indicating that teak is mostly self-incompatible (Zapata and Arroyo, 1978). Fruit set for self- and open-pollinated flowers measured 30 d after pollination differed significantly ($P < 0·01$) from cross-pollinated flowers. Only 2·49% of self-pollinated flowers and 6·54% of open-pollinated flowers set fruit, whereas 14·54% of cross-pollinated flowers set fruit (Table 3). There was no significant difference in percent fruit set among the two trees used for this portion of the study for all types of pollination. The rate of fruit abortion was high during the first 10 d. There was a tendency for a lower percentage of fruit abortion following cross-pollination during the 5 to 20 d period after pollination (Fig. 13).

Seed efficiency and reproductive success with open-pollination

In open-pollination, the seed to ovule (S/O) ratio was 0·33 and the fruit to flower (Fr/Fl) ratio was 0·035. Reproductive success, determined by Fr/Fl multiplied by S/O, was low (0·011). The results of cutting tests and x-ray revealed that most commonly (46·2%) only one of four ovules per flower developed into a mature seed, 30·9% contained two seeds per fruit, 8% contained 3 to 4 seeds and 14·9% of fruits contained no filled seeds (Table 4). There was no significant difference in seeds per fruit among the three trees.

Fruit size during early development stages

Fruit size during early development, up to 10 d after pollination, differed significantly ($P < 0·0001$) among the types of pollination but not among the two trees included in this part of the study. Self-pollination resulted in smaller fruits. Unfortunately, the high abortion rate due to selfing made fruit size data unavailable after 10 d. Mean fruit size resulting from open- and cross-pollination was essentially the same through the first 28 d of development (Table 5).

DISCUSSION

In vitro *pollen germination*

Binucleate pollen generally germinates readily in culture (Mulcahy and Mulcahy, 1983). This was also true for binucleate teak pollen in which germination began within 1 h of pollen being placed in the medium. Using a 14% sucrose medium for *in vitro* pollen germination of teak, Egenti (1974) and Bryndum and Hedegart (1969) reported that pollen collected during the 1200–1400 h period on the day of anthesis had the highest percentage of viability compared to that collected from 0730–1000 h and at 1800 h. They also found that 10% of the pollen was still viable 3 d after anthesis. Using Brewbaker's solution with 10% sucrose, results from our studies show some differences to those of previous studies. Germination was best for pollen collected 1 h earlier (1100 h) than in previous studies, but the pollen also remained viable for 3 d.

FIGS 6 and 7. Epi-fluorescence micrographs of pollinated teak pistils within 24 h of pollination. Pistils were stained with decolorized aniline blue to localize callose. Fig. 6. Within 4 h, a large number of pollen germinate on the stigma papillae and produce pollen tubes that grow into the stylar canal (arrowheads). Fig. 7. Arrested pollen tubes in the ovary 24 h after pollination. A dense bundle of pollen tubes (between arrowheads) was generally seen in the lower stylar transmitting tissue but few grew towards the lower portion of the ovary and entered the micropyles. Bars = 0·5 and 0·2 mm in Figs 6 and 7, respectively. P, Pollen; OV, ovule.

FIGS 8–12. Abnormalities of pollen tubes found at various sites and times following pollination. Fig. 8. Inhibition of a pollen tube on the stigma 12 h after self-pollination. Fig. 9. Reversal of pollen-tube growth (arrowheads) in the style 4 h after self-pollination. Fig. 10. Irregular pollen tube with callose deposition in pollen tube wall (arrowhead) in the upper portion of the ovary 8 h after open-pollination. Fig. 11. Light micrograph showing swelling of pollen tube (arrowhead) in the lower portion of the ovary about 24 h after self-pollination. Fig. 12. Transmission electron micrograph showing the thickened cell wall and small amount of cytoplasm (*) of the tube tip shown in Fig. 11. Bars = 50 mm in Fig. 8; 0·3 mm in Figs 9 and 10; 20 μm in Fig. 11; and 10 μm in Fig. 12. P, Pollen; ST, stigma; PT, pollen tube; OV, ovule; OW, ovary wall.

In general, most pollen collected on the day of anthesis developed normal pollen tubes and exhibited more uniform pollen-tube growth than pollen collected on or after the second day. The highest *in vitro* pollen germination (92·2 %) occurred in pollen released at 1100 h. This indicates that the pollen used for control pollination was highly viable.

TABLE 2. *Number of flowers that had different abnormalities of pollen-tube growth on the stigma and at various positions within the pistil within 24 h following controlled pollination*

Distance through pistil	Types of pollination		
	Self-	Open-	Cross-
Stigma*	4	3	2
50% of style	0	1 R	1 R
75% of style	2 S 1 R	1 R	0
100% of style	0	0	1 R
Upper ovary	0	0	1 I
Lower ovary	3 S	1 I	0
Total number of flowers having abnormal pollen tubes	10 (20·41%)	6 (11·11%)	5 (17·24%)
Total number of flowers observed	49	54	29

* Stigma with pollen adhering but without pollen tube growth. R, Reversing tubes; S, swollen tube tip; I, irregular tube.

TABLE 3. *Number and mean (±s.e.) percentage of fruit set 30 d after controlled pollination*

	Tree number	Types of pollination		
		Self-	Open-	Cross-
Number of inflorescences treated	1	3	4	6
	2	4	5	6
Number of flowers treated	1	30	126	67
	2	52	109	75
Number of fruits set	1	1	2	10
	2	1	10	12
% fruit set	1	3·33	1·59	14·82
	2	1·92	9·17	15·54
Total (% fruit set)		2·49[a] ±1·61	6·54[a] ±4·65	14·54[b] ±2·77

Means of each variable followed by the same superscript are not significantly different ($P < 0·05$) as determined by the Duncan new multiple range test.

Therefore, most of the failure of pollen-tube growth which occurred after control pollination was not due to the lack of pollen vigour and viability, but probably due to incompatibility.

In vitro *and* in vivo *pollen-tube growth in controlled pollination*

There are extreme variations in *in vivo* and *in vitro* pollen-tube growth rate in angiosperms (Hoekstra, 1983). However, there are few reports of *in vivo* or *in vitro* pollen-tube growth in tropical forest trees. Rapid *in vivo* pollen-tube growth has been reported in *Acacia retinotes* in which the pollen tubes reached the ovule within 11 h (Kenrick and Knox, 1985). In other cases growth is slow; it took 10–20 d for pollen tubes of *Eucalyptus woodwardii* (Sedgley and Smith, 1989) to reach the ovules. In our study, the average rate of *in vitro* pollen-tube growth varied from 60–280 $\mu m\ h^{-1}$ which is similar to the majority (19 of the 26) of angiosperm families

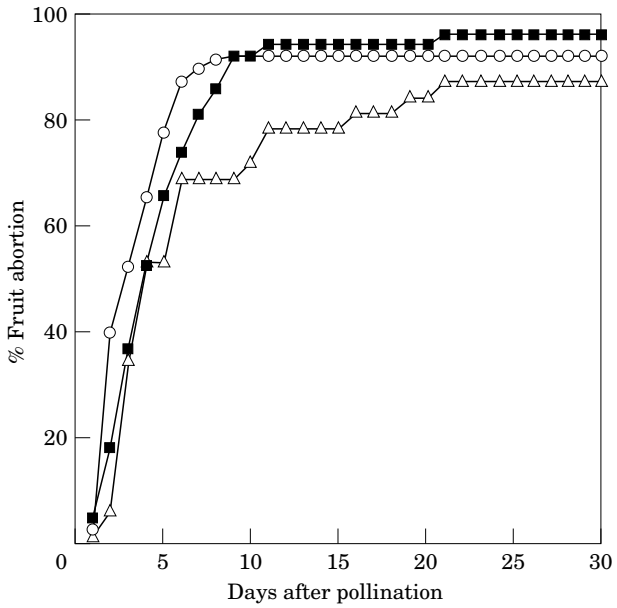

FIG. 13. Effect of self- (■), open- (○), an cross- (△) pollination on fruit abortion for the 30 d after pollination based on 14 inflorescences on each of the two teak trees.

TABLE 4. *Frequency distribution of percentage fruit having 0–4 seeds per fruit 30 d after pollination*

	Numbers of seeds per fruit					
	0	1	2	3	4	Total
Percentage of fruits	14·9[a]	46·2[b]	30·9[c]	6·22[d]	1·78[d]	100
Numbers of fruits	67	208	139	28	8	450
s.e.	3·5	3·7	4·3	2·1	0·69	

Means of each variable followed by the same superscript are not significantly different ($P < 0·05$) as determined by Duncan new multiple range test.

TABLE 5. *Comparison of means (±s.e.) of fruit sizes in mm during early development*

Days after pollinations	Types of pollination		
	Self-	Open-	Cross-
4	2·25±0·07 (20)[a]	2·36±0·06 (20)[b]	2·45±0·08 (19)[b]
7	2·42±0·09 (20)[a]	3·86±0·12 (20)[b]	4·2±0·58 (17)[b]
10	3·07±0·09 (14)[a]	5·71±0·13 (19)[b]	6·41±0·16 (14)[b]
14	N/A	7·03±0·21 (18)	7·46±0·73 (16)
21	N/A	12·09±0·49 (15)	12·94±0·58 (14)
28	N/A	14·15±0·33 (11)	14·17±0·34 (13)

Numbers in brackets represent the number of fruits observed. Means of each variable followed by the same superscript are not significantly different ($P < 0·05$) as determined by the Duncan new multiple range test. N/A, Data not available due to high abortion rate followed self-pollination.

reported by Hoekstra (1983). In our study pollen tubes reached about 1·2 mm in 24 h. This contrasts with studies by Bryndum and Hedegart (1969) for teak where *in vitro*

pollen-tube growth was very fast, up to 9·3 mm with an average of 5·53 mm in 24 h. Although the rate of *in vitro* pollen-tube growth in teak would be considered fast, it was not when compared to the length of pistil. Pistil length averaged 6·55 mm and consequently 24 h after sowing pollen tubes *in vitro* were only about 18 % of the way down the pistil. The *in vitro* growth rates underestimate the *in vivo* rates in teak in which some pollen tubes reached the ovules 4 h after pollination. Our results agree with the study by Brewbaker and Majumder (1961) that *in vitro* growth of binucleate pollen tubes was approx. 10 % of *in vivo* pollen-tube growth.

Rosen and Gawlick (1966) and Rosen (1971) proposed that *in vivo* binucleate pollen-tube growth consists of two phases: autotrophic and heterotrophic. In the first autotrophic phase pollen tubes grow from their own reserves. Pollen grains usually contain enough reserve food to support germination (Johri, 1992). Pollen-tube growth during the first phase is relatively slow and free of callose plugs (Mulcahy and Mulcahy, 1983). Brewbaker (1967) suggested that this phase ends with gamete formation. Pollen-tube growth *in vitro*, or in an incompatible style, may terminate at this point. The second, heterotrophic, phase is observed only *in vivo* within a compatible style. During this phase, pollen tubes grow rapidly and form callose plugs, indicating a shift to heterotrophic nutrition (uptake of substances from the pistil). This may account for the limited teak pollen-tube length *in vitro* using Brewbaker's media. It may not contain all of the nutrients needed for the transition of pollen tubes to the second phase (Read, Bacic and Clarke, 1992).

The continual rapid pollen-tube growth in the stylar canal of teak may be related to movement of the specific proteins from the transmitting tissue, as suggested by Mascarenhas (1993). Hohri (1992) also suggested that a pollen tube growing in a hollow style, as in teak, derives nourishment from the glandular epidermis of the stylar canal and the adjacent tissue becomes depleted of metabolites. Despite these complications, *in vitro* pollen-tube growth measurements reflect some useful pollen properties (Hoekstra, 1983).

Based on our observations of *in vivo* pollen-tube growth, it is likely that the period for the first phase of *in vivo* pollen-tube growth in teak was very short (probably less than 30 min). Within 1 h following pollination, callose plugs were found near the stigma which corresponds to first phase of growth.

The density of pollen deposited on the stigma is reported to affect both pollen germination and rate of pollen-tube growth in some tropical forest trees, including *Leucaena leucocephala* (Ganeshaiah, Shaankar and Shivashankar, 1986). Our study showed that this may not be true for teak. The density of pollen deposited on the teak stigma did not affect the number of pollen tubes entering the micropyle in any pollination trials. Even when the teak stigma was saturated with pollen (up to 38 grains per stigma), the ovule often remained unpenetrated. There was little pollen competition in the teak style, i.e. an increase in pollen load on the stigma resulted in a proportional increase in the number of pollen tubes penetrating to the base of the ovary.

Pollen-tube inhibition and incompatibility in teak

There was no difference in the number or sites of pollen-tube inhibition among open-, self-, and cross-pollinations in teak until pollen tubes reached the embryo sac (Fig. 5). Also, in the previous report on open-(natural) pollination for teak (Tangmitcharoen and Owens, 1997), the site of the final pollen-tube arrest was generally at the ovary, and most pollen tubes did not enter the embryo sac. Other studies in *Eucalyptus woodwardii* (Sedgley and Smith, 1989) and *Medicago sativa* (Cooper and Brink, 1940; Sayers and Murphy, 1966) have shown that the number of penetrated ovules following self-pollination was less than that following cross-pollination.

In angiosperms, self-incompatibility (SI) systems that operate in the ovary, described as late-acting SI systems, were initially assumed to be uncommon in angiosperms (de Nettancourt, 1977). Seavey and Bawa (1986) reported that this notion was erroneous: the scarcity of late-acting SI may have been exaggerated by the preference for studies of breeding systems for small, short-lived, herbaceous plants. Late-acting SI has been reported to be important in the breeding systems of many angiosperms and may be more important in woody angiosperms (Seavey and Bawa, 1986; Sage *et al.*, 1994). They were classified into four categories: (1) ovarian inhibition of incompatible pollen tubes before the ovule is reached; (2) pre-fertilization inhibition in the ovule; (3) post-zygotic rejection of the embryo; and (4) ovular inhibition. Late-acting pollen-tube inhibition has been reported in some hardwood species; *Acacia retinodes* (Kenrick, Kaul and Williams, 1986), *Eucalyptus morrisbyi* (Potts and Savva, 1989), and *E. woodwardii* (Sedgley and Smith, 1989). In these, self- and cross-pollen tubes appear similar through the stigma and style, but self-pollen tubes were inhibited in the ovary. In teak, the late-acting system appears to be associated with inhibition after pollen tubes enter the ovule, however, a post-zygotic stage has not been ruled out. Post-zygotic outcrossing mechanisms have been reported in some tropical woody species including four species of *Eucalyptus* (Griffin, Moran and Fripp, 1987; Ellis and Sedgley, 1992) and mango (Shama and Singh, 1970).

In all pollination trials, a variety of pollen-tube abnormalities were found at various locations in the pistil and at different times after pollination. Swollen tips, reversing tips, forked tips, tapered tips, irregular tubes and spiralling tubes were observed. There was a tendency towards higher pollen-tube abnormalities in self-pollinated flowers than open- and cross-pollinated flowers (Table 2).

Fruit set following controlled pollinations

Low fruit set is typical of many hermaphroditic plant species (Stephenson, 1981; Willson and Burley, 1983; Sutherland and Delph, 1984; Sutherland, 1986, 1987; Guitian, 1993). Zapata and Arroyo (1978) classified the ISI as: > 1 = self-compatible; $> 0{\cdot}2 < 1$ = partially self-incompatible; $< 0{\cdot}2$ = mostly self-incompatible; and 0 = completely self-incompatible. The index of ISI was 0·17 in teak, placing it in the category of mostly self-incompatible. This helps explain the high rate of fruit abortion that occurs

after self-pollination, but some self-pollinated flowers can still develop into fruits.

Dafni (1992) ranked fruit set percent following self-pollination as: 0–3 % (class 0) = self-incompatible; 3–30 % (class 1) = slightly self-compatible; and, > 30 % (class 2) = highly self-compatible. Our study shows the rate of fruit set following self-pollination in teak was 2·49 %, which again classifies teak as a self-incompatible species. Fruit set did not differ significantly between self- and open-pollination which suggests a high incidence of self-pollination in nature or very closely related neighbouring trees. Results from our study support an earlier study by Bryndum and Hedegart (1969) in which less than 1 % of self-pollinated flowers developed into fruits. Evidence from the present and our earlier study (Tangmitcharoen and Owens, 1997) show that teak has late-acting GSI, where the pollen-pistil interaction is genetically controlled by the haploid (gametophytic) genome of each pollen tube as it penetrates the diploid pistil (Sedgley and Griffin, 1989). Low fruit set has also been reported in two other species from the Verbenaceae, *Vitex cooperi* and *Gmilina aborlia* (Bolstad and Bawa, 1982; Bawa, Perry and Beach, 1985), but in these it was not necessarily attributed to self-incompatibility.

In teak, drastic fruit abortion occurred within the first week following control pollination and within 14 d fruit size and fruit set from cross-pollination was generally much greater than from self-pollination. Ackerman (1961) reported that fruit set from self-pollination of *Ziziphus* (chinese jujubes) was less and fruit had a greater tendency to drop prematurely than from cross-pollination. In general, an increase in pollen load by supplementary pollination leads to an increase in fruit set (Sutherland and Delph, 1984; Sutherland, 1987). This was shown in macadamia where an increased number of pollen tubes following cross-pollination increased initial fruit set (Ito, Eyre and Cabral, 1983; Sedgley *et al*., 1990).

Insufficient insect pollinators and their effectiveness appear to be major causes for limited fruit set in teak (Hedegart, 1973; Tangmitcharoen and Owens, 1997). Insects forage mostly among the inflorescences of the same tree or nearby relatives, which contribute to inbred fruits (Hedegart, 1973). This results in a lack of heterozygosity, low fruit set and low germination rate in teak. However, Kertadikara and Prat (1995) suggested that there is high heterozygosity in teak populations and it may be maintained by early elimination of self-material through embryo abortion, and low germination rate.

Low fruit set after cross-pollination in many tropical forest species probably results from three factors (Bawa *et al*., 1985): an artifact of hand-pollination (Bawa *et al*., 1985); a predetermined abortion rate (Bawa and Webb, 1984); and inbreeding depression from close relative cross-pollination (Haber and Frankie, 1982). In our study these factors may have contributed to the relatively high (85·46 %) fruit abortion of teak with cross-pollination. Even in cross-pollinated flowers, only $30 \pm 6{\cdot}1$ % ($n = 95$) of the flowers had pollen on the stigma and fruit abortion occurred more often if hand-pollination was performed on the flowers which developed at the end of the flowering period in an inflorescence, in particular the outermost flowers of the inflorescence. Also the trees used in our study may be close relatives since they were located in the same plantation.

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