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Floral Biology, Pollination, Pistil Receptivity, and Pollen Tube Growth of Teak (*Tectona grandis* Linn f.)

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Teak flowers are weakly protandrous and pollen is shed within a few hours of flower opening. Pollen is tricolpate and 29 μm in diameter. The papillate stigma is of the wet type and is receptive from 1100–1300h. The style is hollow throughout its length. Nectar and pollen are the major floral rewards for pollinators. The major pollinators are *Ceratina* sp. which carry teak pollen on most parts of their bodies, especially the specialized hair structures (scopal brushes) on the tibia. The most effective pollination period in terms of flowers pollinated and pollen per flower is between 0900 and 1300h. At 1300h the number of pollen per flower is the highest, ranging from 1–36 (average 7). Pollen tubes grow very fast. Within 2 h after pollination 8 % of the pollen tubes have reached the micropylar end of the ovule and pollen tubes first enter the embryo sac at 8 h. Only one to two pollen tubes enter the micropyles of a flower. Although 78 % of flowers were pollinated in open-pollination, the low fruit set (3·5 %) suggests that there are factors other than pollination limiting fruit set. The main factor appears to be a high amount of selfing, and self-incompatibility occurs when pollen tubes are arrested at the lower portion of the ovary.

Key words: *Tectona grandis*, floral biology, pollen tube growth, pollination, receptivity, pollinators.

INTRODUCTION

Tectona grandis Linn f. (teak), a member of the family Verbenaceae, order Laminales (Trope, 1921), is of interest as a timber species because of its unique wood properties and the value of its wood (Anon., 1956). Teak is a deciduous tree throughout most of its range and is phenologically classified, according to Longman and Jenik (1987), as a periodic-growth deciduous type. It is native to South-East Asia and occurs naturally only in the Indian Peninsula, Myanmar, Northern Thailand and Northwestern Laos along the northern Thai border (Troup, 1921; Mahaphol, 1954; Anon., 1956; Kermode, 1957; Ko Ko Gyi, 1972; Kaosa-ard, 1977). Teak is one of the most extensively planted tree species in the tropics; both as a native and as an exotic. Despite the extensive planting programmes little is known about its reproductive biology. The first studies of the reproductive biology, mainly on flowering and fruiting, were begun about 30 years ago at the Teak Improvement Centre (TIC) in northern Thailand (Bryndum and Hedegart, 1969). However, little attention was paid to the pollen, the pollination mechanism, pollen and stigma–style relations, pollen tube growth, or the behaviour of insect pollinators and their important role in the reproductive success of teak.

Investigations carried out at the TIC indicated that fruit set in teak from open pollination was only 0·4–5·1 % (average 1·3 %) (Hedegart, 1973). This low fruit to flower ratio is generally found in hermaphroditic plants (Sutherland, 1986) which exhibit self-incompatibility. Teak is primarily an out-crossing species, but self-pollination is possible (Bryndum and Hedegart, 1969; Hedegart, 1973). The extent of self-incompatibility in teak varies from 96–100 % (Hedegart, 1973), and commonly less than 1 % of self-pollinated flowers develop into fruits (Hedegart, 1976). Low fruit set in nature may be largely due to a high incidence of self-pollination and a high level of self-incompatibility, but several other causes, such as resource limitation and position of fruit within inflorescences, may also be involved (Bawa and Webb, 1984). There is no report about the mechanism and the site at which self-incompatibility occurs in teak. Self-incompatibility has also been reported in *Gmelina arborea* L., another member of the Verbenaceae (Bolstad and Bawa, 1982). In this species no self-pollinated flowers develop into mature fruits, although many of the fruits develop to different sizes before they abort.

Kedarnath (1974) reported protandry in teak but without specific comment on the exact times of pollen dispersal and stigma receptivity. In a preliminary study of stigma receptivity, Egenti (1978) reported that the stigma was still receptive 1 d after anthesis. This was contrary to the study by Hedegart (1973), in which the optimum pollination period was only a few hours (1130–1300 h) during the day of flower opening. Siripatanadilox (1974) also reported that the stigma was receptive at 1130h. There is no report on stigma development during the receptive period or style structure in teak. Heslop-Harrison and Shivanna (1977) classify the stigma of the Verbenaceae as wet and papillate. A stigma is categorized as wet on the basis of a free-flowing surface exudate secreted by stigmatic cells (Dumas *et al.*, 1978; Sedgley 1981; Knox *et al.*, 1989). The anatomical organization of the style may be hollow or solid (Hanf,

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1936) but this is not known for teak. The stigma papillae and the transmitting tissue of both hollow and solid styles have secretory cells which produce the medium (Knox, 1984) which guides the pollen tubes on their route from the stigma to the ovule (Weber, 1994).

Many studies show that teak is insect-pollinated (Horne, 1961; Cameron, 1968; Bryndum and Hedegart, 1969; Egenti, 1974; Kedarnath, 1974). Observations at the TIC indicated that two bee species, *Ceratina hieroglyphica* Sm., of the Anthophoridae and *Heriades binghami* Dover (*H. partula* Bingham) of the Megachilidae are important teak pollinators. In Nigeria, Egenti (1981) found a relationship between fruit production and frequency of insect visits to teak flowers.

It is evident that fruit production in teak is very low when compared to the abundance of flowers commonly produced. However, the causes of low fruit production are uncertain, in part because many aspects of the reproductive biology of teak are unknown or incomplete. The purpose of the present study is to supply some of this missing information by reporting on teak flower structure and development during the prefertilization period, concentrating on the time of receptivity, floral nectaries, development of the stigma papillae, insect visitations, the pollination mechanism including pollen deposition, pollen-tube growth and the site of incompatibility. Subsequent embryo, seed and fruit development and abortion are covered in another study from our laboratory (Palupi, 1996).

MATERIALS AND METHODS

Plant material and study site

The study was conducted in 1992 at Muak-lek, Saraburi, Thailand, (14° 40′ N and 101° 17′ E) at about 200 m elevation. The daily mean maximum and minimum temperatures, and mean temperatures per month in 1990–1993 averaged 32·21, 20·97, and 26·58 °C, respectively. The relative humidity averaged 78 % and the annual rainfall was 1135 mm. Three *T. grandis* trees were selected from a teak plantation based on accessibility, flowering performance and isolation. Scaffolding was erected to a height of 8 m around each tree. The study began in Jun. 1992 and abundant flowers were produced during the Jun.–Aug. rainy season.

Microscopy

Specimens to be used for scanning electron microscopy (SEM) were fixed in formalin-acetic acid-alcohol (FAA) and subjected to a dehydration series to 100 % ethanol. They were then critical-point dried, mounted on aluminum stubs, sputter-coated with gold and observed using a JEOL JS M-35 SEM at 15 kv. Specimens to be used for transmission electron microscopy (TEM) were fixed in 2·5 % glutaraldehyde in 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 structure and pollen shedding

A flower was open and receptive for less than 1 d. On the day of receptivity, flowers in several inflorescences were tagged with coloured thread and anthers were periodically collected to examine morphological changes and to determine the period of pollen shedding. Both dehydrated and hydrated pollen grains were examined using LM and SEM to determine pollen morphology. Dehydrated pollen grains were removed from the anthers and directly mounted on stubs, coated with gold, and observed using SEM. To measure pollen size, dehydrated pollen grains were dry mounted on glass slides. Hydrated pollen was fixed in FAA, rinsed with water and placed on a slide with a cover slip. Hydrated and unhydrated pollen grains were measured by means of an eyepiece micrometer using LM. Three hundred hydrated and unhydrated pollen grains from each of three trees were measured.

Floral receptivity

To determine the morphological changes of individual flowers during receptivity flowers were tagged with coloured thread, and fifteen flowers from each of five inflorescences from each of the three trees were collected at −4, 0, 4, 8, 12, 16, 20, 24, 48, and 72 h from flower opening (0 h). Flowers were observed using a dissecting microscope and the characteristics were recorded. Pistils during the early and late receptive periods were stained with periodic acid Schiff's reagent (PAS) modified from Pearse (1972) for localization of nectariferous tissue. To determine stigma receptivity flowers fixed in FAA were examined using the SEM, and resin embedded sections were observed using LM and TEM procedures. The development of the stigma during the receptive period was observed.

Insect visitors to flowers

Using a hand net, the insects visiting teak flowers were collected for identification at 0800–1000h, 1000–1200h, 1200–1400h and 1400–1600h for 8 d in Jul. Diversity, abundance, timing of visitation, and behaviour of insects were recorded. Collected insects were killed in a glass container containing cotton saturated with CCl_4 and sent to the Entomology and Zoology Division, Ministry of Agriculture and Cooperatives, Bangkok, Thailand for identification. The identified insects were then examined under the dissecting microscope to assess their comparative efficiency for carrying pollen. To identify pollen on their bodies some insects of each species carrying pollen were examined using SEM at 15 kv.

Monitoring of pollen tube growth

To determine *in vivo* pollen tube growth, flowers that had been tagged and pollinated were collected at 14 different

times (2, 4, 6, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44 and 48 h) after each flower had become receptive (1100h). Pistils were longitudinally sliced to remove the hairy lateral surfaces of the ovary and fixed in ethanol-acetic acid (3:1 v/v) for 24 h. After rinsing with water, pistils were cleared in 8N NaOH at room temperature for 2–4 d, or until most tissues became transparent. They were then rinsed in water and stained with 0·1% aniline blue in 0·1 K_3PO_4 modified from Dumas and Knox (1983) and Kendrick and Knox (1985). The number of pollen tubes and the rate of pollen tube growth in the style were examined using fluorescence microscopy. To observe the penetration of pollen tubes, styles and the transmission tissue within were observed using a cryo-fracturing procedure modified from Tanaka (1989). Styles were excised as small pieces, fixed in 2·5% glutaraldehyde in 0·075 M PO_4 buffer (pH 7·2) for 2 h at room temperature, rinsed in 0·075 M PO_4 buffer and placed in 10, 25, then 50% dimethyl sulfoxide (DMSO). They were then rapidly frozen with liquid nitrogen and cracked using liquid-nitrogen-chilled forceps. The frozen tissues were immediately placed into 50% DMSO, rinsed in 0·075 M PO_4 buffer, placed in 0·1% osmium tetroxide at 20 °C for 1 d then postfixed with 2% osmium tetroxide at 4 °C for 3 h. They were then rinsed in water several times, dehydrated in an alcohol series, critical-point dried, coated with gold and observed with the SEM at 20 kv.

Pollen production, pollination success, pollen–ovule ratio and fruit set

To determine the number of pollen grains per flower, one mature anther per flower was removed from five unopened flowers from five inflorescences from each of two trees. Anthers were gently squashed on a 1 mm graticule slide, stained with aceto-carmine, covered with a 12 mm circular coverslip and pollen grains were counted. To determine the number of pollen grains per stigma, stigmas were removed from flowers tagged at flower opening and stained with aceto-carmine at 0, 2, 4, 6, 8 h after flower opening. Pollination success was calculated as the percentage of pollinated flowers. The pollen–ovule ratios were determined by dividing the average number of pollen grains per flower by the average number of ovules per flower. Pollination efficiency was determined by dividing the number of pollen grains per stigma by the number of pollen grains produced per flower. To determine fruit set, ten inflorescences from each of three trees were flagged and the number of flowers at pollination and the number of fruits remaining 2 months after pollination were counted.

Statistical analysis

Means $\pm$ the standard error of the mean were calculated for all measurements. Arc-sine transformations were applied to the percentage of fruit set and the percentage of pollinated flowers before analysis of variance (ANOVA) was carried out. The among tree variation in pollen size, the number of pollen grains per flower, pollination success, and fruit set were assessed by ANOVA. The Duncan new multiple range test at $P < 0{\cdot}05$ was used to compare the means if there was a significant difference among the variables.

RESULTS

Floral morphology

Teak flowers are hermaphroditic and arranged in large panicles containing 2700 ± 240 ($n = 30$) flowers. There was a highly significant difference in number of flowers per inflorescence among trees ($P < 0{\cdot}001$). Only 1–3% of the flowers in an inflorescence bloom each day, thus blooming took 1–2 months for the entire inflorescence, depending on its size. The actinomorphic flowers have six whitish petals making up a corolla with a diameter of $6{\cdot}31\ \text{mm} \pm 0{\cdot}07$ ($n = 30$). The lower half of the corollas are undivided forming a tube to which six stamens are attached (Fig. 1).

Each anther has two microsporangia, each bearing two chambers (Figs 2 and 3). The epidermal cells of the mature anther expand (Fig. 2) and, based on cryofractured specimens, appear to contain secretory substances (Fig. 4). The pistil has a long ($6{\cdot}55\ \text{mm} \pm 0{\cdot}11$, $n = 31$), narrow bifurcate style and a hairy ovary containing four ovules. The forked stigma (Fig. 10) is of the wet papillate type with unicellular papillae (Figs 5 and 18). Transmitting tissue is of the hollow type (Fig. 6) and extends from the cell beneath the papillae of the stigma (Fig. 19) to the loculi (Fig. 28).

Pollen shedding and pollen structure

Approximately 1 h after the flower opens cells along the sides of the anthers break down forming a slit. As the anthers are moved by wind or brushed by insects, mature pollen is released (Fig. 4). An anther contains 2100 ± 170 ($n = 10$) pollen grains. Some pollen remains in the anther for approx. 3 h after anther opening (0800–1100h). Pollen dispersal increased in wind. Anthers began to become brown with a bent filament at 1500h and collapsed by 1700h.

Pollen is medium tricolpate, but varies somewhat in size and shape (Table 1) depending on the amount of hydration. Dehydrated pollen is semiangular, ranging in size from 12–29 μm ($\mu = 20\ \mu$m) as seen in polar view, and oval, perprolate to prolate, ranging in size from 24·24–48 μm ($\mu = 40\ \mu$m) in equatorial view. Shortly after landing on the stigma, pollen hydrates and expands. Hydrated pollen is spherical to oblate or suboblate in both polar and equatorial views, and ranges in size from 16·8–36 μm ($\mu = 29\ \mu$m). There was a highly significant difference among trees ($P < 0{\cdot}001$) in size, in both polar and equatorial views of dehydrated pollen, but no significant difference in diameter of hydrated pollen. Pollen coat substances (pollen kitt) were occasionally found on the maturing teak pollen surface, in particular on pollen collected during the early receptive period. The degree of hydration had no influence on pollen germination; poorly and highly hydrated pollen (based on size and shape) germinated and produced pollen tubes (Fig. 5).

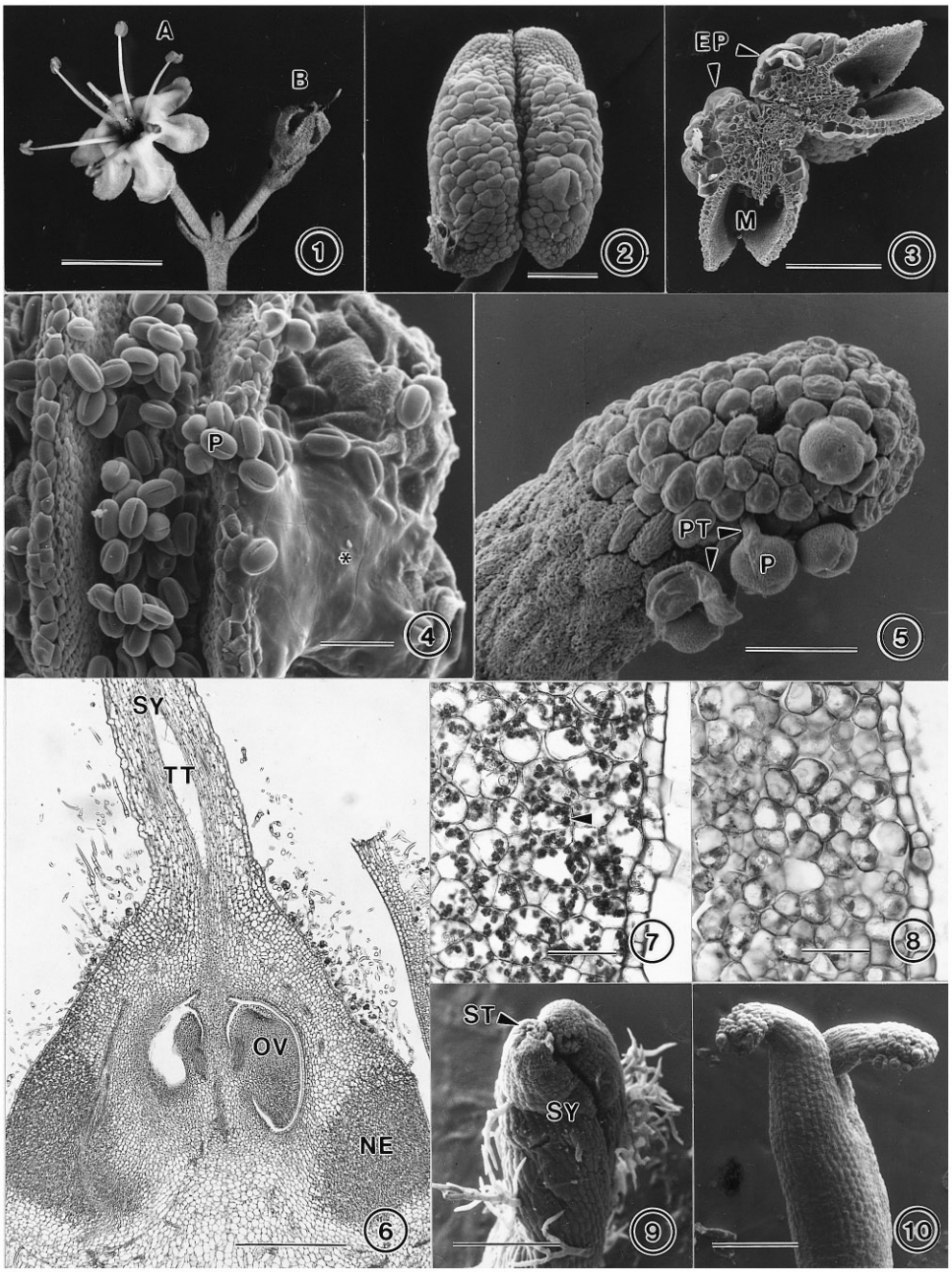
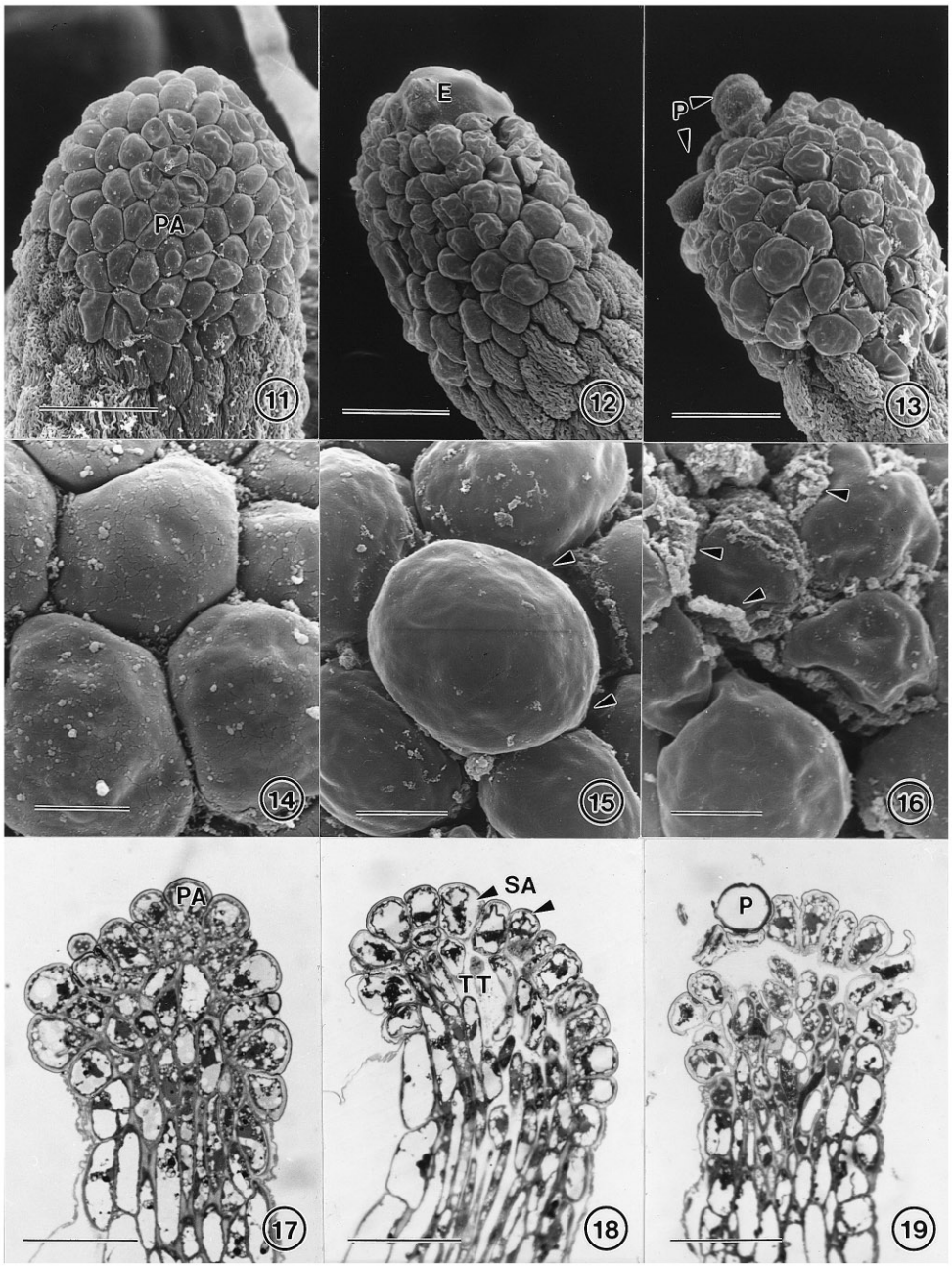


FIG. 1. Teak (*Tectona grandis*) flowers, showing six stamens and petals and straight style at receptivity (A); and (B) 1 d after receptivity after the corolla abscised. Bar = 5 mm. FIGS 2–4. Scanning electron micrographs (SEM) of mature anthers of *Tectona grandis*. FIG. 2. Longitudinal view of the partially open anther at 1300h, showing swelling of the epidermal cells which appear to contain secretory substances. Bar = 0·4 mm. FIG. 3. Transverse section of anther as in FIG. 2 showing two microsporangia, each bearing two chambers. Bar = 0·3 mm. FIG. 4. Inside of dehisced anther showing pollen and the secretion (*). Bar = 0·04 mm. FIG. 5. SEM of stigma papillae during receptivity with germinated pollen showing varying degrees of hydration. Pollen tubes appear to have entered between the loose papillae. Bar = 50 μm. FIGS 6–8. Light micrographs of longitudinal sections of floral nectaries stained with periodic acid-Schiffs. FIG. 6. Floral nectaries at the base of the ovary. Bar = 0·4 mm. FIG. 7. Nectariferous tissue during the pre-receptive period characterized by darkly staining cells containing large amounts of starch (arrowhead). Bar = 0·03 mm. FIG. 8. Nectariferous tissue during post-receptive period. Starch has been hydrolyzed leaving large vacuolate cells. Bars = 0·03 mm. FIGS 9 and 10. SEM micrographs during receptivity when style is relatively straight. FIG. 9. Forked stigma but lobes remain together. Bar = 0·3 mm. FIG. 10. Splayed stigma. Bar = 0·3 mm. *P*, pollen; EP, epidermal cell; M, microsporangia; PT, pollen tubes; NE, nectary; SY, style; TT, hollow transmitting tissue; OV, ovule; ST, stigma.

Receptivity and stigma development

The phenology of individual teak flowers during the day of receptivity is shown in Table 2. Individual flowers are weakly protandrous with anthers starting to dehisce at 0800h, approx. 3 h before peak stigma receptivity begins (1100h) and when the corolla is completely open. The bent style is the first organ to emerge. It extends fully between



FIGS 11–16. Scanning electron micrographs (SEM) of *Tectona grandis* stigmas. FIG. 11. Pre-receptive stigma at 0900h. Bar = 60 μm. FIG. 12. Receptive stigma at 1300h showing some exudate. Bar = 60 μm. FIG. 13. Post-receptive stigma with pollen adhering at 1500h. Papillae are starting to dry and collapse. Bar = 60 μm. FIG. 14. Incompletely expanded papillae of pre-receptive stigma. Bar = 10 μm. FIG. 15. Turgid papillae of receptive stigma showing spaces (arrowhead) between. Bar = 10 μm. FIG. 16. Degenerating papillae of post-receptive stigma showing collapsed papillae and large spaces between which contain remnants of exudate (arrowheads). Bar = 10 μm. FIGS 17–19. Light micrographs of longitudinal sections of stigmas as shown in FIGS 11–13. FIG. 17. Pre-receptive stigma showing enlarging unicellular papillae. Bar = 60 μm. FIG. 18. Receptive stigma showing papillae with pericytoplasmic spaces (arrowhead) and beginning of hollow transmitting tissue. Bar = 60 μm. FIG. 19. Post-receptive stigma showing collapsing papillae with space below (arrowhead) and adhering pollen. PA, papillae; E, exudate; P, pollen; TT, hollow transmitting tissue; SA, space.

1100h and 1300h and exceeds the length of the anthers (Fig. 1). The corolla begins to abscise at about 1900h, or earlier in windy conditions. About 95% of the flowers shed the corolla by 2300h.

Nectar appears from 0500h to 1700h in the lower half of the corolla where the floral tube forms. Sectioned pistils stained with PAS show many floral nectaries located around the base of the ovary (Fig. 6). Cells in nectariferous tissue during the pre-receptive period had dense cytoplasm with large amounts of starch (Fig. 7). The starch hydrolyzed and disappeared during the receptive period leaving large vacuolate cells (Fig. 8).

It is difficult to determine the receptive period by the shape of the style, which remained relatively straight during

TABLE 1. Tectona grandis *pollen size and shape before and after hydration in polar and equatorial view*

Pollen type	Polar view		Equatorial view	
	size (μm)	shape	size (μm)	shape
Dehydrated ($n = 900$)	19·94 ± 0·14	semiangular	39·79 ± 0·35	oval-prolate to perprolate
Hydrated ($n = 900$)	28·84 ± 0·12	circular	same as polar view	circular-oblate to suboblate

TABLE 2. *The phenology of individual* Tectona grandis *flowers during the day of receptivity*

Time of day	Events
0400h	flower closed, style coiled
0500h	nectar appears
0700h	flower opens
0800h	anthers open
1100h–1300h	peak receptive period – corolla completely open – style straight – stigma reflexed and turgid – hydration of pollen on stigma
1500h	post-receptive – stigma tip dry and collapsed
1700h	anthers collapse, nectar disappears
1900h	corolla begins to shed

the day of receptivity, or the stigma between the pre- and post-receptive periods in which the two forks may stick together (Fig. 9) or splay (Fig. 10). However, there were some morphological changes in the papillate stigma. During the pre-receptive period, the stigma enlarged slightly but papillae did not expand completely (Figs 11, 14 and 17) and some remained relatively wrinkled. During the receptive period, from approx. 1100h to 1300h, the stigma enlarged, papillae became more turgid (Figs 12, 15 and 18), secretions were occasionally seen on the surface and some papillae had pericytoplasmic spaces where secretions may be present (Fig. 18). During the post-receptive period, the stigma turned dark-yellow and dry, and papillae became wrinkled and collapsed (Figs 13, 16 and 19). Remnants of the exudate were found on some stigmas (Fig. 16).

Insect visitors to flowers

Thirty-seven insect species belonging to the Hymenoptera, Diptera, Lepidoptera, Hemiptera and Coleoptera were collected from teak inflorescences during eight observation days (Table 3). Most were observed in the morning (0900h–1100h). Flies and bees, particularly the small carpenter bees (*Ceratina* sp.), were the most common insects found; they foraged for pollen and nectar all day. The number of pollen grains on insect bodies was quite variable among insect groups. Generally only Diptera and Hymenoptera had pollen on their bodies, and Hymenoptera had more pollen. Lepidoptera (butterflies) and Formicidae (ants) played little or no part in the transfer of teak pollen. The SEM study revealed pollen other than teak on the long proboscis of butterflies.

Ceratina sp. had the highest pollen loads and pollen grains on all parts of their hairy bodies. They collected pollen by foraging on several newly open flowers in one inflorescence and travelled among inflorescences and trees. However, most tended to forage and stay on the same tree for a long period of time. Heavy pollen loads were also located on their legs, particularly on the specialized, dense scopal hairs of the tibia and tarsus (Fig. 20). In addition to teak pollen, other pollen (*Acacia*. sp) was occasionally present (Fig. 21). SEM studies revealed a rough surface on the pollen exine (Fig. 22) and long-hairs of the tibia (Fig. 21) which may enhance pollen deposition on the insect bodies. *Ceratina* sp. had no sticky substances on their bodies.

Most honey bees (*Apis mellifera*) visiting teak flowers carried foreign pollen; however, they occasionally carried teak pollen on their hind legs, especially the tibia (Fig. 23). Unlike carpenter bees, honey bees have a pollen moistening behaviour. Pollen, once collected, became hydrated and expanded (Fig. 24). The sticky substances may cause pollen to accumulate on their hind legs but would not allow effective pollen transfer.

Pathway of pollen tubes and rate of pollen-tube growth

The pathway of pollen-tube growth to the embryo sac is shown in Fig. 28. Pollen landing on the stigma quickly hydrates and pollen tubes emerge from the pollen aperture adjacent to the stigma surface within 2 h. Pollen tubes pass through the intercellular spaces between papillae (Fig. 5) then grow along the surface of the hollow transmitting tissues (Figs 25–27) of the style. In the transmitting tissue, pollen tubes vary in width and number of callose plugs (Figs 29 and 30). Callose plugs usually form sporadically in the pollen tube all along the style length (Figs 29 and 30). As a result the intensity of pollen tube and callose plug fluorescence varies among pollen tubes and along the style length. Pollen tubes at the upper part of the style lose fluorescence while tubes in the lower part still fluoresce. Abnormalities of pollen-tube growth such as meandering tubes, irregular tubes, forked and swollen tips (Fig. 30) were found for most collection times. Abnormalities may occur anywhere along the style length but are more common in the lower portion of the style. Most pollen tubes grow through the style and reach an ovule. A few become arrested in the lower portion of the ovary (Figs 31 and 32) and only 1–2 pollen tubes per flower reach an embryo sac (Figs 33–35).

TABLE 3. *Insect visitors to* Tectona grandis *flowers collected at ASEAN Forest Tree Seed Centre, Saraburi, Thailand during 8 observation d in July*

Insect visitors Order Family	Species	Relative* amount of teak pollen /insect	Estimated† number of insects observed	Time of visitation
Hymenoptera				
Anthophoridae	*Ceratina* sp.	4	3	0800h–1700h
Vespidae	*Polistes* sp.	2	2	1000h–1400h
	Polistes sagitarrius Sauss.	2	2	1000h–1200h
	Polistes stigma Fabr.	2	2	1000h–1200h
	Vespa affinis (L.)	2	2	0800h–1000h
	Vespa velutina Lepeltier	2	2	1200h–1400h
Formicidae	—	0	1	1000h–1600h
Apidae	*Apis mellifera*	3	2	0800h–1200h
Eumennidae	*Eumenes conica* Fabricius.	2	2	1400h–1600h
	Eumennes esuriens Fabricius.	2	2	1400h–1600h
	Eumenes orchitectus Smith.	2	2	1400h–1600h
	Eumenes petiolata Fabricius.	2	2	1400h–1600h
	Eumenes sp.	2	2	1200h–1600h
Diptera				
Calliphoridae	*Stomorhina lunata* (Fab.)	1	4	0800h–1700h
	Lucilia sp.	1	3	1000h–1600h
Tachinidae	*Dolichocolon* sp.	1	3	1000h–1600h
Sarcophagidae	*Sarcophaga* sp.	1	3	1000h–1600h
Syrphidae	*Eristalinus arvorum* (Fab.)	1	2	1000h–1600h
Otitidae	*Chrysomyza* sp.	1	2	1000h–1600h
Lepidoptera				
Hesperidae	*Iambrix salsala* salsala Moore.	0	1	1400h–1600h
	Oriens sp.	0	1	1400h–1600h
	Pelopides sp.	0	1	1400h–1600h
Lycaenidae	*Euchrysops paudava* Horsf.	0	1	1400h–1600h
	Nacaduba sp.	0	1	1400h–1600h
Nymphalidae	*Naptis columella* martabana Moore.	0	1	1000h–1600h
	Precis almana almana (L.).	0	1	1400h–1600h
Papilionidae	*Precis lemonias* L.	0	2	1000h–1200h
	Atrophaneura aristolochiae aristolochiae Fabr.	0	1	0800h–1600h
Pieridae	*Catopsilia pomona* pomona-f hilaria stoll.	0	1	1400h–1600h
	Catopsilia pomona pomona-f pomona Fabr.	0	1	1200h–1400h
Satyridae	*Delias hyparate* ciris Fruhst.	1	1	0800h–1000h
	Eurema sp.	1	2	0800h–1200h
Symtomidae	*Ypthima baldus* baldus Fabr.	0	1	1400h–1600h
Hemiptera	*Ypthima* sp.	0	1	1400h–1600h
Flatidae	*Amata Sperbius* Fabr.	0	2	1000h–1600h
Membracidae				
Coleoptera	—	0	1	1400h–1600h
Scarabaeidae				
	Tricentrus sp.	0	1	1400h–1600h
	Glycyphana horsfiedi Hope.	0	1	1200h–1600h

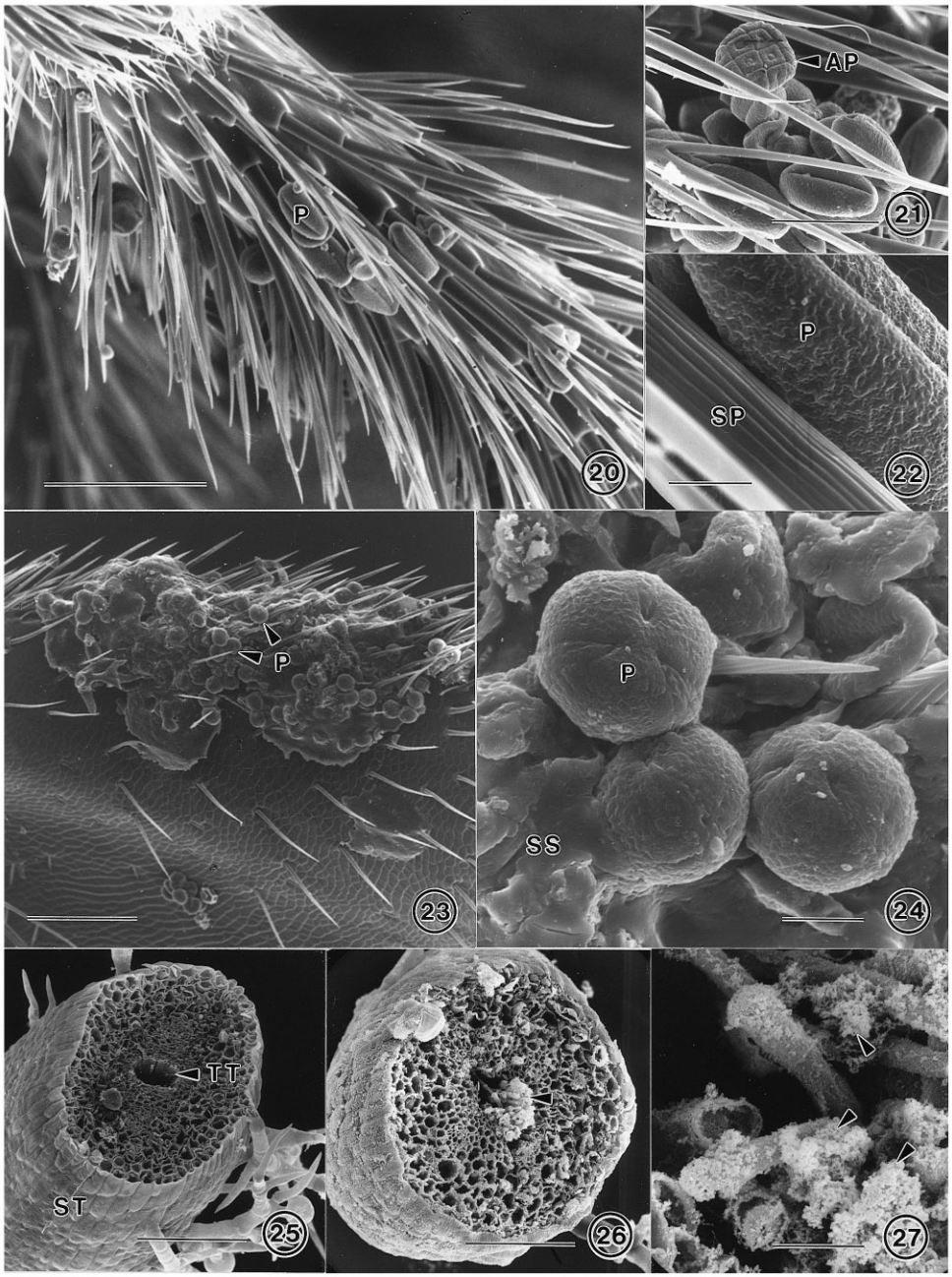
* Relative amount of teak pollen per insect; 0, none; 1, very few grains (individual pollen grains periodically scattered on the bodies of insects); 2, few grains (small numbers of pollen grains can be found individually or in small clusters); 3, many pollen grains (pollen grains found in clusters); 4, abundant (clusters and individual pollen grains are noticeable even with the naked eye).

† General range of number of insects observed: 1, occasional (1–4); 2, low (5–10); 3, medium (15–25); 4, high (55–65 or almost always present).

The rates of pollen-tube growth in open pollinated flowers after the start of the receptive period (1100h) are shown in Fig. 36. Over 90% of pollen tubes penetrate between papillae. At 2 h after pollination, 8% of the pollen tubes reach the micropylar end of the ovule. There is little pollen-tube growth between 2 and 6 h after pollination. Pollen tubes first appear in embryo sacs (1·06% $\pm$ 0·69) at 8 h. At 12 h, approx. 62% of the pollen tubes reach an ovule and 30% reach the lower portion of the ovary. At 24 h, approx. 70% of pollen tubes reach an ovule, or the lower portion of the ovary. Over 40% of the pollen tubes enter into a micropyle, but only 3–8% grow to an embryo sac.

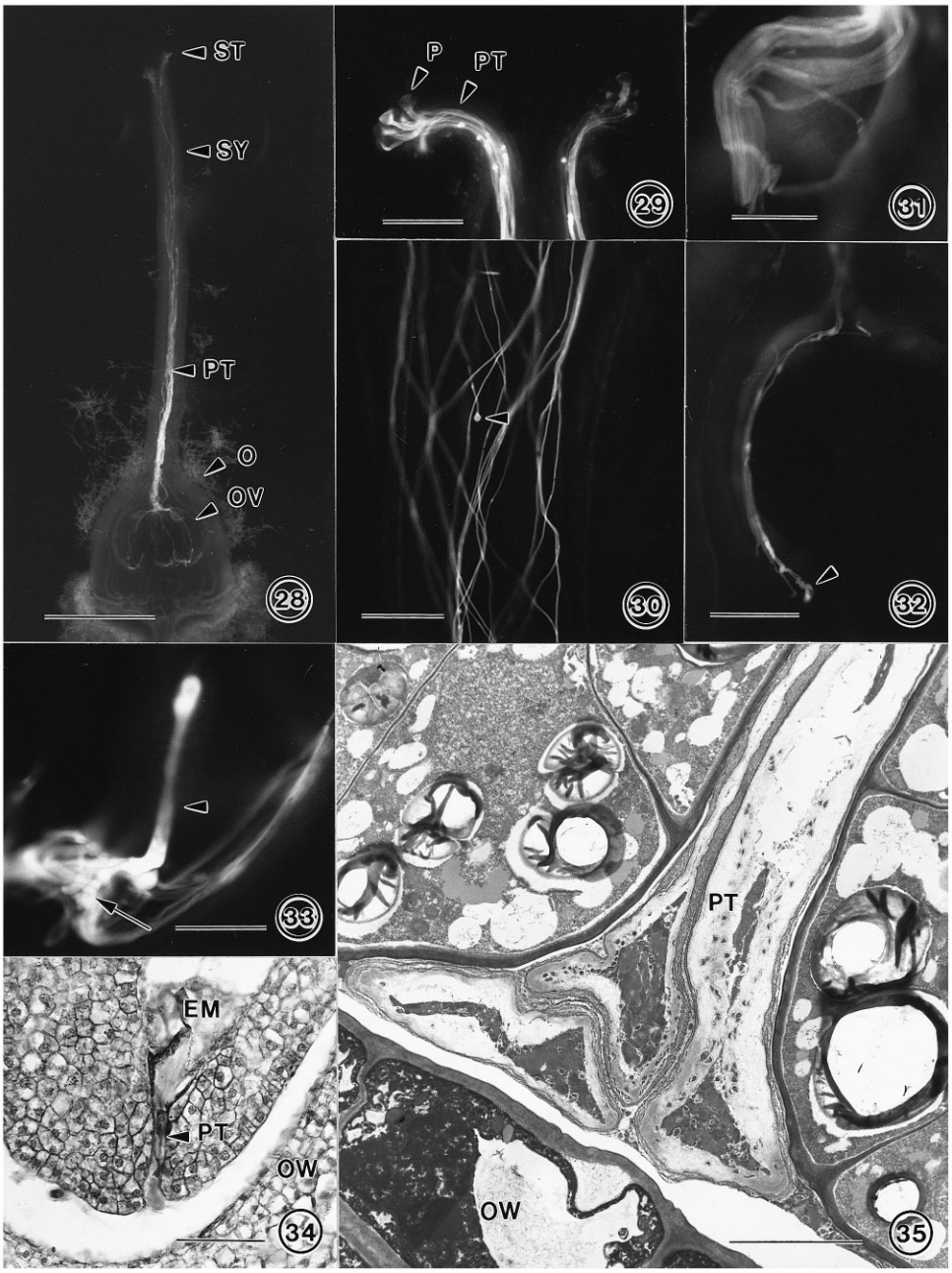
Pollination success and pollen–ovule ratio

The number of pollen grains reaching the stigma (pollination success) gradually increased during the day of receptivity. There were highly significant differences ($P < 0{\cdot}001$) in the number of pollen grains landing on the stigma, both among trees, and at different times during the day of



FIGS 20–22. SEM micrographs of pollen on tibia of carpenter bee (*Caratina* sp.). FIG. 20. Teak pollen carried by the specialized dense scopal hairs. Bar = 0·1 mm. FIG. 21. Close-up showing dry teak and *Acacia* pollen among scopal hairs. Bar = 40 μm. FIG. 22. Close-up showing rough exine on an unhydrated teak pollen and a scopal hair without sticky substances. Bar = 5 μm. FIGS 23–24. SEMs of teak pollen on honey bee (*Apis mellifera*) tibia. FIG. 23. Teak pollen adhering to tibia with sticky substances. Bar = 0·2 mm. FIG. 24. Close-up of hydrated pollen adhering to corbiculae of tibia with sticky substances. Bar = 15 μm. FIGS 25–27. Cryo-SEMs of transversely sectioned mid-portions of mature styles at 1300h on the day of receptivity. FIG. 25. Style of unpollinated flower showing the empty stylar canal. Bar = 0·2 mm. FIG. 26. Style of pollinated flower showing several pollen tubes (arrowhead) in the canal. Bar = 0·2 mm. FIG. 27. Close-up of pollen tubes in FIG. 26 showing remnants of secretions (arrowheads). Bar = 10 μm. AP, *Acacia* pollen; P, teak pollen; SP, scopal hair; SS, sticky substances; ST, style; TT, hollow transmitting tissue; PT, pollen tube.

receptivity (Fig. 37). About 7% of flowers were pollinated at the time of flower opening (0700h) but there was only about one pollen grain per pollinated flower. There was a significant increase in pollinated flowers from 0700h to 0900h when approx. 50% of flowers were pollinated and from 0900h to 1300h when approx. 80% of flowers were pollinated. The number of pollen grains per stigma varied among trees ($P \leqslant 0{\cdot}001$), and increased significantly ($P < 0{\cdot}001$) from 0700h to 1100h when there were about three grains per stigma, and from 1100h to 1300h when there were



FIGS 28–32. Epi-fluorescence micrographs of open-pollinated pistils 24 h following pollination. Pistils were stained with decolourized aniline blue to localize callose. FIG. 28. Pistil showing pathway of pollen tubes from style to ovules. Pollen tubes in the lower half of the style stain more intensely for callose and callose plugs. Bar = 1 mm. FIG. 29. Close-up of stigma from FIG. 28 showing entrance of pollen tubes into the stylar canal shortly after pollination. Bar = 0·25 mm. FIG. 30. Close up of pollen tubes in the upper half of the style. Intensity of fluorescence shows variation in size of pollen tubes, amount of callose, and swelling of tube tip in the stylar canal. Bar = 0·05 mm. FIG. 31. Arrested pollen tubes with tapered tips and faint fluorescence in the upper portion of the ovary. Bar = 0·05 mm. FIG. 32. Arrested pollen tubes in the lower portion of the ovary. Bar = 0·1 mm. FIGS 33–35. Pollen tubes entering the embryo sac about 24 h after pollination. FIG. 33. Fluorescence micrograph showing pollen tubes near the micropyle (arrow) with only one tube growing towards the embryo sac (arrowhead). Bar = 0·07 mm. FIG. 34. Light micrograph showing pollen tube entering the micropyle. Bar = 0·07 mm. FIG. 35. Transmission electron micrograph showing the micropylar region of the ovule shown in FIG. 34. Bar = 5 μm. ST, stigma; SY, style; O, ovary; OV, ovule; P, pollen; PT, pollen tubes; SW, swelling tip; EM, embryo sac; OW, ovary wall.

about six grains per stigma. There was no significant increase in the number of pollen grains per stigma after 1300h. Therefore, the most effective pollination period was between 0900h and 1300h in terms of flowers pollinated and the number of pollen grains per flower. At 1300h approx. 80 % of flowers were pollinated and on average there were seven pollen grains per stigma.

The mean number of pollen grains produced per flower was 13000 ± 1100 ($n = 10$). There was no significant difference in the number of pollen grains produced per

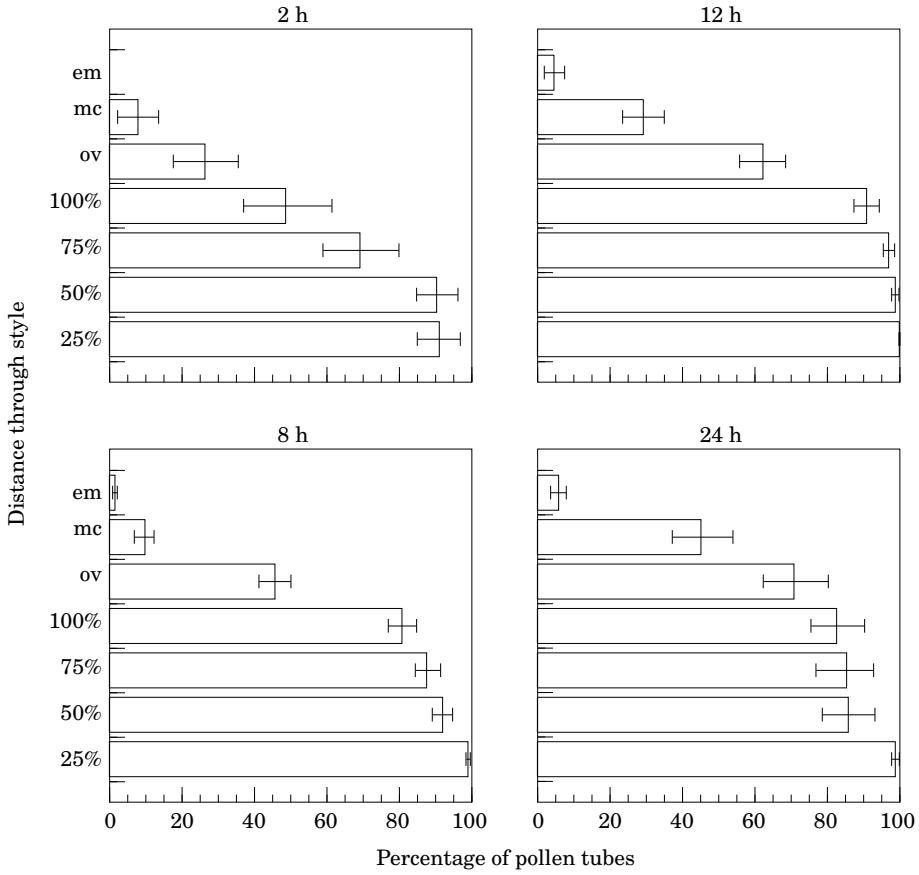


FIG. 36. Percentage of pollen tubes at different positions in the pistil at 2 h ($n = 10$), 8 h ($n = 65$), 12 h ($n = 36$), and 24 h ($n = 24$) after the start of the receptive period (1100h). Horizontal lines represent the standard error. 25%, 50%, 75%, and 100% refer to percent of the style length; ov, ovule; mc, micropyle; em, embryo sac.

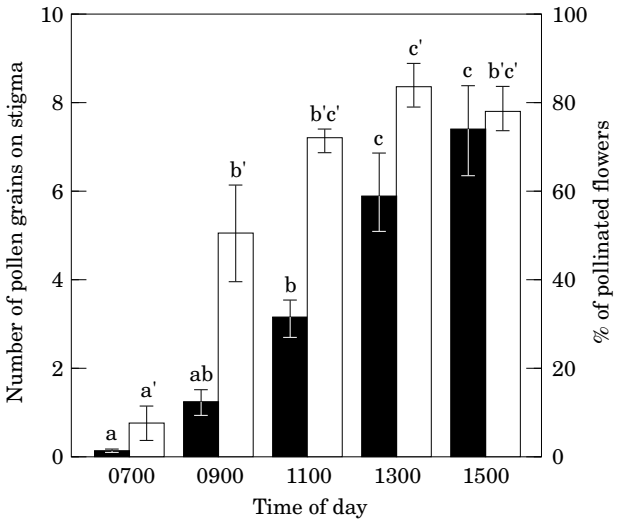


FIG. 37. Mean number of pollen grains per the stigma (■) of open-pollinated flowers and mean percentage of pollinated flowers (□) on the day of receptivity on 60 flowers from each of three trees. Along a line, means of each variable shown by the same letter are not significantly different at $P < 0{\cdot}05$ as determined by Duncan's new multiple range test. Vertical bars represent standard errors.

flower. All pollen was shed from a flower in one day. Two days after the day of receptivity, unpollinated flowers abscise. The pollen-ovule ratio was 3253/1, and the percentage of fruit set was $3{\cdot}51 \pm 0{\cdot}28$ ($n = 30$). There were highly significant differences among trees ($P < 0{\cdot}001$) in these parameters.

DISCUSSION

Floral morphology and receptivity

Teak has a hermaphroditic flower with little spatial and temporal separation of male and female functions; the style slightly exceeds the length of the anthers and the anthers begin to dehisce 3 h before peak stigma receptivity. Protandry makes it possible for most pollen to be dispersed before the papillate stigma becomes most receptive. Protandry occurs frequently in outcrossing angiosperms (Stout, 1928; Garnock-Jones and Malloy, 1982; Lloyd and Webb, 1986; Dudash, 1990) and is assumed to be a common method selected for avoidance of self-fertilization (Richards, 1994). Lloyd and Webb (1986) reported that protandry may also serve to prolong pollen presentation, avoid pollen-stigma interference, and optimally position pollen for dispatch and reception. In teak, however, the protandrous flowers still allow self-pollination.

In teak, flowers which bloom when the infloresence first emerges are usually bigger and produce more pollen and nectar, making these flowers more attractive to insect visitors. Fruit abortion occurs more commonly in the flowers which develop at the end of flowering in an inflorescence, in particular the outermost flowers of the inflorescence. This suggests that pollination success may be greater in flowers developing early and during the peak of the flowering season, than in those which develop at the end of the flowering season. However, there are many other factors, such as position of developing fruits within the inflorescence (Bawa and Webb), which affect fruit development.

Floral nectaries of teak consist of densely staining, starch-filled cells, located in orange-coloured tissues around the base of the ovary. The starch was hydrolysed and nectar was secreted from 0500h to 1500h. This exceeded the most effective pollination period based on number of pollen grains on the stigma and the percentage of pollinated flowers (Fig. 37) which occurred from approx. 0900h to 1300h. Nectar commonly contains sugars, proteins, amino acids, lipids, antioxidant organic acids, and a variety of other substances in very small amounts, including alkaloids, glycosides, saponins, and phenolics (see Baker and Baker, 1975).

Teak has a closed stylar system as is found in many dicotyledons (Knox, 1984). It contains two major parts, the stigma and transmitting tissue. Heslop-Harrison and Shivanna (1977), reported the stigma in the Verbenaceae to be wet and papillate; our study confirmed this for teak. The stigma has specialized papillae for pollen reception which differ from the transmitting tissue cells in shape and content of cytoplasmic organelles (Knox, 1984). The teak stigma produces a small amount of secretion but this was not analysed. The stigmatic secretions reportedly contain lipids, amino acids and perhaps sugars (see Baker, 1973) and are thought to prevent drying of the stigmatic surface and to provide a suitable medium for pollen germination (Baker, Baker and Opler, 1973). These authors also report that these sticky secretions may be smeared onto the bodies of some insects as they contact the surface of the stigma. Pollen grains dislodged from the anthers may adhere to the secretions.

Teak stigma maturation occurs over 6 to 8 h and the style remains relatively straight throughout the day of receptivity. Small amounts of stigmatic secretion were occasionally observed using the SEM from 1100h to 1300h. Therefore, the peak receptive period in teak is characterized by expansion of the style and stigma, increased turgidity of the unicellular papillae, and the presence of a stigmatic secretion, and is bracketed by nectary secretions. It is likely that the stigma secretion comes partly from cells beneath the papillae or from the transmitting tissue and is exuded between the loosely fitting papillae and partly from the papillae themselves, which then collapse.

The transmitting tissue of teak is of the hollow type and the space is about the same width throughout its length. SEM observations of transverse sections of teak styles containing pollen tubes show remnants of a secretion in the hollow transmitting tissue. Hollow styles containing secretions have also been reported in *Trimezia* (Bystedt and Venningerholz, 1991), *Ornithogalum* (Tilton and Horner, 1980), *Dendrobium* (Slater and Calder, 1990), and *Trifolium* (Heslop-Harrison and Heslop-Harrison, 1982). This secretion, and that of the micropyle, may contain lipids, phenolics, polysaccharides, pectins, and proteins but may vary in chemical composition depending on the species (Weber, 1994). It has been suggested that stigma and transmitting tissue secretions have many functions, such as attraction and nourishment for floral visitors (Lord and Webster, 1979), pollen adhesion to pollinators (Kandasamy and Kristen, 1987), pollen-stigma recognition (Clarke *et al*., 1979; Knox, 1984), pollen-tube growth (Sanders and Lord, 1992) and pollen-tube penetration of ovules (Franssen-Verheijen and Willemse, 1993).

According to Wodehouse (1935), pollen grains in the size range found for teak (20–40 μm) are adapted for easy liberation from the anthers by moderate wind; transport; and adherence both to the insect visitors and to the stigma. The morphology of hydrated teak pollen is similar to that described by Subramanian and Seethalakshmi (1984). Although dehydrated teak pollen size varied among the sample trees, no significant difference was found in the size of hydrated pollen. Pollen kitt was occasionally found on mature teak pollen, however it usually disappeared in most pollen observed using the SEM. These substances are largely lipid and may function to protect the pollen from dehydration or act as a binding agent, holding grains together in groups to attract and adhere to insect pollinators (Bedinger, 1992); it disappears after pollen hydration (Heslop-Harrison, 1979).

Pollinators

Faegri and Pijl (1979) suggested that in insect-pollinated plants, nectar and pollen are the major rewards and are presented only at certain times. This appears true for teak. It is likely that nectar is the chief attractant in teak because it is produced in large volumes and over a long period of time (0500h–1500h), whereas pollen is presented in relatively small quantities over a short time (0800h–1100h). Insects that are primarily pollen collectors are usually thought to be more effective pollinators than those that are nectar collectors (Jay, 1986). However, nectar foragers have been found to be effective pollinators in almond orchards (Estes, Atmos and Sullivan, 1983). Hodges (1995) found that an increase in nectar production by *Mirabilis multiflora* resulted in an increase in flower visits and significantly increased pollen removal from the anthers and deposition on the stigma.

Teak floral size and architecture, i.e. the small flat corolla forming a landing platform of medium size (6–7 mm), facilitates landing of small insects. Teak flowers produce a relatively unpleasant scent, especially under sunny conditions, which may attract certain insects, e.g. flies. The malodorous smell and the whitish corolla of teak flowers may not attract many kinds of bees when other, more perfumed and more colourful flowers are available. In this study, 25 bees were captured compared to 70 flies. However, few of the bees carried abundant teak pollen.

As in other studies of teak by Mathew, Koshy and Mohanadas (1987) and Egenti (1981), the majority of insects visiting teak flowers are Diptera, Hymenoptera, and Lepidoptera; and insect activity was highest during the morning (0800h–1200h) coinciding with peak pollen presentation and nectar secretion. Most members of the order Hymenoptera found in this study, other than *Ceratina* spp. and *Apis mellifera*, feed on floral nectar and pollen but may not be considered as effective pollinators because they were present in small numbers and carried only small amounts of pollen.

Carpenter bees, (*Ceratina* sp.) are small (4–6 mm long), but more effective pollinators than the more numerous flies visiting teak flowers. They were also found on teak at the TIC, Thailand (Hedegart, 1976) and regularly visit teak flowers, although not in large numbers (21 captured). Carpenter bees have a short proboscis in comparison to their body length, and are forced to alight on the anthers and corollas, thereby collecting pollen on most parts of their bodies. It is likely that these bees effect primarily self-pollination because they tend to stay on a single flower for some time prior to moving to another flower in an inflorescence on the same tree. Rarely do they move among trees. Honey bees (*Apis mellifera*) have been recognized as effective pollinators in many tropical trees (Cruden *et al*., 1990; Sedgley *et al*., 1992; Carthew, 1993; Ish-Am and Eisikowitch, 1993; Visuthitepkul and Moncur, 1993). They forage on teak only in the morning (0900h–1000h) but are much less effective pollinators than carpenter bees because they are present in small numbers and few carry teak pollen grains. They also carry pollen packed in oil secretions which may make transfer to the stigmas difficult. The oils may be produced in certain plant families (i.e. orchids) (Friedrich, 1985) which grow near the teak. Butterflies and most flies feed on floral nectar rather than pollen, and thus do not purposely contact anthers in the flowers. This may limit the amount of teak pollen they pick up. Therefore, flies which are the main flower visitors for the majority of the flowering season, and honeybees and butterflies which visit infrequently, are not regarded as important pollinators. Ants and beetles, although present on teak, are not effective pollinators because they do not carry teak pollen, and the metapleural gland secretions spread over the bodies of most ants during grooming are toxic to pollen (Beattie *et al*., 1985).

Pollen-tube growth and incompatibility

In angiosperms, pollen-tube growth through the style to the ovule may take hours to days. In *Acacia retinodes* it takes 11 h for both self- and cross-pollen tubes to reach the ovules (Kenrick and Knox, 1985), whereas in *Banksia coccinea* R. Br. (Proteaceae) it takes 6 d (Fuss and Sedgley, 1991). In teak, pollen-tube growth was fast. At 2 and 8 h after open-pollination (1300h), approx. 50 and 81%, respectively, of pollen tubes had grown to the base of the style. The fast pollen-tube growth of teak may be an advantage in that it minimizes exposure to unfavourable abiotic factors, such as rain and wind, which commonly occur at pollination and cause abundant loss of unfertilized flowers.

Self-incompatibility can act in the stigma, style or ovary (Seavey and Bawa, 1986). In teak, gametophytic self-incompatibility occurs with some pollen tubes being inhibited in the style, but most are inhibited in the ovary. The fluorescent studies of pollen tubes in the pistil demonstrated that the stigma was of little importance in pollen recognition and rejection. Also outcrossing did not appear to be controlled by pollen-tube growth in the style. Less than 20% of pollen tubes were inhibited before they reached the lower end of the style. Transmitting tissue is fairly large and about the same width throughout the style length, suggesting that there is no or little competition among pollen tubes for space and little direct contact with stylar tissues which may reduce incompatibility reactions. Generally, a larger number of pollen tubes ($7 \pm 1{\cdot}1$) reach the ovary than the number of ovules (four), but only 1–2 pollen tubes enter the four micropyles. The final inhibition then occurs within the ovule; 70–80% of pollen tubes reach the upper part of ovule but only 50% reach the lower portion of the ovary.

Pollen-limited and climatic factors in relation to fruit set and seed set

In teak, pollination success was 78%, but there was low fruit set, only 3·5%, suggesting that the numbers of flowers pollinated is not the major limiting factor. High levels of self-pollination would result in abundant pollen and pollen tubes, but flower abortion due to a lack of fertilization. These occurred in teak. However, other unfavourable abiotic factors can affect fruit and seed set.

Temperature is important in determining the effective pollination period, stigma receptivity (Burgos, Egea, and Dicenta, 1991), ovule longevity (Eaton, 1959; Postweiler, Stösser and Anvari, 1985), pollen germination (Escobar, Valledor and Rallo, 1983), and pollen-tube growth both *in vitro* (Escobar *et al*., 1983) and *in vivo* (Cuevas, Rallo, and Rapoport, 1994). All of these may affect fertilization and fruit set. The most favourable temperature for maximum fruit set in *Olea europaea* L. was 25 °C, when there was faster pollen tube growth, more abundant and earlier fertilization, and higher fruit set; whereas at 20 °C pollen tube growth was slower, resulting in delayed and reduced fertilization and lower fruit set. Extreme temperatures can cause seed abortion, thus eliminating or diminishing fruit set (Sedgley and Annells, 1981; Cuevas *et al*., 1994). Fruit set in *Olea europaea* was completely inhibited when high temperature occurred during the flowering period (Cuevas *et al*., 1994). In teak, flowering occurs during the rainy season and insect activities on a rainy day are less than on a sunny day. Also, teak stigmas are most receptive at mid-day (1100h–1300h). High temperature on a sunny day may cause drying of the stigmatic surface resulting in less effective pollination or pollen germination, thus reducing fruit set.

Seed set per fruit is pollen-limited or pollinator-limited when small amounts of pollen are deposited on stigmas (Bierzychudek, 1981; Spira and Pollak, 1986). For instance, Heard (1993) reported that *Macadamia integrifolia* required approx. 150 insect visits per raceme to ensure adequate

pollination. Primack and Silander (1975) demonstrated that three or four visits by honey bees to *Oenothera fruticosa* L. flowers are required to assure maximum seed set. In teak, although *Ceratina* sp. regularly visited flowers, the number of pollen grains reaching the stigma varied from 1–36 ($\mu = 6{\cdot}7$). Open-pollinated teak flowers receive less than two pollen grains per ovule and approx. 42 % receive only zero to three pollen grains per flower, which is less than the number of ovules (four) per flower. Therefore, although 78 % of flowers are pollinated the number of pollen grains per ovule may be too low for good seed set.

To ensure good seed set, excess pollen relative to the number of ovules is necessary to allow for pollen-tube competition (Spira *et al*., 1992). Many workers suggest that pollen competition may be an important component of natural selection through gametophytic selection (Mulcahy, 1979; Snow, 1986; Mulcahy and Mulcahy, 1987; Walsh and Charlesworth, 1992). Shaanker and Ganeshaiah (1990) found that pollen deposition patterns regulate the seed number per fruit in multi-ovulated species. Stigmas of multi-ovulated species generally receive more than enough pollen to fertilize all the ovules in an ovary. Several investigators have found that seeds produced under intense pollen-tube competition have significantly better germination, seedling growth, and seedling survival than those produced with little or no pollen-tube competition (Mulcahy and Mulcahy, 1987). Also, an increase in pollination increased pollen germination in *Betula pubescens* (Holm, 1994). The same studies also report that the length of the longest pollen tube per style increases with increasing number of pollen tubes in the same style. In Thailand, the germination percentage of teak fruits is low, 14–40 % (Kaosa-ard, 1983). This may result from little pollen-tube competition due to insufficient pollination and high levels of self-pollination, as has been shown here.

The amount of pollen produced by a flower reflects the probability that a sufficient number of pollen grains will reach a stigma (Cruden, 1977). In teak, pollen production is prolific as shown by high pollen-ovule ratio (P/O = 3253). According to Cruden (1977), teak would be classified as obligate xenogamy (the highest outcrossing level). However, teak is less efficient in pollen transfer than many autogamous species. This indicates that in teak one major problem is pollen transfer, probably due to insufficient or ineffective pollinators. Other factors dealing with fruit and seed development are also important and will be covered in another study (Palupi, 1996).

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LITERATURE CITED

Anon. 1956. Country report on teak forestry (Burma). In: *Country reports on teak 11*. Rome: Food and Agriculture Organization (F.A.O.)

Baker HG. 1973. Evolutionary relationships between flowering plants and animals in American and African forests. In: Meggers BJ, Ayensu ES, Duckworth WD, eds. *Tropical forest ecosystems in Africa and South America: A comparative review*. Washington, DC: Smithsonian Institution Press, 145–159.

Baker HG, Baker I. 1975. Nectar-constitution and pollinator-plant coevolution. In: Gilbert LE, Raven PH, eds. *Coevolution of animals and plants*. Austin: University of Nijmegen, 100–140.

Baker HG, Baker I, Opler PA. 1973. Stigmatic exudates and pollination. In: Brantjes NBM, ed. *Pollination and dispersal*. Nijmegen: University of Nijmegen, 47–60.

Bawa KS, Webb CJ. 1984. Flower, fruit and seed abortion in tropical forest trees: implications for the evolution of paternal and maternal reproductive patterns. *American Journal of Botany* **71**: 736–751.

Beattie AJ, Turnbull C, Hough T, Jobson S, Knox RB. 1985. The vulnerability of pollen and fungal spores to ant secretions: evidence and some evolutionary implications. *American Journal of Botany* **72**: 606–614.

Bedinger P. 1992. The remarkable biology of pollen. *Plant Cell* **4**: 879–887.

Bierzychudek P. 1981. Pollinator limitation of plant reproductive effort. *American Naturalist* **117**: 838–840.

Bolstad PV, Bawa KS. 1982. Self-incompatibility in *Gmelina arborea* L. (Verbenaceae). *Silvae Genetica* **31**: 19–21.

Bryndum K, Hedegart T. 1969. Pollination of teak (*Tectona grandis* Linn f.). *Silvae Genetica* **18**: 77–80.

Burgos L, Egea J, Dicenta F. 1991. Effective pollination period in apricot (*Prunus armeniaca* L.) varieties. *Annals of Applied Biology* **119**: 533–539.

Bystedt P-A, Venningerholz F. 1991. The transmitting tract in *Trimezia fosteriana* (Iridaceae). 2. Development of secretory cells in stigma, style, and ovary. *Nordic Journal of Botany* **11**: 345–357.

Cameron AL. 1968. Forest tree improvement in New Guinea, 1. Teak. 9th Commonwealth Forest Conference, New Delhi.

Carthew SM. 1993. An assessment of pollinators visitation to *Banksia spinulosa*. *Australian Journal of Ecology* **18**: 257–268.

Clarke A, Gleeson P, Harrison S, Knox RB. 1979. Pollen-stigma interaction: identification and characterization of surface components with recognition potential. *Proceedings of the National Academy of Sciences* (*USA*) **76**: 3358–3362.

Cruden RW. 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* **31**: 32–46.

Cruden RW, Baker KK, Cullinan TE, Disbrow KA, Douglas KL, Erb JD, Kirsten KJ, Malik ML, Turner EA, Weier JA, Wilmot SR. 1990. The mating systems and pollination biology of three species of *Verbena* (Verbenaceae). *Journal of Iowa Academy Science* **97**: 178–183.

Cuevas J, Rallo L, Rapoport HF. 1994. Initial fruit set at high temperature in olive, *Olea europaea* L. *Journal of Horticultural Science* **69**: 665–672.

Dudash MR. 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianceae): a comparison in tree environments. *Evolution* **44**: 1129–1139.

Dumas C, Knox RB. 1983. Callose and determination of pistil viability and incompatibility. *Theoretical and Applied Genetics* **67**: 1–10.

Dumas CM, Rougier M, Zandonella P, Ciampolini F, Cresti M, Pacini E. 1978. The secretory stigma in *Lycopersicon peruvianum* Mill.: Ontogenesis and grandular activity. *Protoplasma* **96**: 173–187.

Eaton GW. 1959. A study of the megagametophyte in *Prunus avium* and its relation to fruit setting. *Canadian Journal of Plant Science* **39**: 466–476.

Egenti LC. 1974. Preliminary studies on pollinators of teak (*Tectona grandis* L.f.). Research Paper No. 29. Research Paper Forest Series, Federal Department of Forest Research, Nigeria.

Egenti LC. 1978. Pollen and stigma viability in teak (*Tectona grandis* L.f.). *Silvae Genetica* **27**: 29–32.

Egenti LC. 1981. Aspects of pollination ecology of teak (*Tectona grandis* Linn f.) in Nigeria. Pollinators and fruit production. In: Krugman SL, Katsuta M, eds. *Proceedings of the symposium on flowering physiology*, IUFRO XVII World Congress, Kyoto, Japan, 27–31.

Escobar FR, Valledor GG, Rallo L. 1983. Influence of pistil extract and temperature on *in vitro* pollen germination and pollen tube growth of olive cultivars. *Journal of Horticultural Science* **58**: 219–227.

Estes JR, Amos BB, Sullivan JR. 1983. Pollination from two perspectives: the agricultural and biological sciences. In: Jones CE, Little RJ, eds. *Handbook of experimental pollination biology*. New York: Van Norstrand Reinhold, 173–183.

Fægri K, van der Pijl L. 1979. *The principles of pollination ecology*. Oxford: Pergamon Press.

Franssen-Verheijen MAW, Willemse MTM. 1993. Micropylar exudate in *Gasteria* (Aloaceae) and its possible function in pollen tube growth. *American Journal of Botany* **80**: 253–262.

Friedrich GB. 1985. *Insects and flowers*. New Jersey: Princeton University Press.

Fuss AM, Sedgley M. 1991. Pollen tube growth and seed set of *Banksia coccinea* R.Br. (Proteaceae). *Annals of Botany* **68**: 377–384.

Garnock-Jones PJ, Malloy BP. 1982. Protandry and inbreeding depression in *Hebe amplexicaulis* (Scrophulariaceae). *New Zealand Journal of Botany* **20**: 401–404.

Hanf M. 1936. Vergleichende und entwicklungsgeschichtliche Untersuchungen über Morphologie and Anatomie der Griffel und Griffeläste. *Beihefte zum Botanischen Zentralblatt* **54A**: 99–141.

Heard TA. 1993. Pollinator requirements and flowering patterns of *Macadamia integrifolia*. *Australian Journal of Ecology* **41**: 491–497.

Hedegart T. 1973. Pollination of teak (*Tectona grandis* L.). *Silvae Genetica* **22**: 124–128.

Hedgegart T. 1976. Breeding systems, variation and genetic improvement of teak (*Tectona grandis* L.f.): In Burley J, Styles BT, eds. *Tropical trees, variation, breeding and conversation*, Linnean Society Symposium Series 2. New York: Academic Press.

Heslop-Harrison J. 1979. Pollen walls as adaptive systems. *Annals of the Missouri Botanical Garden* **66**: 813–829.

Heslop-Harrison J, Heslop-Harrison Y. 1982. Pollen-stigma interaction in the Leguminosae: constituents of the stylar fluid and stigma secretion of *Trifolium pratense* L. *Annals of Botany* **41**: 1233–1258.

Heslop-Harrison Y, Shivanna KR. 1977. The receptive surface of the angiosperm stigma. *Annals of Botany* **41**: 1233–1258.

Hodges SA. 1995. The influence of nectar production on hawkmoth behavior, self pollination, and seed production in *Mirabilis multiflora* (Nyctaginaceae). *American Journal of Botany* **82**: 197–204.

Holm SO. 1994. Pollination density-effects on pollen germination and pollen tube growth in *Betula pubescens*. Ehrh. in northern Sweden. *New Phytologist* **126**: 541–547.

Horne JEM. 1961. Teak in Nigeria. *Nigerian Forestry Information Bulletin* (New series) **16**: 16.

Ish-Am G, Eisikowitch. 1993. The behaviour of honey bees (*Apis mellifera*) visiting avocado (*Persea americana*) flowers and their contribution to its pollination. *Journal of Apicultural Research* **32**: 175–186.

Jay SC. 1986. Spatial management of honey bees on crops. *Annual Review of Entomology* **31**: 49–65.

Kandasamy & Kristen. 1987.

Kaosa-ard A. 1977. *Physiological studies of sprouting of teak* (Tectona grandis *Linn.f.*) *planting stumps*. PhD Thesis, Department of Forestry, ANU. Canberra, Australia.

Kandasamy MK, Kristen U. 1987. Developmental aspects of ultrastructure, histochemistry and receptivity of the stigma of *Nicotiana sylvestris*. *Annals of Botany* **60**: 427–437.

Kaosa-ard A. 1983. Teak improvement program annual report, No. 15–16. Teak Improvement Centre (TIC), Bangkok, Thailand.

Kedarnath S. 1974. Genetic improvement of forest tree species in India. *India Journal of Genetics. Proceedings of 2nd General Congress Sarrao*, 34A New Delhi.

Kenrick J, Knox RB. 1985. Self-incompatibility in nitrogen-fixing tree, *Acacia retinodes*: quantitative cytology of pollen tube growth. *Theoretical and Applied Genetics* **69**: 481–488.

Kermode CWD. 1957. Teak. In: *Tropical silviculture 2*: 168–192. Rome: Food and Agriculture Organization (F.A.O.)

Knox RB. 1984. Pollen-pistil interactions. In: Linskens HF, Heslop-Harrison J, eds. *Cellular Interactions. Encyclopedia of Plant Physiology. New Series* **17**: 508–608. Berlin: Springer-Verlag.

Knox RB, Kenrick J, Jobson S, Dumas C. 1989. Reproductive function in the memosoid legume *Acacia retinodes*: ultrastructural and cytochemical characteristics of stigma receptivity. *Australian Journal of Botany* **37**: 103–124.

Ko Ko Gyi. 1972. *An investigation of factors relevant to development of teak plantations in South-East Asia with particular reference to Burma*. MSc Thesis, ANU, Canberra.

Lloyd DG, Webb CJ. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms. I. Dichogamy. *New Zealand Journal of Botany* **24**: 135–162.

Longman KA, Jenik J. 1987. *Tropical forest and its environment*. 2 Edn. Harlow: Longman Scientific and Technical.

Lord EM, Webster BD. 1979. The stigmatic exudate of *Phaseolus vulgaris* L. *Botanical Gazette* **140**: 266–271.

Mahapol S. 1954. *Teak in Thailand*. Royal Forest Department, Thailand.

Mathew G, Koshy MP, Mohanadas K. 1987. Preliminary studies on insect visitors to teak (*Tectona grandis* (Linn f.) inflorescence in Kerala, India. *Indian Forester* **113**: 61–64.

Mulcahy DL. 1979. The rise of the angiosperms: a genocological factor. *Science* **206**: 20–23.

Mulcahy DL, Mulcahy GB. 1987. The effect of pollen competition. *American Scientist* **75**: 44–50.

Palupi ER. 1996. *Reproductive biology of teak* (Tectona grandis *Linn. f.*) *in East Java, Indonesia*. MSc Thesis, University of Victoria, Canada.

Pearse AG. 1972. *Histochemistry – Theoretical and applied 1*. Edinburgh: Churchill Livingstone, 659–660.

Postweiler K, Stösser, Anvari SF. 1985. The effect of different temperatures on the viability of ovules in cherries. *Scientia Horticulturae* **25**: 235–239.

Primack RB, Silander JA. 1975. Measuring the relative importance of different pollinators to plants. *Nature* **255**: 143–144.

Reynolds ES. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *Journal of Cell Biology* **17**: 208–212.

Richards AJ. 1994. Plant breeding systems. New York: Chapman and Hall.

Richardson KC, Jarrett L, Finke EH. 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Technology* **35**: 313–323.

Sanders LC, Lord EM. 1992. A dynamic role for the stylar matrix in pollen tube extension. In: Russell SD, Dumas C, eds. *International review of cytology 140. Sexual reproduction in flowering plants*. San Diego: Academic Press, 297–318.

Seavey SR, Bawa KS. 1986. Late acting self-incompatibility in angiosperms. *Botanical Review* **52**: 195–219.

Sedgley M. 1981. Ultrastructure and histochemistry of the watermelon stigma. *Journal of Cell Science* **48**: 137–146.

Sedgley M, Annells CM. 1981. Flowering and fruit-set response to temperature in the avocado cultivar 'Hass'. *Scientia Horticulturae* **14**: 27–33.

Sedgley M, Khen CV, Smith RM, Harbard J. 1992. Insect visitors to flowering branches of *Acacia mangium* and *Acacia auriculiformis*. In: Carron LT, Aken KM, eds. *Breeding technologies for tropical Acacias*. The Australian Centre for International Agricultural Research (ACIAR) Proceedings **37**: 51–56.

Shaanker RU, Ganeshaiah KN. 1990. Pollen grain deposition patterns and stigma strategies in regulating seed number per pod in multi-ovulated species. In: Bawa KS, Hadley M, eds. *Reproductive ecology of tropical forest plants*. Man and the biosphere series 7, Parthenon Publishing, 165–178.

Siripatanadilox S. 1974. Development of teak flower (*Tectona grandis* Linn f.). *Forest Research Bulletin* **31**: Faculty of Forestry, Kasetsart University, Thailand.

Slater AT, Calder DM. 1990. Fine structure of wet, detached cell stigma of the orchid *Dendrobium speciosum* Sm. *Sexual Plant Reproduction* **3**: 61–69.

Snow AA. 1986. Pollination dynamics in *Epilobium canum* (Onagraceae): consequences for gametophytic selection. *American Journal of Botany* **73**: 139–151.

Spira TP, Pollak OD. 1986. Comparative reproductive biology of alpine biennial and perennial gentians (Genetiana: Gentianaceae) in California. *American Journal of Botany* **73**: 39–47.

Spira TP, Snow AA, Whigham DF, Leak J. 1992. Flower visitation, pollen deposition, and pollen-tube competition in *Hibiscus moscheutos* (Malvaceae). *American Journal of Botany* **79**: 428–433.

Spurr AR. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research* **71**: 173–184.

Stout AB. 1928. Dichogamy in flowering plants. *Bulletin of the Torrey Botanical Club* **55**: 141–153.

Subramanian K, Seethalakshmi TS. 1984. A preliminary note on pollen in teak in relation to fruit-set. *Indian Forester* **110**: 1023–1029.

Sutherland S. 1986. Floral sex ratios, fruit set, and resource allocation in plants. *Ecology* **67**: 991–1001.

Tanaka K. 1989. High resolution electron microscopy of the cell. *Biology of the Cell* **65**: 89–98.

Tilton VR, Horner HT. 1980. Stigma, style, and obturator of *Ornithogalum caudatum* (Liliaceae) and their function in the reproductive process. *American Journal of Botany* **67**: 1113–1131.

Troup RS. 1921. *The Silviculture of Indian Trees*. Oxford Press.

Visuthitepkul S, Moncur MW. 1993. Floral biology of Petford *Eucalyptus camaldulensis* Dehnh. In: Drysdale RM, John SET, Yapa AC, eds. *Proceedings of the International Symposium on Genetic Conservation and Production of Tropical Forest Tree Seed*, Chiang Mai, Thailand: ASEAN-Canada Forest Tree Seed Centre Project, Muak-lek, Thailand, 182–189.

Walsh NE, Charlesworth D. 1992. Evolutionary interpretations of differences in pollen tube growth rates. *The Quarterly Review of Biology* **67**: 19–37.

Weber M. 1994. Stigma, style and pollen tube pathway in *Smyrnium perfoliatum* (Apiaceae). *International Journal of Plant Science* **155**: 437–444.

Wodehouse RP. 1935. *Pollen grains*. New York: McGraw-Hill.