ASEAN
Forest Tree Seed Centre Project

A Manual

Technique for controlled hand-pollination of teak (*Tectona grandis* Linn. f.)

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Cover Photograph: Controlled pollination by brushing an anther against the stigma of a receptive flower.

Copies of this publication can be obtained free of charge from:

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Contents

Glossary of terms used in the in text

Definition marked *, #, and @ are adapted from the Chambers Biology Dictionary, W & R Chambers Ltd and Cambridge University Press, 1989; The Concise Oxford Dictionary of Botany, Oxford University Press, 1992; and the Penguin Dictionary of Botany, Market House Books Ltd, 1984, respectively.

	Term	Definition
	Actinomorphic	Radially symmetrical.
@	Allogamy	(Syn cross-fertilization, exogamy) Fusion of female and male gametes derived from genetically dissimilar individuals of the same species.
*	Autogamy	Fertilization involving pollen and ovules from (1) the same flower or sometimes (2) the same plant or genetically identical individuals (same genet or clone).
@	Cross-pollination	The transfer of pollen from the anthers of one individual of the same species.
*	Geitonogamy	Fertilization involving pollen and ovules from different flowers on the same individual plant (ramet) or from the same clone (genet).
@	Hermaphroditic	Having both male and female reproductive parts in the same flower.
@	Panicle	A racemose inflorescence in which the flowers are formed on stalks (peduncles) arising alternately or spirally from the main axis. The term is also sometimes used to describe any type of complex, branched inflorescence.
@	Papillate	A projection from a cell, usually of the epidermis. Papillae are often swollen and covered with wax. In xerophyte, a plant adapted to dry habitat, where growth may be limited by water shortage.
@	Pedicel	The stalk attaching individual flowers to the main axis (peduncles) of the inflorescence. The pedicel may act as a temporary storage organ for sugars prior to seed development.
	Perprolate	Elliptic shape (more oval than prolate).
	Prolate	Elliptic shape.

#	Protandry	The maturation of the male reproductive organs before those of the female. For example, in many members of the Legumenosae the pollen is released from the anthers before the stigma in the same flower is receptive.
#	Raceme	A racemose inflorescence in which the flowers are formed on individual pedicels on the main axis (peduncle).
@	Racemose inflorescence	An inflorescence in which meristematic activity continues at the apex of the main stem and primary laterals and flowers are developed from the axillary meristems. Different types of racemose inflorescence include panicle, corymb, raceme, umbel, and spike.
#	Self-pollination	The transfer of pollen from anther to stigma of the same tree (either of the same flower or of a different flower but always of the same individual).
@	Tricolpate	Describing a pollen grain having three colpi (oblong to elliptic germinal apertures), as is commonly found amongst most dicotyledon species.

1. Introduction

Teak (*Tectona grandis* Linn.f.) is one of the most highly sought after species in the international timber trade. Native to India, Burma, Thailand, and Laos, it is also widely planted in Indonesia and, more recently, in Malaysia. Teak plantation establishment has been expanding rapidly, resulting in an increased demand for high-quality seed.

To meet this demand for high-quality seed in Thailand, the Royal Forest Department (RFD) has begun an intensive tree improvement program based on the extensive seed orchards and seed production areas established in northern Thailand since 1965. Although approximately 2 016 ha of seed orchard exist, the demand for improved quality seed and planting stock far exceeds supply.

The clonal orchards were established by bud grafting selected individuals from native stands throughout the range in Thailand. To date, no effort has been made to breed and test these selected clones. However, the RFD now plans to test the over 300 clones in the orchards, and strategies have been designed to identify the selections by molecular techniques and to test the breeding population using full-sib and half-sib mating. To accomplish the breeding portion, effective and cost-efficient techniques for controlled pollination are necessary.

This manual on controlled hand-pollination is based on studies of controlled pollination of teak conducted from 1992 to 1996 at two locations, a plantation near the ASEAN Forest Tree Seed Centre (AFTSC) in Saraburi Province and a teak seed orchard at Payao in northern Thailand, and from a review of existing literature. The manual is designed to provide field staff with the current knowledge of teak flowering and to serve as a "how-to" guide for controlled hand-pollination of teak. It should be of benefit to all ASEAN members involved in teak improvement programs and related research.

2. Background information

2.1 Distribution and phenology of teak

Teak is indigenous to northern Thailand, ranging from 16 to 20° N latitude and from 97 to 101° E longitude, and is now widely planted throughout northern and central Thailand. In its native range, it is a component of the northern mixed deciduous forest of which the dominant species are teak, *Xylia kerii*, *Pterocarpus macrocarpus*, *Afzelia xylocarpa*, *Lagerstroemia calyculata*, and several bamboos (Kaosa-ard 1993). In Thailand, flowering in teak usually starts at the age of 8-10 years (Hedegart 1976) and is generally frequent and abundant.

The phenology of teak varies across the natural range depending on the climatic conditions. In India, for example, new leaves appear in June and July followed by the flowering period during September and October. Fruit development ends in November and maturation in March. Fruit shed occurs during April and May (Seth and Kaul 1978). In Indonesia, the flowering period occurs in February and March, followed by fruit development in April and May. Fruits mature during June through August and fruit is shed in September (Palupi

1996). At the Teak Improvement Centre (TIC) in northern Thailand, teak flowering occurs during July and August and the fruits mature from November through February with seed fall occurring in March and April (Hedegart 1976).

The source of the planted stand growing at the AFTSC in Muak-Lek, and used for this study, was northeast Thailand. In Muak-Lek, leaf shedding occurs November through January and the trees remain leafless through the hot, dry season. The new leaves appear in April through June. The trees begin to flower a month earlier (in June) than in the north. Flowering continues through the first half of the rainy season and ends in August. The fruit matures from September to February and the fruit is shed during the hot, dry season from March to April. Bryndum and Hedegart (1969) have suggested that since flowering occurs during the rainy season, the heavy rains are likely to have a deleterious effect on pollination.

2.2 Floral morphology

Unlike most other tree species, teak begins fructification with an inflorescence emerging on the main axis of a young tree (Syrach-Larsen 1966). The inflorescence is a large panicle (Figure 1) containing up to a few thousand flower buds. As only 1-3% of the flowers in an inflorescence bloom each day, anthesis for the entire inflorescence occurs over a 1 to 2 month period, depending on the size. Flowers are small (6-8 mm in diameter), whitish, actinomorphic, and hermaphroditic. The lower half of the corolla is undivided, forming a

Figure 1. *Large panicle of teak inflorescence showing high rate of fruit abortion, which commonly occurs in self- and open pollination.*

tube to which 6-7 stamens are attached (Figure 2). The pistil is composed of a hairy ovary containing 4 ovules and a long (6.55 mm), narrow, bifurcate style with a forked stigma. The forked stigma is of the wet papillate type with unicellular papillae (Bryndum and Hedegart 1969, Tangmitcharoen and Owens 1997).

Figure 2. *Teak flowers (left) at receptivity showing six stamens and petals and straight style; and (right), one day after receptivity after corolla abscised.*

2.3 Receptivity and stigma development

The phenology of individual teak flowers during the day of receptivity is provided in Box 1. Individual flowers are weakly protandrous. Anthesis begins at 0700 h with anthers starting to dehisce at 0800 h, approximately 3 hours before the corolla is completely open and stigma receptivity begins (1100 h). The peak receptive period for teak flowers is 1100 to 1300 h. The bent style is the first organ to emerge as the flowers open. It fully extends between 1100 and 1300 h and exceeds the length of the anthers. Corollas begin to abscise about 1900 h, or earlier in windy conditions, and about 95% of the open corollas are shed by 2300 h.

Box 1. The phenology of individual *T. grandis* flowers during the day of receptivity.

Time of day	Events
0400	flowers closed, style coiled
0500	nectar forms within the lower part of the corolla
0700	flowers begin to open
0800	anthers begin to dehisce
1100-1300	peak receptive period •corolla completely open •style straight •stigma reflexed and turgid •hydration of pollen on stigma
1500	post-receptive period -stigma tip dry and collapsed
1700	anthers collapse, nectar disappears
1900	corolla begins to shed

Source: Tangmitcharoen and Owens (1997)

2.4 Pollen morphology and pollen germination

Pollen is medium tricolpate, but varies somewhat in size and shape depending on the amount of hydration. Dehydrated pollen is semiangular, ranging in size from 12-29 µm (average 19.94 µm) as seen in polar view, and oval, perprolate to prolate, ranging in size from 24.24-48 µm (average 39.79 µm) in equatorial view. Shortly after landing on the stigma, pollen hydrates and expands due to secretion presented by the stigma papillae. Hydrated pollen is spherical to oblate or suboblate in both polar and equatorial views, and ranges in size from 16.8-36 µm (average 28.84 µm). Pollen kitt (a sticky coating) is often found on the mature teak pollen surface, particularly during the early receptive period (1100 h). Pollen released at 1100 h (4 hours after anthesis) has the highest viability (92.2%). Pollen viability gradually decreases after 1100 h. Three days (84 hours) after flower opening, pollen is no longer viable (Tangmitcharoen and Owens, in press).

2.5 Pollination and fruit production

Many studies have reported that teak is insect-pollinated (Horne 1961, Bryndum and Hedegart 1969, Cameron 1968, Egenti 1974, Kedarnath 1974). In Thailand, two bee species, *Heriades binghami* and *Ceratina hirrglyphica* are reported to be the main pollinators, although various kinds of flies and butterflies are also implicated (Bryndum and Hedegart 1969). Tangmitcharoen and Owen (1997) found that the major pollinators are *Ceratina sp.*, which carry teak pollen on most parts of their bodies, but especially on the tibia. They collect pollen by foraging on several newly opened flowers in one inflorescence and travel among inflorescences and trees. Most, however, tend to forage and stay on the same tree for a long period of time.

In large teak plantations, low populations of pollinators and self-pollinations are thought to be the causes of poor pollination success. In trees located near roads, in roadside plantations,and in agricultural fields, fruit production can be high, possibly as a result of a greater number of pollinators, favorable site conditions (Suangtho and Lauridsen 1990), and a higher incidence of cross-pollination.

In general, fruit production following open pollination is low compared to that obtained in controlled cross-pollination. Based on investigations carried out at the TIC during 1962-72, natural open pollination resulted in only 0.4 - 5.1% (average 1.3%) of flowers developing into fruits. Studies on controlled pollination in teak were pioneered by Bryndum and Hedegart (1969) who described a procedure for controlled pollination using small bags to isolate single flowers and large bags stretched over a bamboo frame to isolate an entire inflorescence. The first success of controlled hand-pollination was reported by Hedegart (1973). He indicated that with hand-pollination, the percentage of flowers developing into fruits increased from 6 to 60% (average 20%).

2.6 Fruit and seed development

Palupi (1996) found that fertilization occurs within 24 hours of pollination. The early endosperm develops soon after fertilization and fills the whole embryo sac within 1 week after pollination. The zygote starts to develop into a proembryo 3 days after pollination.

The major cause of fruit and seed abortion is failure in endosperm development. Undeveloped early endosperm causes zygote abortion, and a lack of, or abnormal, endosperm development results in embryo abortion. Palupi also suggested that failure in endosperm development may be attributed to selfing. Teak ovules reach their maximum size 8 to 11 weeks after pollination, which coincides with the maximum fruit diameter. The maximum embryo size is reached 14 to 16 weeks after pollination when the cotyledons become thick and fill the ovule cavity. Fruit moisture content is declining during this period, indicating the end of the seed development stage and the beginning of the maturation stage.

The period from flowering to fruit maturation in Thailand varies between 4 and 7.5 months, which is similar to that in northern India (7-8 months) (Seth and Kaul 1978), and that in Indonesia (6 months) (Palupi 1996). In all three locations, flowering occurs during the rainy season and fruit reaches maturation in the dry season. In northern Thailand, fruits develop to full size approximately 50 days (about 7 weeks) after pollination and another 70-150 days (10-21 weeks) is required for maturation, for a total of about 120-200 days (17-28 weeks) (Hedegart 1976).

2.7 Types of pollination

Natural or open pollination implies no human interference, while controlled pollination requires some human input. Though controlled pollination can be conducted in various ways depending upon the objectives, hermaphroditic flowers are generally subjected to the following treatments:

Type of pollination	Treatments
Natural (open)	no human manipulation
Controlled	• flowers isolated from foreign pollen by bagging • flowers bagged and emasculated (anthers removed)
Self —autogamous	flowers not emasculated, pollen from the same flower applied
—geitonogamous	flowers emasculated, pollen from different flowers of the same tree applied
Cross —allogamous	flowers emasculated, pollen from another tree applied

3. Materials and supplies required for hand-pollination

Controlled hand-pollination activities are fairly complex and are usually conducted in areas, such as clonal seed orchards, where immediate access to equipment, materials and supplies is limited. Advanced planning and purchase of required materials and supplies is critical to ensure a smooth operation. Access to flowers must be ensured and pollination supplies must be available and prepared.

Figure 3. *Access to flowers of a 40-year-old teak tree using steel scaffolds which should be erected soon after emerging of flower bud (July).*

3.1 Reaching the flowers with scaffolding

To conduct controlled pollinations, it is necessary to have easy access to the flowers and to be able to spend time safely and reasonably comfortably within the flowering crown. Fixed scaffolding is probably the most effective method. Scaffolding can be made of bamboo or steel. Bamboo is an inexpensive material; however, it is rather difficult to erect and usually deteriorates after about 1 year of use. On the other hand, steel scaffolding (Figure 3) is more expensive initially, but is easy to erect, is very secure, and does not deteriorate with time. It is more economical in the long run. The number of sections required depend on the height and form of the tree, intensity of inflorescence in the canopy, and size of inflorescence. Each section costs around $50 US and provides about 1.7 m of height. An adjustable base, which allows easier and more secure scaffold installation, is highly recommended.

3.2 Isolating the flowers with pollination bags

To exclude natural pollinators and ensure only the chosen pollen is available to the flowers to be bred, the flowers must be isolated with pollination bags. Of the various types of pollination bags, cellophane has been found to be the most appropriate (Appendix). It can also be obtained easily from an ordinary stationery store.

3.3 Identifying and marking flowers for pollination

Inflorescences and flowers within inflorescences must be identified and well marked or tagged so that there is no doubt about the pedigree of the fruit when it is mature. The tags must be unique and durable so that the identity of the pollen parent is known throughout the process. Color-fast, light weight, pliable yet durable crochet string has been found to be suitable. The yarn comes in spools of many colors. The string should be cut into approximately 6-in. lengths to ease tagging work. If colors are limited, a combination of two or more pieces of the same color or a combination of colors can be used to increase the number of individual labels.

3.4 Handling and manipulating flowers with forceps

Teak flowers, particularly the stamens, anthers, pistils, and stigmas are very small and fragile. The recommended way for handling and manipulating the flowers is by using forceps. Forceps should be approximately 4-5 in. long with a fine point and should be nonsticking. Cole-Parmer forceps, catalogue number E-06440-00, are suitable and are available from: Pennyful (Thailand) Co.Ltd., 245/23 Soi Watdaowadung, Jaransanitwong Rd., Bangplad, Bangkok 10700. Tel (662) 424 3512, Fax (662) 433 7356.

3.5 Observing pollen on stigma

Observation of pollen adhering to the stigma may be required to ensure the success of pollen application and to investigate the success of emasculation to ensure no contamination with self-pollen on each stigma. The use of a 10x magnifying glass in the field will help determine pollination success. Flowers may be collected and taken to the field office where the use of a zoom stereo microscope will provide a clear image of the stigmatic surface. This, however, seems expensive and may not be readily available. A WILD M7A stereo microscope with stepless magnification changer (62x magnification maximum)may be used instead (Wild Heerbrugg Ltd CH-9435 Heerbrugg, Switzerland).

4. Planning the pollination activities

Controlled pollinations conducted within breeding programs are very time sensitive. The window for pollination is often short so advanced planning of all aspects is critical. A mating or breeding plan which identifies clones to be tested must be in place; ramets must be selected; access to adequate numbers of inflorescences and flowers achieved; and staff training in the pollination techniques must be provided.

4.1 The breeding plan

The strategies for the breeding and testing of clones within the tree improvement program must be clearly defined. Conducting controlled hand-pollinations is a very time consuming operation and it is likely that only a few clones can be bred and tested each year. To test the entire available population may take several years. The orchards will have to be well mapped with each tree clearly identified by clone and provenance of origin. In addition, it is important that detailed phenology of each clone be available. To date there is no information for pollen storage of teak so controlled pollination will be with fresh pollen. Synchronization of the flowering period of pollen donor and mother tree is obviously an important consideration when selecting clones for each year's pollination program. The annual breeding plan must clearly outline which clones are selected as pollen donors, which clones are selected as mother trees, and which pollen parents are to be crossed with each selected mother tree. To ensure an adequate number of seeds are available for progeny testing, each specific cross has to be repeated many times over the course of a breeding season.

4.2 Selection of ramets

The breeding plan will outline which clones are to be tested each year. Within each clone, a number of ramets are available and choices must be made. Criteria for ramet selection will vary depending on materials and supplies available. The following should be considered when choosing ramets:

- pollen parents and mother trees should be reasonably close as travel between the two will be required
- ease of accessibility to the trees and flowers, i.e. near road or trail and on reasonably level ground to facilitate scaffold construction
- sufficiently mature with a history of flowering in the recent past so that adequate numbers of inflorescences are likely to be produced. It may be that more than one

ramet of each clone will be required to ensure an adequate number of inflorescences. The use of substantial branches on mature ramets is essential to complete fruit maturation. Small branches of recent grafts may not be capable of providing sufficient nutrients throughout the 4 to 7.5 months of fruit development.

- crown size and height of tree should be reasonable to facilitate access.

4.3 Timing and erection of scaffolding

Scaffolding must be erected on both pollen donor tress and mother trees so flowers for pollen can be collected and controlled hand-pollination can be conducted. Although the clones and the ramets will have been selected in advance, it is recommended that scaffolding be erected after the appearance of sufficient inflorescences to complete the pollination work. Setting up scaffolding prior to visible inflorescences is risky since some selected ramets may not produce sufficient flowers to meet needs.

The scaffolding should be erected and placed so that an adequate number of inflorescence (10 to 20) are easily accessible, and the climbing and pollination work can be conducted in an unhindered fashion. Wooden platforms should be installed at appropriate levels on the scaffolding to provide some comfort, and the scaffold structure should be firmly attached to surrounding trees with guy-wires if height exceeds 7 m (4 sections) to ensure complete safety. Guy-wires should be well flagged to prevent injury.

4.4 How much can be accomplished in a day?

Due to the short daily receptive period (1100-1300 h - see Box 1), one person can expect to hand-pollinate only about 30-60 flowers a day depending on experience. Approximately 7-15 flowers per inflorescence open each day. Thus, around five inflorescences on a single mother tree should be selected for every day of pollination. Selection and marking of inflorescences and flowers within inflorescence will be completed in the early morning. As only 1-3% of the flowers in an inflorescence bloom each day, pollination work will likely be done every day for about 1 month to complete the breeding plan. Inflorescence can be repeatedly used, due to newly opening flowers; however, if sufficient inflorescences are available, it is better to use new inflorescences each day to avoid damaging the previously pollinated flowers.

4.5 Preparation of pollination bags

Experience has indicated that cellophane is the most appropriate material for making pollination bags. Cellophane is available in sheets of 31 x 34 in. and costs only $0.50 US each. To make pollination bags, the cellophane sheets are cut in 2 pieces (31 x 17 in.) and each piece is folded to produce a bag of 15.5 x 17 in. The two sides are held together with transparent tape. A few smaller sized bags should also be prepared for use on smaller panicles.

The number of pollination bags to be prepared will depend on the number of trees to be tested and the number of inflorescences per tree available for pollination on a daily basis.

The cellophane pollination bags can be used twice but are prone to breakage with greater use. The required number of bags per tree will be about 75 (5 inflorescences x 30 days ÷ 2). It is best to ensure that more than enough pollination bags are available for the pollination program.

4.6 Record keeping

Keeping accurate and detailed records of all pollination activities and having excellent orchard (clone) maps is an absolute necessity. The truism "if in doubt, throw it out" should never be heard. Detailed records, i.e. a diary, should be kept on a daily basis. Initially, all activities and problems encountered, as well as weather, should be recorded. During the period of pollination, details on daily pollen donors, pollen collection time, mother trees selected, inflorescence and flowers chosen, cross-pollinations made with identifying string colors, time of emasculation and pollination, weather at the time of pollination, and any problems (e.g. with equipment or supplies) should be recorded as well. A well-kept diary may help to explain variation in seed set, will ensure the pedigree of all genetic material (mature fruit), and will be of great benefit in subsequent years to reduce operational problems.

4.7 Training staff in pollination techniques

Hand-pollination of teak is meticulous and tedious work. Staff cannot become skilled and effective quickly. It is necessary to clearly demonstrate the technique both in the lab and in the field prior to pollination activities. Special emphasis and care must be placed on the emasculation, bagging, and pollen application procedures. Intensive training at the beginning will lead to successful pollinations.

4.8 Pollination success

Tangmitcharoen and Owens (in press) suggested that pollination success was greater in flowers which opened during the early and peak flowering seasons than in those which opened at the end of the flowering season. As such, to catch the early flowering season, all the prepartions, including the breeding plan, selection of ramets, staff training, equipment and supplies, must be completed in advance and be fully operational as the trees begin to flower.

5. The hand-pollination technique

The following tasks are listed in order of daily occurrence:

5.1 Selecting inflorescence and flowers on the mother tree

As pollination success is greater during the early and peak flower seasons, pollination should be done only on those flowers which bloom in these seasons, i.e. only on those flowers on the innermost or proximal portion of the inflorescence. The most distal flowers on an inflorescence should not be pollinated as little success will occur. Selection of flowers on an inflorescence should be done prior to flower opening, i.e. by 0700 h.

Figure 4. *Marking flowers using colored string tied to the pedicel of an unopened flower. Marking should be completed by 0700 h.*

5.2 Marking flowers

Individual flowers must be marked to record the pedigree of the cross. As recommended earlier, flowers should be marked, tagged, or labelled using colored string. At the time of selection in the early morning (by 0700 h) on the day of pollination, the appropriate colored string is tied to the pedicel (Figure 4) of each unopened flower that is to be pollinated. Different colors are used to identify both pollen parent and day of pollination. Tagging should be done carefully and lightly to avoid damaging fragile flowers.

5.3 Emasculating treatments

Anthers of flowers to be used as female parents have to be removed prior to bagging to eliminate the possibility of self-pollination. Using fine forceps, the undehisced stamens are removed from the newly opened flowers between 0600 and 0700 h on the day of pollination. Emasculation should be conducted gently and carefully to avoid dispersion of pollen from anther to stigma of the same flower.

Figure 5. *Pollinated flowers are enclosed in a cellophane bag to exclude all potential pollinators. The bag should be removed the following day.*

5.4 Installing pollination bags

The pollination bags of various sizes have been prepared in advance. The purpose of pollination bags is to isolate the flowers that are to be pollinated that day from pollinators so there is no chance of contamination. The cellophane pollination bags are placed over the group of open flowers (approximately 7-15 flowers per inflorescence) to exclude all potential pollinators (Figure 5). Pollination bags should be attached as soon as possible after emasculation and marking of flowers to prevent pollen contamination from any insect visitors. If more than one bag is contemplated (maximum 2) on an inflorescence, great care must be taken to avoid breaking inflorescences and the pollination bags should be positioned on opposite sides. Plastic wrapping string is used to secure the bag to the inflorescence and to close the open end of the bag.

5.5 Collecting flowers from the pollen donor

Though pollen released at 1100 h has the highest viability (92.2%) (Tangmitcharoen and Owens in press), flowers should be collected as they open, i.e. 0800 - 0900h. As dry windy weather will cause pollen to dehisce, early collection of pollen-donor flowers ensures that the pollen will remain in the anthers. Early collection also provides time to prepare for pollination activities.

Flowers are carefully and gently cut at the pedicel using fine forceps. Removal of flowers should be done gently to avoid dispersion of pollen from the anthers. Ensure that more than enough flowers are collected to pollinate all the flowers on each mother tree. Pollen-donor flowers are used only once so it is necessary to collect at least one pollen-donor flower for each mother-tree flower to be pollinated that day. Collected flowers from the same tree can be stored together in a small plastic container. The container must be handled and stored carefully to avoid contamination by insect visitors or damage from wind and rain. The container can be stored at ambient temperature in the shade without any special treatment since pollen viability remains high until approximately 1500 h (Tangmitcharoen and Owens, in press). The container must be well identified with the pollen parent.

5.6 Pollination treatments

All receptive flowers (7-15) within each bag will be given the some treatment. Within each bag, all the flowers will be controls (pollen excluded), self-pollinated, or cross-pollinated (pollen from donor parents, Section 2.7). During the time the pollination bag is off the flowers, it is important to keep natural pollinators away from the flowers.

5.7 Pollen application at mother tree

Pollen application should be conducted at the peak of the receptive period, i.e. 1100 h (Box 1). Each bag is temporarily removed, one at a time, from the receptive flowers for about 10-15 minutes for hand-pollination. A pollen-donor flower is grasped by the calyx with the forceps. The anthers are then rubbed on the receptive stigmas of the marked flowers to be pollinated (Figure 6). To ensure that sufficient pollen is transferred, all six anthers on a flower are rubbed on the receiving stigma. The pollen-donor flower is then discarded. This operation is repeated using another pollen-donor flower on another marked flower until all open marked flowers in the bag have been pollinated.

5.8 Reinstall pollination bag

After applying pollen, pollination bags are reinstalled. It is necessary to reinstall the bags immediately upon completion of pollen application to each individual group of flowers

Figure 6. *Controlled pollination by brushing an anther against the stigma of a receptive flower.*

to avoid contamination. To prevent build up of heat, which would damage the stigma, the bag and pollinated flowers should not be in contact with one another. After reinstallation, the next inflorescence can be pollinated.

5.9 Removal of pollination bag

Since fertilization occurs within 24 hours after pollination, the bag should be removed the day following pollination.

6. Monitoring of fruit set

After controlled, self- or cross-pollination, the flowers are observed through fruit development until maturity (every day for the first month and every week thereafter). The purpose of regular monitoring is to record fruit set, fruit abortion, and, if possible, the causes of fruit abortion. Generally, the rate of fruit abortion is relatively high during the first two weeks following pollination and possible causes of fruit abortion (insect damage, wind, heavy rain) can usually be determined. During this time, fruit development should be monitored and recorded daily. After fruit reaches full size (50 days), fruit abortion gradually decreases. Observation can be done every week thereafter until fruit maturation. Causes of fruit abortion should be recorded throughout the study.

7. Practical constraints

7.1 Loss of flowers or fruit after pollination

Premature loss of fruit after pollination can occur for different reasons. Common events, causes, and some suggestion to reduce loss are identified in Box 2.

Box 2. Causes and suggestions to reduce premature loss of flowers and fruit

Events	Cause	Suggestion
Broken inflorescences with pollen bag attached	strong wind, heavy rain	• choose light material for making pollination bag, i.e. cellophane • remove bags the day after all of the open flowers in the bag are pollinated • support inflorescence weight by tying inflorescence to main branch
Fruit damage	insects feeding on fruit	• apply insecticide to inflorescence
Unusual loss of pollinated flowers	using the outermost flowers of the inflorescence at the end of flowering season	• use flowers which open during the early and peak flowering seasons • use only flowers which occur in the main body of inflorescence

Ideally, fruit set from cross-pollination should be high. This, however, may not be true for many tropical forest species. Poor fruit set may be caused by three factors: 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 teak, these factors may have contributed to relatively high (80 -85%) fruit abortion with cross-pollination (Hedegart 1973, Tangmitcharoen and Owens, in press).

7.2 Delay of pollination operation due to poor weather

Since the peak of the flowering season of teak occurs during the rainy season, rain is an unavoidable obstacle to pollination work. On a rainy day, hand-pollination cannot be conducted. Cloudy weather may delay flower-opening time, anthesis, and the receptive period. As such, the controlled pollination operation should be flexible to coincide with anthesis and receptivity.

7.3 Worker expertise

Hand-pollination of teak flowers is tedious and time-consuming work. Success of fruit set can vary depending on the attention and expertise of personnel who are conducting pollination. Personnel effectiveness can be enhanced with proper training, good support, and a secure platform on which to work.

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References

Bawa, K.S.; Webb, C.J. 1984. Flower, fruit and seed abortion in tropical forest trees: implications for the evolution of paternal and maternal reproductive patterns. Am. J. Bot. 71:736-751.

Bawa, K.S.; Perry, D.R.; Beach, J.H. 1985. Reproductive biology of tropical lowland rain forest trees. I. Sexual systems and incompatibility mechanisms. Am. J. Bot. 72(3):331-345.

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

Cameron, A.L. 1968. Forest tree improvement in New Guinea, I. Teak. Paper 9th Commonw. For. Conf.. New Delhi, Cameron. 1968.

Dafni, A. 1992. Pollination ecology, a practical approach. IRL Press, at OUP. Oxford, UK.

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

Haber, W.A.; Frankie, G.W. 1982. Pollination of *Leuhea* (Tiliaceae) in Costa Rican deciduous forest: significance of adapted and non-adapted pollinators. Ecology 63:1740-1750.

Hedegart, T. 1973. Pollination of teak (*Tectona grandis* L.) 2. Silvae Genetica 22(4): 124-128.

Hedegart, T. 1976. Breeding systems, variation and genetic improvement of teak (*Tectona grandis* L.f.). Pages 109-124 *in* Burley, J.; Styles, B.T., eds. Tropical trees; variation, breeding and conversation, Linn. Soc. Symp., Series 2. Academic Press, New York, NY. USA.

Horne, J.E.M. 1961. Teak in Nigeria. Nigerian Forestry Infor. Bull. (New series) 16.

Kaosa-ard, A. 1993. Teak in Thailand. *In* Henry Wood, ed. Teak in Asia. Proc. CHINA/ ESCAP/ FAO Regional Seminar on Research and Development of Teak, FORSPA Publ. 4, FAO.

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

Palupi ,E. 1996. Reproductive biology of teak (*Tectona grandis* L.) in East Java, Indonesia. M.Sc. Thesis. University of Victoria, B.C., Canada.

Seth, S.K.; Kaul, O.N. 1978. Tropical forest ecosystem in India: The teak forest in tropical forest ecosystems. Natural Resources Research, XIV, UNESCO, Paris.

Syrach-Larsen, C. 1966. A teak tree show. Nat. Hist. Bull. of the Siam Society, Bangkok, Thailand.

Suangtho, V.; Lauridsen, E.B. 1990. Flowering and seed production in *Tectona grandis* L. *In* Report on the DANIDA training course on Tree Improvement Program. Changmai, Thailand.

Tangmitcharoen, S.; Owens, J.N. 1997. Floral biology, pollination, pistil receptivity, and pollen-tube growth of teak (*Tectona grandis* L.f.). Ann. Bot. (In press)

Tangmitcharoen, S.; Owens, J.N. Pollen viability and pollen-tube growth following controlled pollination and their relation to low fruit production in teak (*Tectona grandis* Linn. f.). Ann. Bot. (In press)

pendix

ious types of pollination bags and their characteristics are listed below, in order of ability for hand-pollination of teak.

terial	Advantages	Disadvantages
Cellophane	• Cheap and disposable • Clear • Pollen-proof • Easy for making and adjusting to desirable size • Very light weight	• Not durable
'aper and ropylene	• Cheap and disposable • Clear (one side) • Pollen-proof	• Not durable under wet weather • Only available in limited sizes and may not fit on different sizes of teak inflorescences • Heavy weight
'aper	• Cheap and disposable • Pollen-proof	• Not durable under wet weather • Not clear, may reduce net photosynthesis and reduce seed production
'ine mesh nets, ride tulle	• Cheap, for multiple use • Less vulnerable to micro-climatic effect inside the bag • Durable in any weather • Different mesh sizes are available	• Not water-proof • Not pollen-proof • Relatively heavy weight, especially if the bags are stretched over bamboo skeletons
'lastic	• For multiple use • Clear and durable under wet weather • Pollen-proof	• Vulnerable to micro-climate effect

ipted from Dafni (1992)

ASEAN Forest Tree Seed Centre Project

The ASEAN Forest Tree Seed Centre (AFTSC) Project is jointly funded by the Canadian International Development Agency (CIDA) and in-kind contributions from ASEAN-member countries.

The executing agency is the Petawawa National Forestry Institute of Forestry Canada.

The host country is Thailand. The Royal Forest Department, Silvicultural Research Division, supports and operates Project headquarters located at AFTSC in Muak-Lek, Saraburi.

Officially launched in 1981, the Project has four main objectives related to improving the management of forests within the ASEAN region through the supply of higher quality seeds and planting stock. These four objectives are:

1) research and development—incorporating applied research on seed and genetic issues into management of the region's forests

2) operational support—supplying seed for research, establishing demonstration projects in seed production and genetic resource conservation, and providing consulting services

3) information dissemination—improving the flow of information within ASEAN-member countries on topics related to forest seeds and genetic resources

4) training—providing relevant training opportunities to forestry personnel in the region.

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