Chapter 6

Grow your own trees

High-quality planting stock is essential for the success of all forest restoration projects that involve tree planting (i.e. for the restoration of degradation stages 3–5). Saplings of all tree species must be grown to a suitable size and must be robust, growing vigorously and disease-free when the season is optimum for tree planting. This is difficult to achieve when growing a large number of different native forest tree species, which will fruit at different times of the year and vary greatly in their germination and seedling growth rates, especially if those species have never been mass-produced in nurseries before. In this chapter, we provide standard tips that are generally applicable for a first attempt to grow native forest trees for a forest restoration program. We also include research protocols that can be used to improve your tree propagation methods, leading to the development of detailed production schedules for each species being propagated.

6.1 Building a nursery

A nursery must provide ideal conditions for the growth of tree seedlings and must protecting them from stresses. It must also be a comfortable and safe place for nursery workers.

Choosing a location

A nursery site should be protected from extremes of climate. It should be:

- flat or slightly sloping, with good drainage (steeper slopes require terracing);
- sheltered and partially shaded (a site protected by existing trees is ideal);
- close to a permanent supply of clean water (but free from the risk of flooding);
- large enough to produce the number of trees required and to allow for future expansion;
- close to a supply of suitable soil;
- accessible enough to allow the convenient transportation of young trees and supplies.

If an exposed site cannot be avoided, a shelter belt of trees or shrubs could be planted, or large containerised trees could provide shelter.

How much space is needed?

The size of the nursery ultimately depends on the size of the area to be restored, which in turn determines how many trees must be produced each year. Other considerations include seedling survival rates and growth rates (which determine how long plants must be kept in the nursery).

Table 6.1 relates the area to be restored each year to the minimum size of the nursery required. These calculations are based on the germination of seeds in trays and their subsequent transplantation into containers, with relatively high survival rates. For example, if the area to be restored is 1 hectare per year, up to 3,100 trees will be needed, requiring a nursery of approximately 80 m^2.

Essential features of a tree nursery

Building a tree nursery need not be costly. Locally available materials, such as recycled wood, bamboo and palm leaves, can all be used to build a simple inexpensive nursery. The essential requirements include:

- a shaded area with benches for seed germination that is protected from seed predators by wire mesh; shade can be provided by commercial materials, but alternatives include palm leaves, coarse grasses and bamboo slats;
- a shaded area where potted seedlings can be grown until ready for planting (the shading should be removable if the young trees are to be hardened here prior to planting);
- a work area for seed preparation, pricking-out etc.;
- a reliable water supply;
- a lockable store for materials and tools;
- a fence to keep out stray animals;
- a shelter and toilet for staff and visitors.

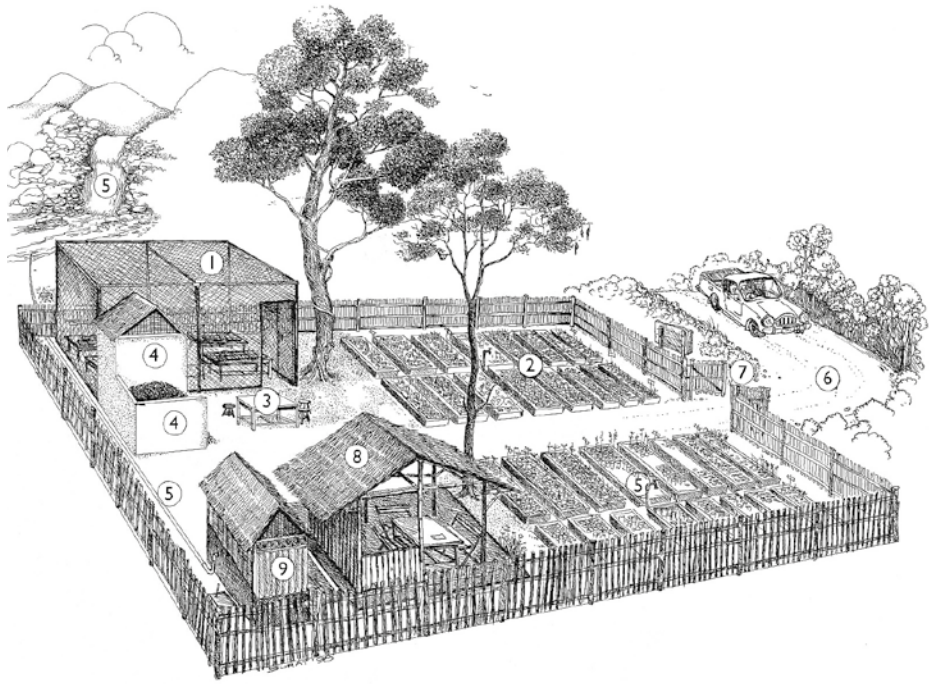
Table 6.1. Relation between the space needed for a nursery and the size of the restoration site.

Area to be restored (ha/year)	Maximum number of trees needed[a]	Seed germination area (m^2)	Standing-down area[b] (m^2)	Storage, shelter, toilet etc. (m^2)	Total nursery area needed (m^2)
0.25	775	3	11	15	29
0.5	1,550	6	22	15	43
1	3,100	13	44	15	72
5	15,500	63	220	15	298
10	31,000	125	440	15	580

[a] Assuming absence of natural regenerants
[b] An additional area of similar size might be required for hardening-off seedlings if it is not possible to remove the shading from the containerised seedlings.

Designing a nursery

A carefully considered nursery layout can greatly increase efficiency. Think about the various activities to be carried out and the movement of materials around the nursery. For example, position the container beds and hardening-off areas near to the main access point, i.e. close to where the trees will eventually be loaded onto vehicles for transport to the restoration site; place the lockable store and media store near the potting area.

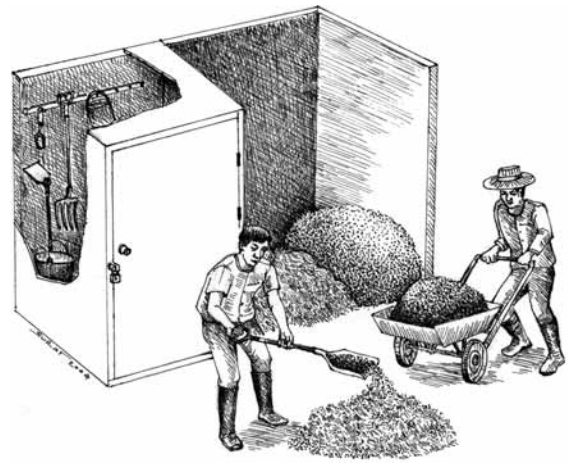


An ideal nursery layout: (1) germination shelter that is protected from seed predators; (2) standing-down area (shade removed); (3) potting work area; (4) media store and lockable equipment store; (5) reliable water supply; (6) easy access; (7) fence to exclude stray animals; (8) shelter from the sun and rain; and (9) toilet.

Nursery tools

Growing trees requires simple, inexpensive equipment. Many of the items illustrated here are readily available in an average agricultural community and could be borrowed for nursery work:

- shovel (1) and buckets (2) for collecting, moving and mixing potting media;
- trowels (3) or bamboo scoops (4) for filling containers with potting medium;
- watering cans (5) and a hose, both fitted with a fine rose;
- spatulas or spoons for pricking-out seedlings;
- sieves (6) for preparing the potting medium;
- wheelbarrows (7) for moving plants and materials around the nursery;
- hoes (8) for weeding and maintaining the standing-down area;
- secateurs (9) for pruning seedlings;
- a ladder and basic construction tools for erecting shade netting etc.



A lockable store for the safe storage of equipment and a media store are essential parts of a tree nursery.

Essential nursery equipment.

6.2 Collecting and handling tree seeds

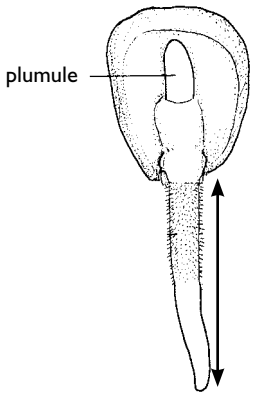
What are fruits and seeds?

The structure that is sown in a germination tray is not always just the seed. For tree species such oaks and beeches (northern hemisphere Fagaceae and southern hemisphere Nothofagaceae), the whole fruit is sown. For, other species, we sow the pyrene, which consists of one or several seeds enclosed within the hard inner wall of the fruit (i.e. the endocarp, which can delay the penetration of the seed embryo by water). So a basic understanding of fruit and seed morphology can be helpful in deciding which pre-sowing seed treatments (if any) are appropriate.

A seed develops from a fertilised egg cell (ovule) that is contained within the ovary of a flower, usually after pollination and fertilisation. Being the products of sexual reproduction, during which the genes of the two parents are combined, seeds are an essential source of genetic diversity within tree populations.

Seeds consist of three main parts: a covering, a food store and the embryo. The seed coat or testa protects seeds from harsh environmental conditions and plays an important role in dormancy. Food reserves, which sustain metabolism during and immediately after germination, are stored in the endosperm or the cotyledons. The embryo consists of a rudimentary shoot (plumule), a rudimentary root (radicle) and seed leaves (cotyledons).

Fruits are derived from the ovary wall. They may be broadly classified as 'simple' (formed from the ovary of a single flower); 'aggregate' (formed from the ovary of a single flower, but with several fruits fused into a larger structure) or 'multiple' (formed from ovaries of several flowers fusing). Each broad category contains several fruit types.



At germination, the radical (first root) and plumule (shoot bud) burst through the outer coat (testa) of the seed fueled by food reserves from the endosperm.

Simple fruits can have either a fleshy pericarp, like that of tomato, or A) a dry covering, such as the pods of legumes. B) Custard apple (*Annona reticulata*) produces aggregate fruits whereas C) jackfruit trees (*Artocarpus heterophyllus*) produce multiple fruits. D) The multiple fruit of fig trees essentially consist of an enclosed infructescence (syconia).

When should seeds be collected?

In all tropical forests, different tree species fruit in every month of the year, so at least one seed collection trip is needed every month. In seasonal tropical forests, fruiting peaks at the end of the dry season and at the end of the rainy season. Reduced numbers of fruiting tree species in the early rainy season means that fewer seed collection trips are needed then.

In parts of Southeast Asia and Central America, the fruiting months of many tree species are well-known, but for many regions, phenology studies are needed to provide this information (see **Section 6.6**). Find seed trees in the forest and monitor them frequently, from flowering onwards, to judge the best time to collect fruits. Collect fruits once they are fully ripe, but just before they are dispersed or consumed by animals. Seeds that are collected too early will be undeveloped and will fail to germinate, whereas those collected too late may have lost viability.

For fleshy fruits, ripeness is usually indicated by a change in the colour of the fruit, usually from green to a brighter colour that attracts seed-dispersing animals. Animals' grazing on the fruits is a sure sign that the seeds are ready for collection. Dehiscent fruits, such as those of some legumes, start to split open when they are ripe. It is usually better to cut fruits from the tree branches rather than to pick them up from the ground.

If you have received appropriate training, climb the tree to cut down ripe fruit. Use a safety harness and never do this alone. A more convenient method of seed collection for shorter trees is to use a cutter mounted on the end of a long pole. Fruits can also be dislodged by shaking smaller trees or some of the lower branches.

The collection of fruits from the forest floor may be the only option for very tall trees. If this is the case, make sure that the seeds are not rotten by cutting them open and looking for a well-developed embryo and/or a solid endosperm (if present). Do not collect fruits or seeds that have signs of fungal infection, teeth marks from animals or small holes made by seed-boring insects. Collect fruits or seeds from the forest floor when the first truly ripe fruits begin to fall.

Seed collection trips require planning and liaison with the people responsible for treating and sowing the seeds because seeds are vulnerable to desiccation and/or fungal attack if they are not processed quickly. Sow the seeds as soon as possible after collection or prepare them for storage as described later in this chapter. Before sowing, do not leave them in damp places, where they might rot or germinate prematurely. If they are sensitive to desiccation, do not leave them in full sun.

Choosing seeds for collection

Genetic variability is essential to enable a species to survive in a changeable environment. Maintaining genetic diversity is therefore one of the most important considerations in any restoration program aiming to conserve biodiversity. It is therefore crucial that the planted trees are not all closely related. The best way to prevent this is to collect seeds from at least 25 to 50 high-quality parent trees locally, and preferably to augment this with some seed from trees located in more distant, eco-geographically matched areas (see **Box 6.1**, p. 159). If seeds are collected from just a few local trees, their genetic diversity may be low, reducing their capacity to adapt to environmental change. Equal numbers of seeds from each seed tree should be mixed together (known as bulking-up) before sowing, to ensure that all the seed trees are represented equally. Once the trees mature within the restored plots, they may inbreed with each other, further reducing genetic variability in subsequent generations. Cross-pollination with unrelated trees can restore genetic diversity, but only where such trees grow close to restoration sites**.**

The number of seeds collected depends on the number of trees required, seed germination percentage and seedling survival rates. Keep accurate records to determine the numbers required in future collections.

Information that should be recorded when collecting seeds

Species number: **Batch number:**

SEED COLLECTION RECORD SHEET

Family:

Species: **Common name:**

Date collected: **Collector's name:**

Tree label no.: **Tree girth:**

Collected from ground [ ] or from tree [ ]

Location: **Elevation:**

Forest type:

Approximate no. seeds collected:

Storage/transport details:

Pre-sowing treatment: **Sowing date:**

Voucher specimen collected [ ]

Notes for herbarium label:

Each time you collect seeds from a new species, give that species a unique species number. Nail a numbered, metal tag onto the tree, so that you can find it again. Collect a specimen of leaves and fruits for species identification. Place the specimen in a plant press, dry it and ask a botanist to identify the species. Use a pencil to write the species name (if known), date and species number on a label and place the label inside the bag with the seeds.

On a data sheet (example below), record essential details about the seed batches collected and what happened to them from collection time until they are sown in germination trays. This information will help to determine why some seed batches germinate well whereas others fail, and thus will improve seed-collection methods in the future. A more detailed seed collection data sheet that might be used for research purposes is provided in the **Appendix** (**A1.3**).

Box 6.1. Gene flow, adaptive genetic diversity and sourcing seed.

Global climate change has profound consequences for tropical forest ecosystems. The evolutionary adaptability of a species, its capacity to survive environmental change, depends on the genetic diversity present among the individuals of the species. Populations of trees that have a wide range of adaptive genetic variation have the best chance of surviving climate change or changes in other environmental factors, such as increased salinity, the use of fertilisers and vegetation redistribution resulting from habitat conversion.

Consider individual trees of a species, each of which might possess different versions or 'alleles' of a gene that encodes a certain protein. If one of these alleles functions better in drier conditions, then the individuals carrying this allele might survive better if rainfall declines and so would be more likely to pass on their version of the gene to subsequent generations. Conversely, trees that carry a different allele of the same gene or of a different gene might survive better if conditions were to become wetter. Consequently, maintaining genetic variability among individual trees that comprise a species population is one of the most important considerations in any restoration program for biodiversity conservation.

Adaptive genetic variation depends on rates of gene mutation, gene flow and other factors. Natural selection increases the frequency of traits, which confer advantages to individuals, at a particular time or place. In the case of tropical trees, it may act at the seedling stage, when saplings have an opportunity to replace a fallen tree in the canopy. It may help tree populations to cope with future climate-induced stress.

Adaptive genetic diversity increases as a result of gene flow, that is when different genes are introduced into a population by pollen or seed from another tree, or population of trees. Gene flow can occur over distances of up to hundreds of kilometres (Broadhurst *et al*., 2008). Habitat fragmentation hinders the dispersal of both pollen and seeds. Furthermore, tree populations that are adapted to the current environmental conditions might not have sufficient adaptive genetic diversity to enable sufficient numbers of their offspring to survive climate change. Forest restoration practitioners must consider whether local gene pools have sufficient adaptive genetic diversity and resilience to meet the challenges of climate change, and to adapt quickly enough as the environment changes. Consequently, there could be a strong case for sourcing a proportion of the seed for restoration projects from areas that are not local in an attempt to mimic natural gene flow.

It has been recommended that seeds for forest restoration projects are collected locally, from 'high quality' parent trees, because local trees are the product of a long history of natural selection that has adapted them genetically to survive and reproduce in the local prevailing conditions. Given the need to maintain high levels of genetic diversity so as to ensure adaptability to climate change, however, locally sourced seed could be supplemented with a small percentage of seeds collected from other areas that have similar environmental and climatic conditions to the planting site. 'Composite provenancing' has been proposed as a way of enhancing natural gene flow (Broadhurst *et al*., 2008). For example, the majority of seeds could be collected from as many local parent trees as is practicable, but also incorporate nearby and eco-geographically matched sources (Sgró *et al*., 2011). A smaller proportion (10–30%) could be sourced from much further afield (Lowe, 2010). The resulting new gene combinations might enable the tree populations to respond to environmental change, which is crucial if natural selection is to act on restoration plantings.

Box 6.1. continued.

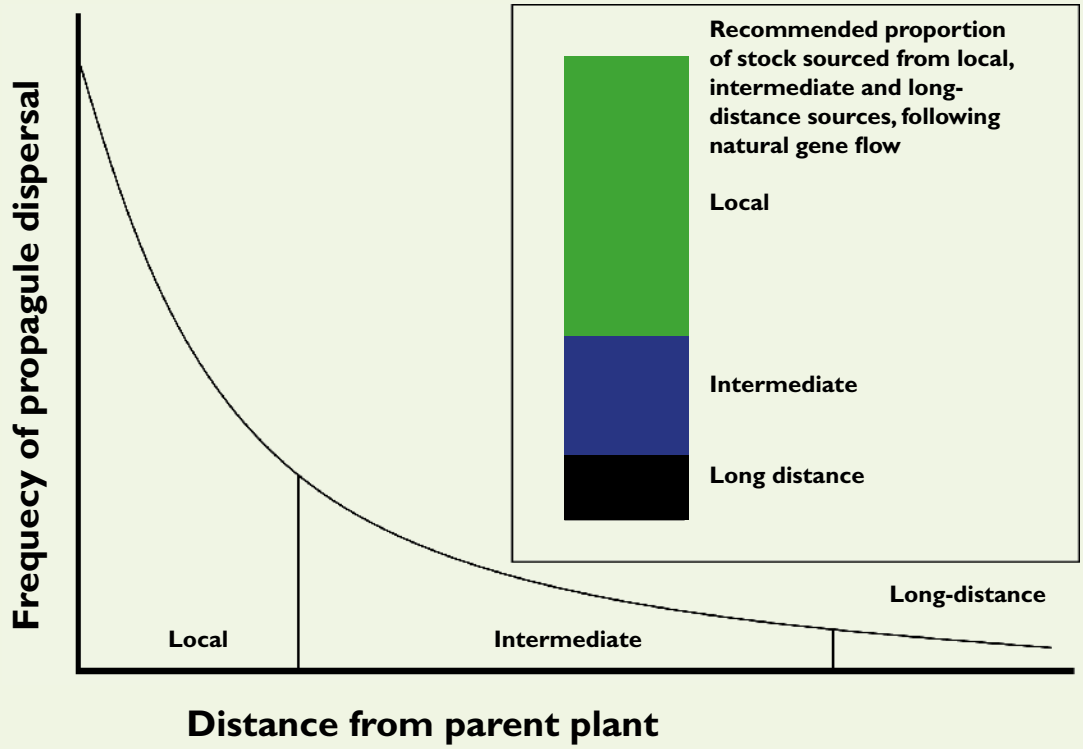


Figure reproduced with kind permission from Sgró *et al.* (2011).

For most species, seed dispersal is local, with much lower proportions of seed being dispersed over intermediate and longer distances. Composite provenancing mimics this dispersal pattern, using a high proportion of locally adapted seed, and lower proportions of seed collected at intermediate (mimicking intermediate gene flow) and distant locations. The seed collected some distance from the restoration site could introduce novel genes into the population.

Extracting seeds from fruits

For most species, the seeds should be removed from the fruits and cleaned before sowing.

With fleshy fruits, remove as much of the fruit pulp as possible with a knife and wash off remaining pulp with water. Soak firm fruits in water for 2–3 days to soften the pulp sufficiently to ease seed extraction. Once the fruit pulp has been removed, the seeds might germinate quickly, so either sow them immediately or process them for storage. Failure to remove fruit pulp encourages fungal infection. In some species, removal of the pulp reveals a woody or stony pyrene containing one or more seeds. If the seeds are to be planted immediately, crack open the tough endocarp to allow water to penetrate into the embryo and trigger germination. Use a vice, hammer or knife to crack the endocarp gently without damaging the seed(s) inside.

Dry dehiscent fruits, such as the pods of trees in the Leguminosae family, often split open naturally, so lay them out in a dry, sunny place until they open and the seeds either fall out on their own or can be easily shaken out.

For dry indehiscent fruits that do not split open naturally, cut pods open or prise them apart with secateurs or other tools. The seeds of some indehiscent fruits, such as samaras and nuts, are not usually extracted and the whole fruits should be placed in germination trays. Fruit appendages, such as the wings of samaras (e.g. *Acer*, *Dipterocarpus*) or the cupules of nuts including acorns or chestnuts, should be removed for easier handling. The germination of seeds that are covered by an aril is nearly always accelerated by scraping the aril.

Ensuring seed quality

It is very important to sow only the highest-quality seeds available. They should have no signs of fungal growth, teeth marks from animals or small holes made by seed-boring insects such as weevils. For larger seeds, dead seeds can be rapidly identified by immersing the seeds in water and waiting for 2–3 hours. Skim off those seeds that remain floating as they have air inside instead of dense cotyledons and a functioning embryo. Sowing poor-quality seed is a waste of time and space, and could encourage the spread of diseases.

Sorting good seed from bad: the good seed sinks (left) the bad seed floats (right).

Seed storage

Although it is usually best to germinate seeds as soon as possible after collection, seed storage can be a useful in streamlining tree production, sharing seeds among nurseries and accumulating seeds for direct seeding. Depending on their physiological storage potential, seeds may be classified as orthodox, recalcitrant or intermediate. The storage behaviour of many species can be found at http://data.kew.org/sid/search.html.

Orthodox and recalcitrant seeds

Orthodox seeds remain viable when dried to low moisture contents (2–8%) and chilled to low temperatures (usually a few degrees above freezing), so they can usually be stored for many months or even years.

Recalcitrant seeds are more common in species from moist tropical habitats and tend to be large and to have thin seed coats or fruit walls. They are very sensitive to desiccation and cannot be dried to moisture contents lower than 60–70%. Furthermore, they cannot be chilled and are relatively short-lived. Therefore, it is very difficult to store recalcitrant seeds for longer than a few days without losing viability.

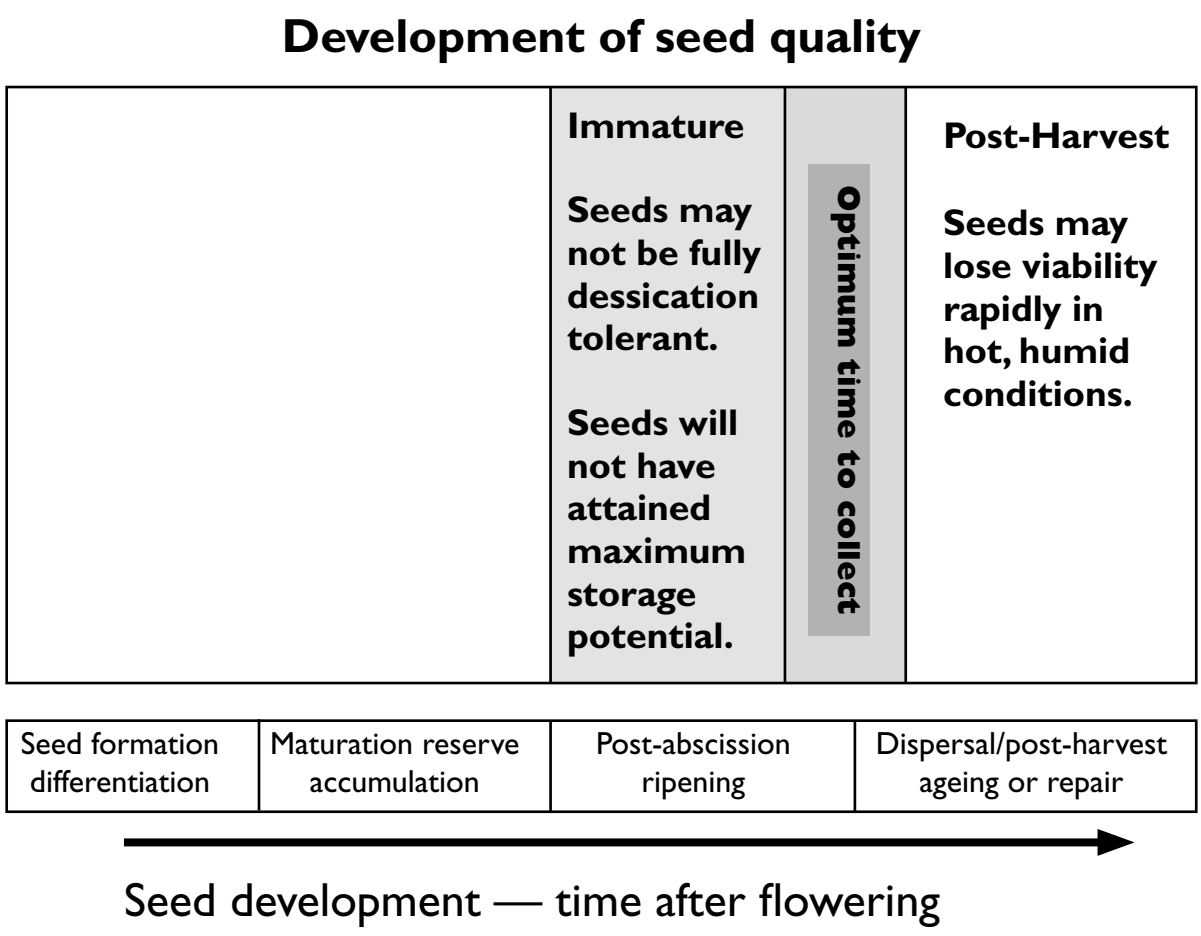
There is also a sub-group of species that have 'intermediate' seeds. These can be dried to low moisture contents, approaching those tolerated by orthodox seed, but they are sensitive to chilling when dried.

Drying and storing orthodox seed

First, determine whether the majority of the seeds are ripe or immature, because individual trees may disperse their seeds at slightly different times. Ripe seeds, which are ready for dispersal, respond best to drying. Immature seeds are generally more difficult to dry.

Immature seeds, either fresh or after drying, do not germinate. They can, however, be ripened and their viability increased markedly by storing them at a controlled humidity and temperature. A relative humidity of 65% is low enough to reduce the chances of mould. Alternatively, store the fruits under as natural conditions as possible, i.e. with the fruit left on stems and seed left in the fruit. Examine a few seeds occasionally to determine when the batch reaches maturity.

Ripe seeds must be handled carefully between collection in the forest and storage or sowing in the nursery. Once the seeds are harvested, they begin to age, particularly if kept at high moisture contents. They may be attacked by insects, mites and/or fungi (if not kept well aerated) or they may germinate.



Development of seed quality with maturation time. (Reproduced with kind permission of the Board of Trustees of the Royal Botanic Gardens, Kew)

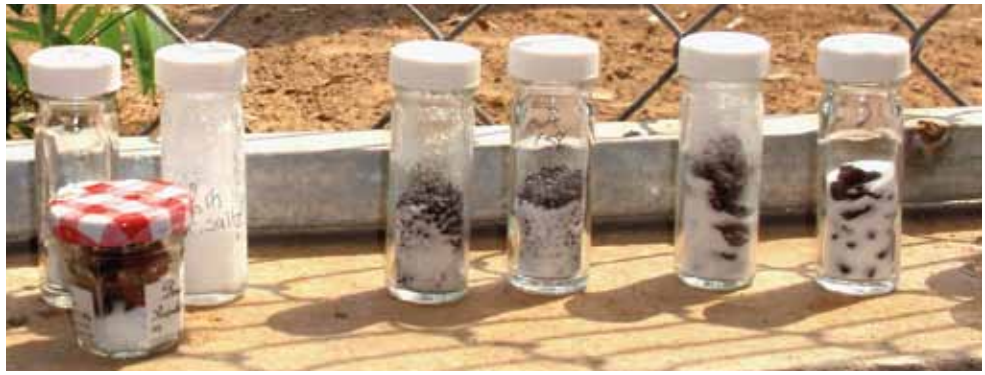
Measuring moisture content

To retain their viability during storage, orthodox seeds must be dried, but how dry is dry enough? To determine if seeds are dry enough for storage, half fill a glass jar with seeds and add a small hygrometer or moisture indicator strip to the jar (Bertenshaw & Adams, 2009a). Wait for the air in the jar to reach a stable humidity in the shade. This is called the equilibrium relative humidity (eRH). **Table 6.2** shows that an eRH% of 10–30% is recommended for the long-term storage of orthodox seeds.

Either a sophisticated and expensive digital hygrometer (left) or simple and cheap dial hygrometers (right) can be used to assess the moisture content of seeds.

Table 6.2. Relation between eRH%, seed moisture content and the survival of seeds in storage.

eRH%	Approximate moisture content (varies with seed oil content and temperature)		Seed survival
	Non-oily seed (2% oil)	Oily seed (25% oil)	
85–100%	>18.5%	> 16%	High risk of mould, pests and disease
70–85%	12.5–18.5%	9.5–16%	Seeds at risk of rapid loss of viability
50–70%	9–12.5%	6–9.5%	Rate of deterioration slower; seeds may survive for 1–2 yrs
30–50%	7.5–9%	5.5–6%	Seeds could survive for several years
10–30%	4.5–7.5%	3–5.5%	Seeds can be kept alive for decades
< 10%	< 4.5%	< 3%	Risk of damage, therefore best avoided



Crude salt test for seed moisture content. The small jam jar (front), the third vial from the right and the far right vial all contain free-flowing salt indicating seeds that are dry enough for storage.

Salt can also be used in a crude test for moisture content. Quarter fill a small glass jar with very dry table salt, add about an equal volume of seeds and shake. If the salt forms clumps, the eRH is higher than 70%. If the salt remains free flowing, then the seeds can be stored, at least in the short term.

An alternative method of determining the moisture content of dried seeds is to weigh a sub-sample of the sun-dried seeds, then put them in an oven at 120–150°C for an hour before reweighing them. If the following calculation gives a values of <10%, the seeds are ready for storage:

$$\frac{\text{(Seeds mass after sun-drying} - \text{Seed mass after oven-drying)} \times 100\%}{\text{Seed mass after sun-drying}}$$

Throw away the sub-sample of seeds used for this test.

Drying seeds

The simplest way to dry seeds is to clean them and leave them in the sun for a few days. Spread the seeds in thin layers on a mat, and turn them regularly with a rake so that they dry quickly and evenly without overheating. Direct sunlight for extended periods reduces seed viability. Shade the seeds during the hottest part of the day and protect the seeds at night or after rain to prevent moisture re-absorption. If practical, transfer the seeds to sealed containers overnight. Once every 24–48 hours, test the moisture content of a sample of the seeds, and continue drying until the eRH falls to 10–30% (equivalent to 5–10% seed moisture content). Drying time will depend on the size of the seed, the structure and thickness of the seed coat, ventilation and temperature.

Seeds drying on a mat in Tanzania. (Photo: K. Gold)

Desiccants

Desiccants are substances that absorb moisture from air. A wide range of desiccants can be used to dry seeds in sealed containers. Silica gel is perhaps the best known, but local products, such as toasted rice and charcoal, are cheaper alternatives. The Royal Botanic Gardens, Kew has developed a seed-drying technique that uses natural or 'lump-wood' charcoal, which is universally available in rural tropical communities (Bertenshaw & Adams, 2009b). First, dry the seeds for 2–3 days under ambient conditions; meanwhile, dry small lumps of charcoal in an oven or in direct sunshine. Place the charcoal in the bottom of a sealable container, then cover it with newspaper and place the seeds on top of the paper. Add a hygrometer or a moisture strip, seal the container and store it in a cool place. Alternatively, put the seeds in cloth bags and hang them in larger containers, such as plastic drums, with charcoal at the bottom. To attain an eRH of 30% use a charcoal:seed weight ratio of 3:1; for an eRH% of 15% use a ratio of 7.5:1.

Charcoal is a cheap desiccant that is widely available in tropical rural communities.

Charcoal in a sealed container or in sealed bags can be used as a natural dessicant. (Photo: K. Mistry)

Once the seeds are dried, store them in air-tight containers under conditions that reduce seed metabolism and prevent the entry (or growth) of pests and pathogens. Containers can be plastic, glass or metal and their seals might be improved with the use of rubber inner-tubes. Fill the containers to the top to minimise the volume of air (and moisture) inside. Efficient sealing of containers is crucial, to prevent the entry of moisture or fungal spores. Even a rise of just 10% eRH can reduce the storage life of the seeds by half. If containers are likely to be opened frequently, store the seeds in small sealed packets within larger containers to minimise the exposure of the remaining seeds to air and moisture. Putting a small sachet of coloured silica gel into the containers will indicate if any moisture is getting into the container.

Storing the containers at ambient temperatures should be sufficient to maintain viability for 12–24 months. Keeping seeds for longer periods may require storage at low temperatures, but this can be expensive and is not usually necessary for forest restoration projects.

Storing recalcitrant and intermediate seed

The storage tolerance of recalcitrant and intermediate seeds varies enormously. Some species have no dormancy at all. Highly recalcitrant seeds die when their moisture content drops below 50–70%, whereas less sensitive ones can remain viable down to 12% moisture content. Chilling tolerance also varies. Keep the storage duration for recalcitrant seeds to an absolute minimum. When storage is unavoidable, prevent desiccation and microbial contamination and maintain an adequate air supply.

For a comprehensive account of seed collection and handling, the reference text "*A Guide to Handling Tropical and Subtropical Forest Seed*", by Lars Schmidt (published by the DANIDA Forest Seed Centre, Denmark, 2000) is highly recommended.

6.3 Germinating seeds

For larger seeds that have hard seed coats, dormancy may be broken by manually cutting the seed coat.

In the nursery, dormancy prolongs tree production time (see **Box 6.2**, p. 168). Therefore, various treatments are commonly applied to shorten dormancy. The treatment used for each species depends on the particular dormancy mechanism(s) present.

A thick, impervious seed coat can prevent water or oxygen reaching the embryo, so one of the simplest techniques to break dormancy is to cut away a small piece of the seed coat with a sharp knife or nail clippers. For smaller seeds, gently rubbing them with sandpaper can be equally effective. These techniques are called scarification. During scarification, care must be taken not to damage the embryo within the seed.

For species with mechanical dormancy, acid treatment is recommended. Acid can kill the embryo, so seeds must be soaked in acid long enough to soften the seed coat but not for long enough to allow the acid to reach the embryo.

When germination is inhibited by chemicals, simply ensuring complete removal of fruit pulp can solve this problem. But if the chemical inhibitors are present within the seed, they must be washed out by repeated soaking. For more on pre-sowing seed treatments, see **Section 6.6**.

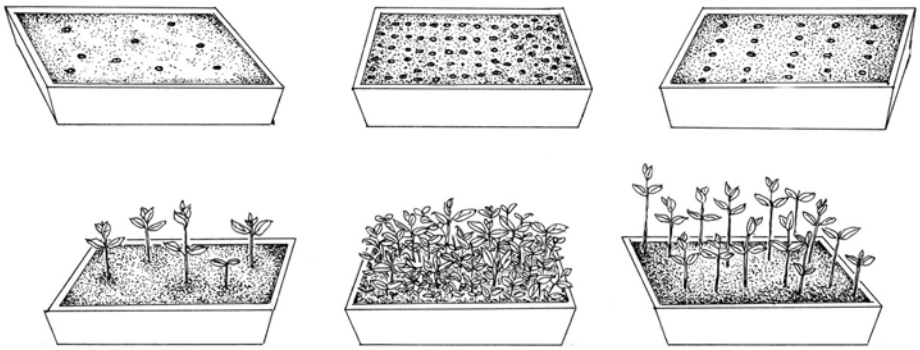
Germination is the most vulnerable time in the long life of a tree.

Sowing seeds

Sow seeds in germination trays filled with a suitable medium. Large seeds can be sown directly into plastic bags or other containers. The advantage of using trays is that they can be easily moved around the nursery, but remember that they can dry out quickly if neglected. Seed trays should be 6–10 cm deep, with plenty of drainage holes in the bottom.

The germination medium must have good aeration and drainage and must provide adequate support for germinating seedlings until they are ready for pricking-out. Seedling roots need to breathe, so the germination medium must be porous. Too much water fills the air spaces in the medium and suffocates seedling roots. It also encourages disease. Compacted soil inhibits both germination and seedling growth.

Mix forest soil with organic materials to create a well-structured medium. Chiang Mai University's Forest Restoration Research Unit (FORRU-CMU) recommends a mixture of two-thirds forest top-soil to one-third coconut husk. A mix of 50% forest soil with 50% coarse sand is more suitable for small seeds, especially those (e.g. *Ficus* spp.) susceptible to damping-off fungi. Include some forest soil in the medium to provide a source of mycorrhizal fungi, which are required by most tropical forest tree species. If forest top-soil is not available, use a mix that includes coarse sand (to encourage good drainage and aeration) and sieved organic matter (to provide texture, nutrients and water retention). Do not add fertiliser to the seed germination medium (except when germinating seeds of *Ficus* spp.), as the seedlings will not require it.



Sowing seeds too far apart (left) is a waste of space, but sowing them too close together (centre) increases the risk of disease.

Sow small to medium-sized seeds on the surface of the medium and then cover them with a thin layer of germination medium (with a depth of approximately 2–3 times the seeds' diameter), which should come to within 1 cm of the tray's rim. Seeds larger than 5 mm in diameter require an equivalent depth of germination medium. This protects the seeds from predators and drying out and prevents them from being washed away during watering. If rats or squirrels are a problem, then cover the germination trays in wire mesh. Place the trays in shade to reduce the drying out and scorching of leaves.

Space the seeds at least 1–2 cm apart (further if the seeds are large) to prevent overcrowding. If the seeds are sown too closely together, the seedlings may be weakened and hence more susceptible to diseases such as damping-off. Water the germination trays lightly, immediately after sowing the seeds and regularly thereafter, using a spray bottle or a watering can with a fine rose to prevent compaction of the medium. Too frequent watering encourages damping-off diseases.

A perfect germination room at Lake Eacham National Park in Queensland, Australia with the germination trays on wire grid benches. The trays at the back are protected by wire cages, which are lowered at night to exclude rats and birds. Note that all of the germination trays are clearly labelled with the species and date of sowing.

Box 6.2. Dormancy and germination.

Dormancy is the period during which viable seeds fail to germinate, despite having conditions (moisture, light, temperature etc.) that are normally favourable for the later stages of germination and seedling establishment. It is a survival mechanism that prevents seeds from germinating during seasons when the seedlings are likely to die.

Dormancy can originate in the embryo or in the tissues that surround it (i.e. the endosperm, testa or pericarp). Dormancy that originates in the embryo can be due to i) a need for further embryonic development (after-ripening); ii) chemical inhibition of metabolism; iii) a block on the mobilisation of food reserves; or iv) insufficient plant growth hormones. Dormancy that is due to the seed coverings can be caused by i) restriction of transport of water or oxygen into the embryo; ii) mechanical restriction of embryo expansion; or iii) chemicals that inhibit germination (most commonly abscisic acid). In many plant species, dormancy results from a combination of several such mechanisms.

Germination consists of three overlapping processes. i) The absorption of water causes swelling of the seed and splitting of the seed coat. ii) Food reserves in the endosperm are mobilised and transported to the embryonic root (radicle) and shoot (plumule), which begin to grow and push against the seed coat. iii) The final stage (and the most precise definition of germination) is the emergence of the embryonic root through the seed coat. In germination trials, this can be difficult to observe as the seeds are buried, so emergence of the embryonic shoot can also be used to indicate germination.

Species number: **Batch number:**

GERMINATION RECORD SHEET

Species:

Date sown: **Number of seeds sown:**

Germinated	Date	Days since sowing
First seed		
Median seed		
Final seed		

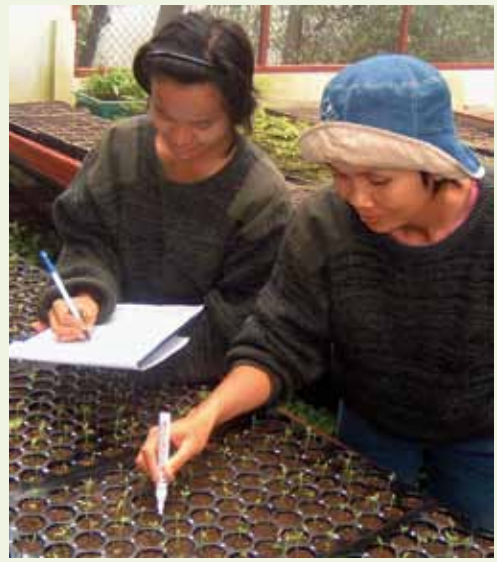
Number germinated: **% Germination:**

Pricking-out date:

No. of seedlings pricked out:

Date	No. Germinated	Date	No. Germinated

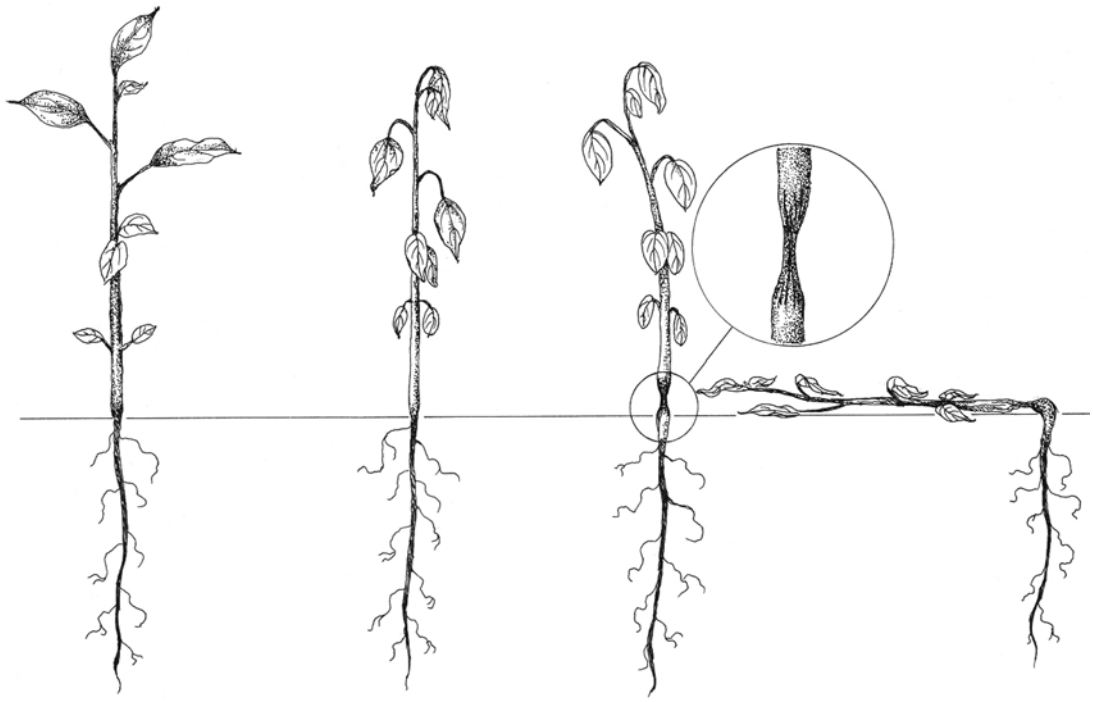
Seed germination is influenced by moisture, temperature and light. Seedlings are at their most vulnerable to disease, mechanical damage, physiological stress and predation just after germination, so take care to protect germinating seeds from infection, drying winds, heavy rain and strong sunshine.



Keeping track of germination gradually improves nursery efficiency over time.

Damping-off diseases

The term 'damping-off' refers to diseases that are caused by several genera of soil fungi, including *Pythium*, *Phytopthera*, *Rhizoctonia* and *Fusarium*, which can attack seeds, pre-emergent sprouts and young seedlings. Pre-emergence damping-off softens the seeds and turns them brown or black. Post-emergence damping-off attacks the soft tissue of recently germinated seedlings just above the soil surface. Infected seedlings appear to be 'pinched' at the base of the stem, which turns brown.



Damping-off diseases, which are caused by various fungi, start with brown lesions appearing on the stem, at or just above the soil surface. The lesions spread and the leaves wilt. Finally, the stem collapses and the seedling dies.

If they become a serious problem, damping-off diseases can be controlled with fungicides such as Captan. The use of chemicals is undesirable, but prompt application of fungicide at the outbreak of disease can mean the difference between saving the tree crop and having to wait another year to collect seeds again.

Remove infected seedlings immediately and destroy them to prevent the disease from spreading. Basic hygiene measures can significantly reduce the incidence of damping-off diseases and are preferable to spraying with a fungicide. These include not sowing seeds too densely, maintaining a well-structured germination medium, not over-watering, ensuring free air movement around the seedlings and disinfecting any nursery tools that have come into contact with soil.

Box 6.3. Propagation of *Ficus* species.

Ficus species play a vital role in tropical forest restoration (see **Box 2.2**) and several species should always be growing in a restoration tree nursery. But propagating them requires a few special techniques. *Ficus* planting stock is best grown from seed: although propagation from cuttings is efficient, the planting stock derived from seed is usually healthier and more vigorous. Stock that has been raised from seed is also more genetically diverse, a crucial consideration for biodiversity conservation projects. But growing fig trees that are large enough for planting from seed can take 18–22 months, so if planting stock is required more urgently, try cuttings.

First make sure that there are seeds in the fig as there are in this female fig of *Ficus hispida*.
(Photo: C. Kuaraksa)

Separate the seeds from the mush inside the fruit and air dry them for a few days.

Collect ripe figs, break them open and see if they contain seeds. Figs of monoecious *Ficus* species contain both male and female flowers, so all of their figs have the potential to produce seeds if they have been visited by pollinating fig wasps (see **Box 2.2**, p. 31). Dioecious figs have separate male and female trees. Obviously, the figs on male trees never contain seeds, so consult a flora to find out if the species that you want to propagate are monoecious or dioecious.

A single fig can contain hundreds or even thousands of miniscule, light brown and hard seeds. Scrape out the mush that contains the seeds from inside the figs with a spoon. Press the mush through a piece of mosquito netting over a bowl of water. Viable seeds will pass through the net and sink. Pour off most of the water and pour the remaining water, along with the seeds (which have sunk to the bottom of the bowl), through a fine tea strainer. Wash the seeds thoroughly and leave them to dry slowly over 1–2 days.

Sprinkle the seeds evenly (aiming for gaps of 1–2 cm) over the surface of a germination medium comprising a 50:50 mix of sand and charred rice husk or similar materials (do not include forest soil in the medium). Do not cover the seeds. Water the trays by hand using a fine spray bottle.

Most species will start to germinate within 3–4 weeks and germination will be complete within 7–8 weeks. Fig seedlings are tiny and grow slowly at first. Adding a few granules of slow-release fertiliser (e.g. Osmocote) just below the surface of the germination medium can accelerate seedling growth, but it can also increase seedling mortality. *Ficus* seedlings are particularly susceptible to damping-off, so remove infected seedlings immediately and apply a fungicide such as Captan if an outbreak occurs. Prick out the seedlings after the second pair of true leaves has expanded (4–10 months after germination) and pot them into standard containers and media.

To produce planting stock from cuttings, follow the method in **Box 6.5**. If propagating a dioecious species, collect an equal number of cuttings from both male and female trees. Apply synthetic auxins to stimulate rooting (Vongkamjan, 2003).

By Cherdsak Kuaraksa

Shade

Germinate all seeds, whether they are from light-demanding or shade-tolerant species, under shade. If practical, provide more shade to shade-tolerant species. As the time for pricking out approaches, reduce the level of shade to that of the growing-on area. If several layers of plastic shade cloth have been used, remove them one at a time.

6.4 Potting

Containers or soil beds?

Trees that have been grown in beds of soil are termed 'bare rooted' because they retain very little soil with the roots when they are dug out for planting. Their exposed roots quickly lose water and are easily damaged. When the root system is reduced but the leaf area remains the same, the roots are unable to deliver enough water to the shoots to maintain transpiration and to retain turgidity of the leaf cells, resulting in wilting and increased mortality. Thus, bare-rooted planting stock often suffers from 'transplantation shock' when planted out in deforested sites, resulting in mortality rates that are much greater than those for trees that have been grown in containers.

With a containerised system, seedlings are transplanted from germination trays into containers in which they grow until they are large enough to be planted out. The containers protect the trees during transportation to the planting site where the whole root ball can be removed from the container, thus minimising transplantation stress.

Choosing your containers

The containers must be large enough to allow the development of a good root system and to support adequate shoot growth. They must have sufficient holes to permit good drainage, and be lightweight, inexpensive, durable and readily available. Containers can be made out of a variety of materials, such as polythene, clay and biodegradable materials. When funding is insufficient to allow for the purchase of containers, try improvising by converting cartons, plastic bottles or old cans (don't forget to add drainage holes); even banana leaves can be folded to make an adequate container.

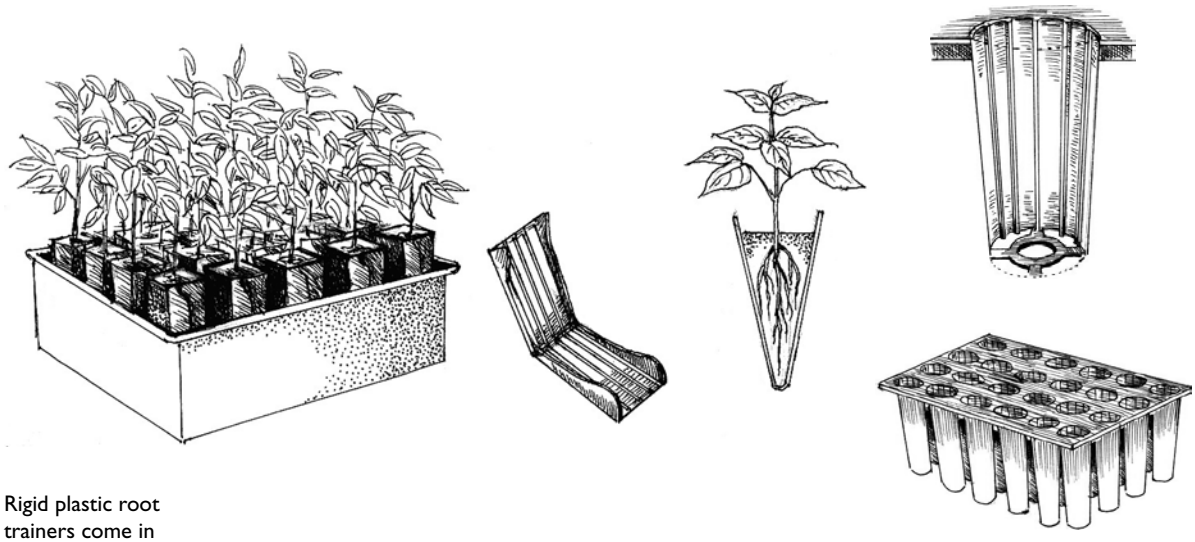
Plastic bags are probably the most commonly used containers. They come in a range of sizes and are strong, lightweight and cheap, and they have been used successfully with a very wide range of species. Large plastic bags are difficult to transport and require a lot of medium, whereas small ones restrict root development. The optimum size is 23 × 6.5 cm, which allows tap roots to reasonable length before they reach the bottom of the bag and begin to spiral.

Plastic bags (23 × 6.5 cm) are cheap but not reusable and can cause root curling of fast-growing tree species.

Plastic bags do have some disadvantages. They can bend easily, particularly during transportation; this can damage the root ball, causing it to crumble during planting. The roots of fast-growing tree species can fill the bags rapidly and begin to spiral around at the bottom. This poor root formation can increase the vulnerability of trees to wind-throw later in life. Roots can grow through the drainage holes into soil beneath, so that roots are severed when the tree is lifted just before planting, causing transplantation shock. Root trainers can reduce this problem.

Root trainers

Root trainers are rigid plastic pots with grooves down the sides that direct root growth downwards, thus preventing root spiralling. Large holes in the bottom allow air pruning (see **Section 6.5**). Although initially more expensive than many other types of container, they can be re-used many times and their rigidity protects the root ball during transportation.



Rigid plastic root trainers come in various designs and sizes.

What makes a good potting medium?

A potting medium consists of coarse and fine soil particles with pores between them that allow aeration and drainage. The medium must provide growing trees with support, moisture, oxygen, nutrients and symbiotic micro-organisms.

Tree roots that are growing in containers have access to only a limited volume of medium. Soil alone is an unsuitable medium because it is easily compacted and the container prevents free drainage, causing water-logging that suffocates the roots. Good drainage is essential, but the medium must also have an organic matter content that is adequate to ensure that the medium remains adequately moist between waterings.

Various materials can be included in potting media, including coarse sand or gravel (washed to remove salts) and forest topsoil. Organic matter can be added in the form of rice husk charcoal, coconut husk, peanut husks and even waste products from agricultural production, such as coffee fruit pulp or pressed sugar cane. Alternatively, try making compost from domestic organic waste. Adding cow dung to the mixture can dramatically increase seedling growth rates because of its rich nutrient content.

Although forest topsoil alone is a poor potting medium, it is an important component of potting mixes because it carries the spores of soil micro-organisms that help trees to grow, such as *Rhizobium* bacteria and mycorrhizal fungi. To prevent compaction, mix forest soil with bulky organic matter or coarse sand. Mixing forest soil with these ingredients 'opens out' the medium and improves drainage and aeration. Whichever materials you choose, they should be cheap and locally available throughout the year.

Table 6.3. Standard potting mix.

Ingredient	Proportion	Beneficial properties	Examples
Forest soil	50%	Nutrients, soil micro-organisms, structural support	Top 15 cm of black forest soil
Coarse organic matter	25%	Air spaces	Peanut husks, leaf litter, domestic compost, tree bark
Fine organic matter	25%	Moisture retention, nutrients	Coconut fibre, charcoal made from rice husks, dried cattle dung

A standard, general purpose medium consists of 50% forest top soil mixed with 25% fine organic matter and 25% coarse organic matter (**Table 6.3**).

Store the potting medium in a moist condition but protected from rain. To prevent the spread of diseases, never re-cycle the potting medium. When disposing of weak or diseased trees, dispose of the potting medium in which they grew well away from the nursery.

When making a potting medium, sieve the materials to remove stones or large clumps and mix them together on a hard, flat surface using a shovel. Large nurseries use electric cement mixers to mix their potting media.

Box 6.4. Wildlings as alternatives to seeds.

Growing a mixed crop of framework tree species from seeds can take 18 months or more because you have to wait for the parent trees to fruit and for the seeds to germinate. So, is there a faster way to produce framework tree saplings? Wildlings are seedlings that are dug up from the forest and cultivated in a nursery. Forest trees usually produce vast numbers of surplus seedlings, most of which die, so digging up a few of them for transfer into a nursery does not harm the forest ecosystem. If wildlings are transplanted from a cool, shady forest directly into an open deforested site they usually die of transplantation shock. So wildlings must first be potted, cared for in a nursery, and hardened off before they are planted out. Researchers at Chiang Mai University's Forest Restoration Research Unit (FORRU-CMU) have determined how to use wildlings to produce framework trees for planting (Kuarak, 2002).

In the forest, locate several suitable parent trees of the required species that fruited heavily the previous fruiting season. It is best to collect seedlings from around many parent trees to maintain genetic diversity. Collect seedlings that are no taller than 20 cm (larger ones have high mortality because of severe transplantation shock) within a 5 m radius of the parent tree (which would otherwise die as the result of competition from the parent tree). The primary consideration when collecting wildlings is to minimise root damage, so dig them up during the rainy season, when the soil is soft. Lever out very young, small seedlings carefully with a spoon or dig up larger seedlings with a trowel, retaining a plug of soil around the roots. Place the seedlings in a bucket with a little water, or use containers made from banana stems.

In the Philippines, containers made from sections of banana plant stems make cheap containers for the transfer of wildlings from forest to nursery.

If wildlings are more than 20 cm tall, consider pruning them just after digging them up to reduce mortality and increase growth rate. Cut back the stem by one-third to one-half, but remember that not all species tolerate pruning, so you might need to carry out some experiments. Make a 45° cut about 5 mm above an axillary bud. Alternatively cut back the larger leaves by about 50%. Secondary roots might need to be trimmed to enable seedlings to be potted easily into 23 × 6.5 cm plastic bags filled with standard potting mix without bending the tap root. Keep the potted wildlings under deep shade (20% of normal sunlight) for about 6 weeks or construct a recovery chamber. Thereafter, follow the same procedures used for care and hardening-off of saplings that have been grown from seed. When compared with growing planting stock from seed, these techniques can shorten the time needed to grow trees to a plantable size by several months to a year and can reduce production costs considerably.

Box 6.4. continued.

A recovery chamber of 1 × 4 m is large enough for 1,225 plants. This example is constructed in a shaded area of the nursery from a simple split bamboo frame. The frame is covered with a polythene sheet, the edges of which are buried in a shallow trench around the structure, thereby sealing the chamber. Humidity builds up in the chamber, preventing transplantation shock. After a few weeks, the chamber is partially opened so that the plants can acclimatise to ambient conditions and eventually the cover is removed completely.

By Cherdsak Kuaraksa

How much potting medium is needed?

To calculate the volume of media needed to fill your containers, measure their radius and height and apply the following formula:

Total volume of medium required = (container radius)^2 × container height × 3.14 × number of containers

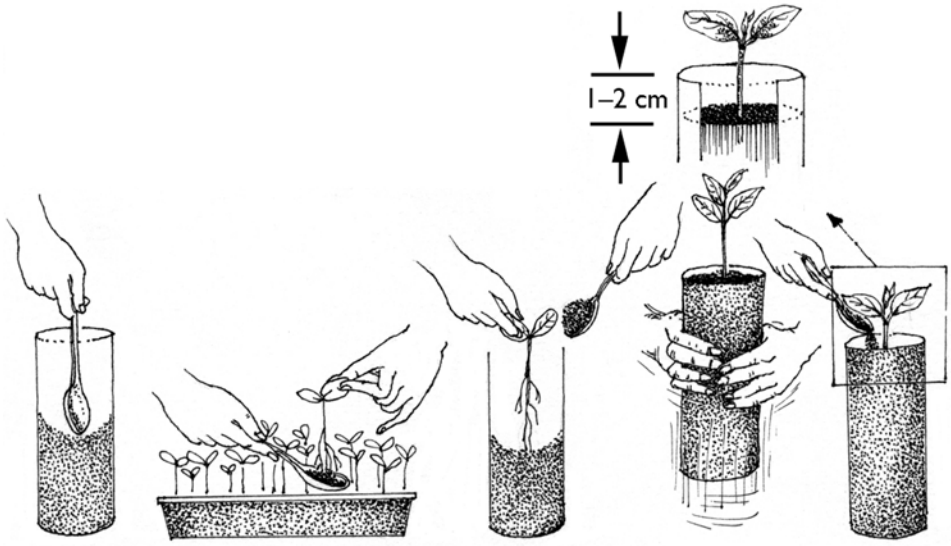
For example, for 2,000 plastic bags of 23 × 6.5 cm, you will need (6.5/2)^2 × 23 × 3.14 × 2,000 = 1,525,648 cm^3 or approximately 1.5 m^3 of medium.

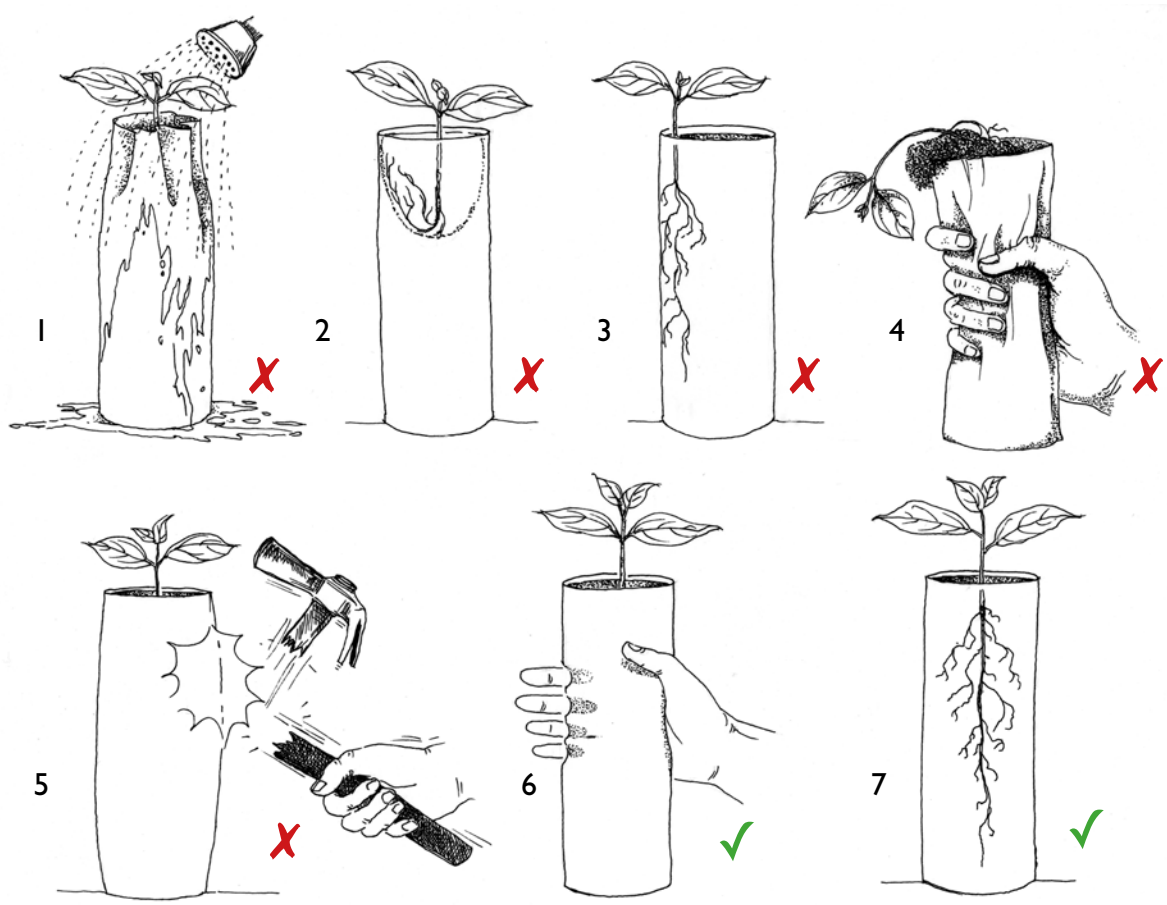
Filling the containers

First, make sure the medium is moist but not too wet: spray it with water if necessary. When pricking-out small seedlings, fill the containers to the brim with medium using a trowel or bamboo scoop. Bang each container on the ground a few times to allow the medium to settle, before topping-up the containers with more medium to 1–2 cm below the container's rim. The medium should not be so compact as to inhibit root growth and drainage, but neither should it be too loose. The consistency of medium within plastic bags can be checked by firmly grasping the bag. The impression of your hand should remain after you let go and the bag should stand up straight, unsupported.

'Pricking-out'

'Pricking-out' (potting) is transferring seedlings from germination trays into containers, a task that should be carried out in shade, late in the day. Seedlings are ready for pricking out when the first 1–3 pairs of true leaves have fully expanded. Fill the containers as previously described. Then use a spoon to make a hole in the medium that is large enough to take the seedling's roots without bending them. Handle the fragile seedlings with care. Gently grasp a leaf (not the stem) of a seedling and slowly prise it out of its germination tray with a spoon. Place the seedling's root into the hole in the potting medium and fill the hole with more medium. Bang the container on the





Potential problems with potting: (1) the medium has settled causing the rim of the plastic bag to collapse and blocking watering; (2) curled roots will make the adult tree susceptible to wind throw; (3) the seedling is not placed centrally; (4) the medium is too soft; (5) the medium is compacted; (6) excellent medium consistency; and (7) the perfectly potted seedling!

ground to settle the medium. Top up until the medium surface is 1–2 cm below the container's rim and the seedling's root collar (the junction between the root and shoot) is at the medium's surface. Then, press the medium to make sure the plant is upright and centrally placed. For larger plants, suspend the roots in a partly filled container and then carefully add medium around the roots.

'Standing down'

'Standing-down' refers to the time that the containerised trees are kept in the nursery from potting until transportation to the planting site. After potting the seedlings, place the containers in a shaded area and water the seedlings. Make sure that plastic bags remain upright and are not squeezed together. At first, the containers can be touching each other (i.e. 'pot thick'), but as the seedlings grow, space the containers a few centimetres apart, to prevent neighbouring seedlings from shading each other.

The containers can be stood down on bare ground, on ground covered by various materials or on raised wire grids. If the containers are stood down on bare earth, tree roots can grow through the holes in the base of the containers into the underlying soil.

Box 6.5. Cuttings as an alternative to seeds.

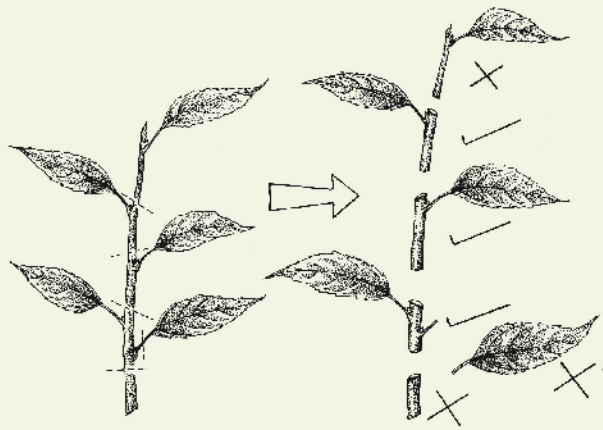
Vegetative propagation is not normally recommended for producing planting stock for forest restoration projects because it tends to reduce adaptive genetic diversity (see **Box 6.1**, p. 159). It may be appropriate, however, for highly desirable, rare, framework tree species whose seeds are difficult to find or germinate. For such species, propagation by cuttings is acceptable provided that the cuttings are collected from as many parent trees as possible.

Trees that are grown from cuttings often mature early — a desirable characteristic for a framework tree species. Low-tech methods can be used to root cuttings. Longman and Wilson (1993) report that most tropical tree species tested to date can be rooted as leafy stem cuttings in low-technology 'poly-propagators' and/or under mist. These authors also provide a comprehensive review of techniques, but bear in mind that little work has been done on the vegetative propagation of the vast majority of tropical tree species that are to be useful for the restoration of tropical forest ecosystems.

A study of vegetative propagation at Chiang Mai University's Forest Restoration Research Unit (FORRU-CMU) provided the following recommendations for rooting cuttings of framework species, using a simple method based on plastic bags (Vongkamjan, 2003).

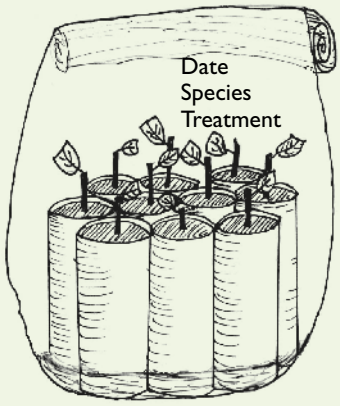
Cut medium-sized, vigorous juvenile shoots (leafy shoots can often be found on stumps after chopping or burning), from as many parent trees as possible, with a sharp, clean pair of secateurs or a knife. Place the cuttings in plastic bags with a little water and take them to a nursery immediately. In the nursery, trim the cuttings into 10–20 cm lengths. Remove the lower, woody parts and the fragile apical section. If each node has a leaf or bud, single nodes can be used, but for shoots that lack buds and have short internodes, cuttings can include 2–3 nodes.

Cut back the leaves transversely by 30–50%. Cut the bases of the cuttings with a sharp propagation knife into a heel shape just below a node.



Hormone treatments are usually required to stimulate the cuttings to root. Each species responds differently to the various hormone preparations that are available, so some experimentation will be necessary. Products that contain auxins, either indole-3-butyric acid (IBA) or naphthalene-1-acetic acid (NAA), in various concentrations are most likely to be effective. These products are usually powders, which should be dusted lightly onto the bases of the cuttings. Some rooting powders also contain a fungicide such as 'Thiram' or 'Captan' which helps to discourage disease. Follow the instructions on the packet.
For more advice on rooting cuttings of tropical trees see: www.fao.org/docrep/006/AD231E/AD231E00.htm#TOC

Box 6.5. continued.



Bags within bags can be used to maintain 100% humidity while the cuttings grow roots.

Mix 50% sand with 50% rice husk charcoal to make a rooting medium and place it in small, black, plastic bags. Push the bases of the cuttings into the medium. Water the medium and press it to make it firm around each cutting. Put groups of 10 small bags into larger plastic bags (20 × 30 cm). Add one litre of water and seal the larger bag, resulting in an atmosphere of 100% humidity that will keep the cuttings alive until the roots grow and are able to supply sufficient water to the cuttings' shoots. Label each bag with the species name and starting date. Keep records of how many cuttings develop roots and shoots. Top up the bags with water weekly and remove dead cuttings and dried leaves. When the cuttings show vigorous root and shoot development, transplant them into 23 × 6.5 cm plastic bags and care for them as described in **Section 6.5**.

By Suphawan Vongkamjan

When the trees are lifted for planting, these roots will break, suddenly reducing the supply of water from the root to the shoot. This can cause the plant to go into shock before it even reaches the planting site. Therefore, the containers must be lifted every few weeks, and any protruding roots pruned back before they can penetrate the soil. Covering the standing down beds with very coarse gravel can help to prevent this problem. Roots growing into the gravel find no nutrients and little moisture, and are gradually killed by exposure to air. Covering the standing down area in plastic sheets also prevents roots from penetrating the soil, but non-porous plastic can obviously create drainage problems.

(A) Standing down on bare earth works well, but the young trees require constant attention to prevent roots growing into the soil under the pots. In this nursery, bamboo guard rails are used to keep the plants upright. (B) Covering the ground with gravel and then a porous sheet ('weed-mat') prevents roots from growing into the underlying soil. In this nursery, an automatic sprinkler system waters the plants, which are grown in square, rigid, reusable, plastic pots.

The ultimate (and most expensive) solution is to stand down containers on raised wire grids. Roots that grow out from containers are exposed to air and either stop growing or die. This is called air pruning (see **Section 6.5**). It encourages root branching within containers and the formation of a dense root ball, which increases the survival chances of trees after planting out.

Five-star accommodation for trees. The trees sit in wire grid trays on a frame that is raised off the ground, allowing the air pruning of roots. Removable shade netting permits control of lighting conditions.

6.5 Caring for trees in the nursery

Shade requirements

After pricking-out, place the seedlings under about 50% shade to prevent scorching of the leaves and wilting. Shade netting, graded according to the percent shade cast, can be bought at most agricultural supplies stores. Hang it on a frame 0.5–2.5 m above the ground. If shade netting is unavailable or too costly, local materials such as coconut palm leaves, thin strips of bamboo or even dried grass are also effective, but take care not to provide too much shade with these materials. More than about 50% shade will produce tall, weak trees that are susceptible to diseases. Even when well-established in containers, trees remain vulnerable to high temperatures and full sunlight. Consequently, they are usually grown under light shade until they are ready for hardening-off.

Watering

Each container holds a relatively small amount of water, so seedlings can dry out rapidly if watering is interrupted for more than a day, especially in a dry season. By contrast, over-watering can saturate the potting medium, which suffocates the roots, and this can be just as damaging to plant growth as dehydration.

Water the trees early in the morning and/or late in the afternoon to avoid the heat of the day. If there is any doubt about the reliability of the water supply, install a system of water tanks as a reserve supply. Nursery workers who are responsible for watering should make a record on a calendar each time watering is carried out.

Hand watering allows more control than is provided by automated sprinklers.

Large commercial nurseries often use a system of sprayers that are inter-connected with pipes, allowing effortless watering whenever the tap is turned on, but such systems are expensive. In nurseries producing many different tree species with different water requirements, watering by hand using a watering can or a hose with a fine rose attached is recommended. This allows nursery workers to assess the dryness of each batch of trees and to adjust the amount of water delivered accordingly.

Some training is usually required to enable the person responsible for watering to judge how much water to provide. During the rainy season, it may be possible to go several days without watering the saplings in an open nursery. By contrast, in a dry season, it may be necessary to water the saplings twice in a day. The saplings are ready for water when the soil surface is starting to dry out. The presence of mosses, algae or liverworts on the surface of the potting medium indicates that the seedlings are being given too much water; they should be removed and watering reduced. Weeds can compete aggressively for water, so should be removed from containers.

Extra care is required when watering germination trays: a very fine rose must be used and the watering should be carried out in a sweeping motion to avoid damage to the seedlings.

Fertiliser

Trees require large amounts of nitrogen (N), phosphorus (P) and potassium (K), moderate amounts of magnesium, calcium and sulphur and trace amounts of iron, copper and boron and other mineral nutrients to sustain optimal growth. The potting medium might supply adequate quantities of these nutrients, especially if rich forest soil is being used, but the application of additional fertiliser can accelerate growth. Your local agricultural extension service or agriculture college might be able to analyse the nutrient content of the medium you use and advise you on fertiliser requirements.

The decision to apply fertiliser depends not only on the availability of nutrients in the potting medium but also on the growth rate required, or the appearance of the seedlings. Plants that have symptoms of nutrient deficiency, such as yellowing leaves, should receive fertiliser. Fertiliser should also be applied when it is necessary to accelerate growth to ensure that the plants are ready for transplantation by the planting season.

The application of 10 granules of slow-release fertiliser every 3–6 months can mean the difference between seedlings growing tall enough by planting day and having to keep them in the nursery for another year.

The use of slow-release fertiliser granules at a rate of about 1.5 g per litre of potting medium is recommended. At FORRU-CMU, good results have been achieved for many species by adding about 10 granules of 'Osmocote' NPK 14:14:14 (about 0.3 g) to the surface of the medium in each container every 3 months. 'Nutricote' is also widely available, and recommended. Although slow-release fertilisers are expensive, only very small quantities are applied every 3–6 months, and hence the labour costs of applying them are very low.

Alternatively, ordinary fertiliser can be used, either as solid fertiliser mixed in the potting medium (a rough guide is 1–5 g per litre of medium) or dissolved in water. Dissolve roughly 3–5 g of fertiliser per litre of water and apply with a watering can. Then, water the saplings again with fresh water to wash any fertiliser solution from the leaves. This treatment must be repeated every 10–14 days, so it requires much more time and labour than using slow-release granules.

Do not apply fertiliser i) to rapidly growing species that will reach a plantable size before the optimal planting time (as they will outgrow their containers), ii) to species in the family Leguminosae, or iii) immediately before hardening-off (as new shoot growth should not be encouraged at that time). The overuse of fertiliser can result in the 'chemical burning' of plants and can kill beneficial soil micro-organisms such as mycorrhizal fungi.

Mycorrhizal fungi

Mycorrhizal fungi (literally 'fungus root') form mutually beneficial 'symbiotic' relationships with plants. They form extensive networks of fine fungal hyphae that radiate out from tree roots into the surrounding soil. The fungi transfer nutrients to trees from a much greater volume of soil than can be exploited by the trees' root systems alone. In return, the fungi gain carbohydrates (as an energy source) from the trees. There are two main types of mycorrhiza that associate with trees: ectomycorrhiza and vesicular arbuscular mycorrhiza (VAM). Ectomycorrhiza form a sheath of fungal threads around the outside of tree roots that extends between the plant's cells but does not penetrate them. All dipterocarps, some legumes, many conifers and a few broadleaved trees (e.g. oaks) have ectomycorrhiza. VAM live within roots and actually penetrate the root cells. They can be found on the vast majority of tropical trees but we know relatively little about the diversity of mycorrhiza in tropical forests or about their role in maintaining the complexity of tropical forest ecosystems. It is therefore impossible to prescribe detailed actions for the use of mycorrhiza in forest tree nurseries.

We do know, however, that mycorrhizal inoculation can increase the survival and growth of nursery grown trees after they are planted out, especially on highly degraded land that has been without native vegetation or top soil for several years (e.g. mined land). When forest soil is included in the potting medium, most native forest tree species become naturally infected with mycorrhizal fungi and application of commercially produced mycorrhizal inoculae has no significant advantage (Philachanh, 2003).

Weed control

Weeds that are present in the nursery can harbour pests, and their seeds may spread into containers. Grasses, herbs and vines should all be removed from the nursery grounds before they can flower. Weeds that colonise containers compete with tree seedlings for water, nutrients and light. If not dealt with when small, weeds can be difficult to remove from containers without damaging the roots of tree seedlings. Check containers frequently and use a blunt spatula to remove weeds while they are still small. Weed in the morning, so that any remnant weed fragments dry out in the heat of the day. Wear gloves when dealing with thorny or noxious weeds. Also remove any mosses or algae that are growing on the medium surface. Obviously, herbicides cannot be used to control weeds in tree nurseries.

Take care to avoid poisonous snakes or insects in the dense foliage of a batch of container-grown tree seedlings.

More weeds than trees? Weed the nursery regularly to prevent the build-up of weeds in the containers.

Diseases

Disease prevention

Diseases can occur even in the best-maintained nurseries and there are three main causes:

- **fungi** — although some species are beneficial, others cause damping-off, root-rots and leaf-spots (blights and rusts);
- **bacteria** — most are harmless, but some cause damping-off, canker and wilts; and
- **viruses** — most do not cause problems, but some cause leaf-spots.

Prevention is better than cure, so keep containers, tools and work surfaces clean by washing them in a solution of domestic bleach. Follow the manufacturer's instructions for dilution, taking care to avoid getting bleach onto your skin or into your eyes. Thoroughly wash rigid plastic containers when re-using them. Do not recycle plastic bags or medium.

Rigid plastic pots can be re-used provided they are properly cleaned, but plastic bags must be disposed of well away from the nursery to prevent the build-up of pathogens.

Detecting and controlling disease

Constant vigilance is needed to prevent disease outbreaks. Ensure that all of the nursery staff learn how to recognise the symptoms of common plant diseases and that all the young trees are inspected at least once a week. To prevent disease spread, make sure that the plants are not being over-watered, that there is adequate drainage within and beneath the containers, and that the plants are well-spaced to allow air movement around them and to prevent direct transfer of pathogens from individual seedlings to their neighbours. Use disinfectant to wash tools or rubber gloves that come into contact with diseased plants.

If a disease outbreak occurs, remove infected leaves or dispose of diseased plants immediately. Burn them well away from the nursery. Do not recycle the medium or plastic bags in which they grew. If using rigid containers, wash them with disinfectant and dry them in the sun for several days before re-using them. Inspect the plants daily until the outbreak is over.

Routine spraying with chemicals should not be necessary. Chemicals are expensive and they are a health hazard if not handled properly. If it is necessary to spray an infected batch of plants, first try to identify the type of disease (fungal, bacterial or viral) and select an appropriate chemical. For example, Iprodione is active against fungal leaf-spots, whereas Captan is particularly effective against damping-off fungi.

When using any fungicides, read the health warnings on the packet and follow all the protective precautions recommended.

Where diseases become prevalent, consider pasteurising the potting medium by heating it in the sun. This will kill most pathogens, pests and weed seeds, but it could also kill beneficial soil micro-organisms so consider re-inoculating the medium with mycorrhiza.

Pest control

Most insects are harmless or indeed beneficial, but some can rapidly defoliate young trees or damage their roots causing death. Not all pests are insects: nematode worms, slugs and snails, and even domestic animals can all cause problems.

The most important pests include leaf-eaters, such as caterpillars, weevils and crickets; shoot borers, particularly beetle and moth larvae; juice-suckers, such as aphids, mealy bugs and scale insects; root-eaters, such as nematode worms; cutworms, the larvae of certain moths; and termites, which also destroy nursery structures. In addition to eating the plants, pests can transmit diseases.

Inspect the trees for pests regularly and carefully to ensure that an infestation cannot develop. Remove harmful animals or their eggs by hand, or spray the saplings with a mild disinfectant. If this fails to prevent infestation, then spray the saplings with an insecticide. Prevention is better than cure as most insecticides are poisonous to humans. It is therefore essential to read the labels on insecticide packaging and follow the instructions carefully, observing all the health precautions recommended by the manufacturer. Select the most appropriate chemical for the particular pest species present. For example, 'Pirimicarb' is active against aphids, 'Aldrin' can be used to control termites, and 'Pyrethrin' is a more general insecticide.

Not all pests are small — this nursery in western Thailand is protected from elephants by an electric fence.

Not all pests are small. Dogs, pigs, chickens, cattle and other animals can wreak havoc in a tree nursery in just a few minutes. So, where such animals occur, make sure that the plants are protected within a sturdy fence.

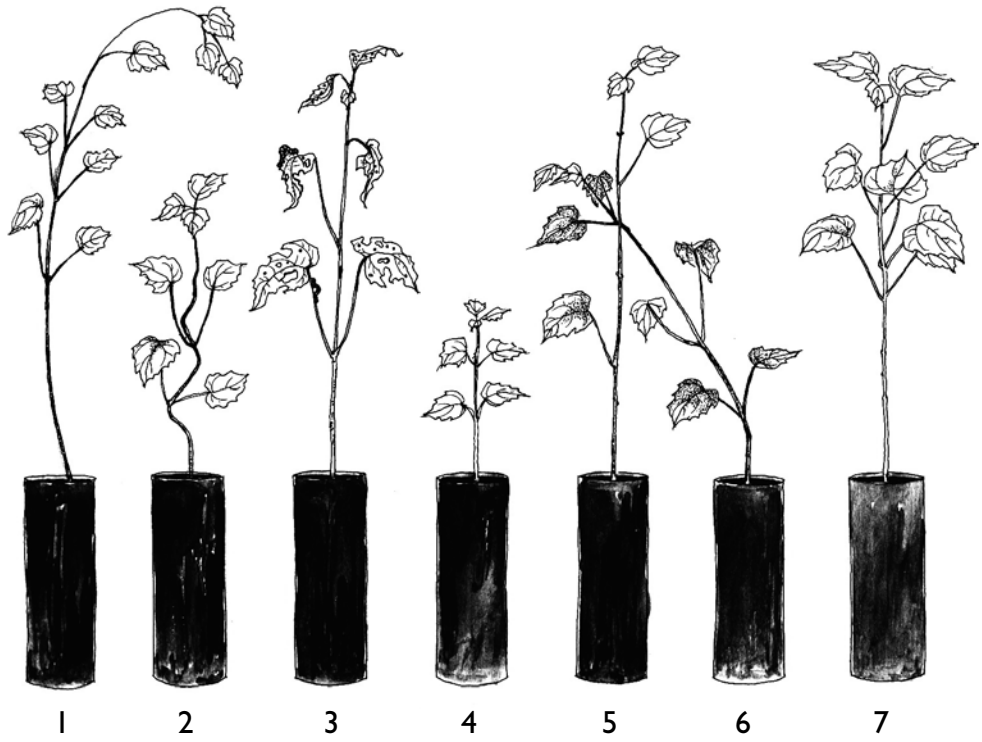
Quality control by grading

Grading is an effective method of quality control. It involves arranging the growing trees in order of size, while at the same time removing stunted, diseased or weak ones. In this way, only the most vigorous and healthy trees are selected for hardening-off and planting-out and thus post-planting survival is maximised. When the nursery is full, the smallest and weakest plants can be identified easily and removed to make room for new more vigorous plants.

Grading is the best form of quality control.

Carry out grading at least once each month. Root pruning and disease inspection can be carried out at the same time. Wash hands, gloves and secateurs in disinfectant frequently to prevent the spread of diseases from one block of plants to another. Dispose of poor-quality plants by burning them, well away from the nursery, and do not recycle the medium or plastic bags in which they were grown. Nursery workers are sometimes reluctant to dispose of poor quality plants, but keeping them is a false economy, as they waste space, labour, water and other nursery resources that would be more efficiently provided to healthy plants. Poor-quality plants are susceptible to disease, and therefore pose a health risk to the entire nursery stock.

The nursery manager must produce high-quality trees that will perform well when planted out in the harsh conditions typical of deforested sites. Both the shoot and root systems of the young trees should be healthy and in balance with each other. This reduces transplantation stress, tree mortality and the risk of having to replant the following year. It is a false economy and a waste of time to plant poor-quality trees.



1. Unbalanced root and shoot growth: the shoot is too long and thin and may well break during handling. Prune back well before planting time.
2 A malformed stem compromises future growth, plants that have such stems need to be disposed of.
3 Plants that have been attacked by insects should be burnt and surviving plants sprayed with insecticide to prevent the infestation from spreading.
4 Dispose of plants whose growth is stunted growth when compared with other plants of same age.
5 This plant is losing its leaves, possibly as a result of disease; it should be burnt.
6 This container was knocked over and spent some time lying on its side, resulting in a non-vertical stem – dispose of such plants.
7 The perfect plant is well balanced, disease-free and straight; with adequate care and rigorous grading, all of the plants in your nursery should look like this.

A healthy root system

Root systems are far more crucial to the survival of trees than shoot systems. A plant can survive and re-sprout after losing its shoot, but not after losing its roots. The root system must constantly supply water and nutrients to the shoots. Root growth is affected by the container, the potting medium, the watering regime and by pests and diseases. By planting time, the root systems of containerised trees must:

- form a compact root ball that does not fall apart when the tree is removed from its container;
- be densely branched with a balance between thick, supporting roots and fine ones that absorb water and nutrients;
- not be spiralling at the base of the container;
- be able to support the shoot system;
- be infected with mycorrhizal fungi and (if the tree is a legume) with nitrogen-fixing bacteria; and
- be free of pests and diseases.

If the containers are stood down on bare earth, lift them frequently and prune back protruding roots using a clean pair of secateurs (do this in the late afternoon to minimise moisture loss). Alternatively, inhibit root growth beyond the containers by standing down the trees on gravel or on raised wire-grid benches, which allows for air pruning of the roots (see **Section 6.4**).

Root pruning encourages both root-branching within the pot and the formation of a compact root ball, thus increasing the probability of survival after planting out.

Size of saplings at planting time

The actual height of the saplings at planting is less important than their capacity to produce vigorous new growth. Some fast-growing pioneer tree species can be planted out when only about 30 cm tall; for *Ficus* species, the recommended size is 20 cm tall (Kuaraksa & Elliott, 2012). For slower-growing climax forest tree species, it is better to plant trees of around 40–60 cm tall. Small saplings have much higher post-planting mortality rates than larger ones because of competition with weeds, but very large saplings are much more susceptible to transplantation shock and more difficult to transport.

Shoot pruning

Shoot pruning is necessary for plants of fast-growing species that must be kept in the nursery for a long time. Such trees can become too large for their roots to support or too cumbersome to handle during transportation and planting. The stems of tall saplings are easily broken when they are being moved. In some species, pruning encourages branching. This is a desirable characteristic because spreading crowns shade out weeds and rapidly close the canopy. Never prune shoots in the month before planting out

because pruning will promote the growth of new leaves just as the saplings are about to be stressed by transplantation. Immediately after planting, the root system might not be able to take up enough water to supply new leaves, so anything that stimulates bud break shortly before planting out should be avoided. Some species do not respond well to pruning or become highly susceptible to fungal infections after pruning. So before attempting to prune large numbers of saplings, experiment with a few to test the effects of pruning.

Hardening-off

Weaning, or 'hardening-off', prepares saplings for the difficult transition from the ideal environment in the nursery to the harsh conditions of deforested sites. If they are not hardened to the hot, dry, sunny conditions of planting sites, the planted trees suffer transplantation shock and mortality rates are high.

About 2 months before planting, move all of the saplings to be planted to a separate area in the nursery and gradually reduce the shade and the frequency of watering. Light-demanding trees should stand in full sunlight for their final month in the nursery. Shading should be reduced but not removed for shade-bearing species that will not be planted in full sun.

Gradually reduce watering by approximately 50% to slow shoot growth and to ensure that any newly forming leaves will be relatively small. During hardening, water saplings just once in the late afternoon instead of twice (early morning and late afternoon). Water saplings that are normally watered once a day once every other day. Do not reduce watering to the point at which the leaves wilt, as that would stress and weaken the saplings. Regardless of the normal schedule, water the saplings as soon as any wilting is observed.

Record keeping

Learning from experience is only possible if both nursery activities and the performance of each species are recorded accurately. Records are essential to prevent new nursery workers from repeating the mistakes of previous ones. They are also used to assess the productivity and achievements of the nursery (e.g. numbers of species or saplings grown) and for the development of species production schedules.

Record keeping and labelling seed trays facilitates efficient nursery management.

Label the seed trays and plants in the nursery with species names, batch numbers and dates of seed collection and pricking-out. Use the record sheet in the **Appendix** (**A1.6**) to record when and where each batch of seeds was collected and which seed treatments were applied, together with germination rates, growth rates, diseases observed and so on. Finally, record when and to where the saplings were dispatched for planting.

6.6 Research for improving native tree propagation

The standard protocols outlined above are sufficient to get you started on growing a wide range of native forest tree species. But as you gain experience, you will want to refine these techniques and to develop individual production schedules for each species being propagated, thereby improving the efficiency and cost-effectiveness of your nursery. Here, we provide a few basic research procedures to help you produce high-quality, vigorous, and disease-free saplings of the required size, by the optimum planting time, as rapidly and cost effectively as possible. This is achieved by conducting basic controlled experiments to test treatments that either accelerate or slow down seed germination and/or seedling growth.

Selection of species for research

Guidelines for selecting candidate framework species and nurse plantation species were provided in **Sections 5.3** and **5.5**, respectively. It is very likely that propagation protocols will have already been well-researched for any commercially valuable species. So, start by doing a literature search to find out what is already known about the species you want to grow and where the gaps in knowledge lie.

Recognising and identifying trees

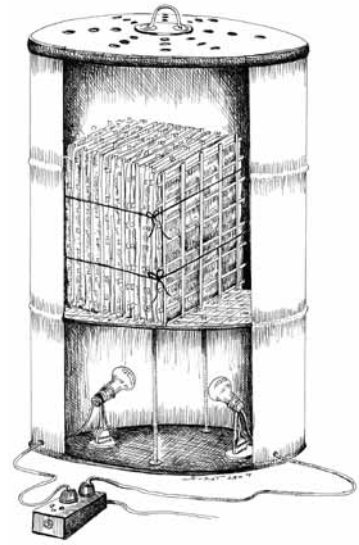
At the start of a restoration research program, not all of the scientific names of the tree species to be grown will be known, so it is useful to assign a species number to every tree species from which seeds are collected: the first species to provide seeds becomes S001, the second S002 and so on. Subsequent seed batches that are collected from the same species are labelled with the same 'S' number, but are assigned their own batch number. So 'S001b1' would be the first batch of seeds collected from species no. 1, and 'S001b2' would be the second batch of seeds collected from species no. 1, either from the same tree on a different date or from a different tree of the same species. Nursery staff often remember species numbers more easily than scientific names and, with a little experience, the numbers will be used more consistently than local names. List all species and their 'S' numbers on a board in the nursery and keep it up-to-date. Then label every seed germination tray and block of containerised seedlings with their 'S' and 'b' numbers.

Display a list of 'S' numbers, alongside local and scientific names, so that all nursery staff know which species they are working with.

All species numbers must be matched with scientific names. Local, vernacular names can also be noted, but they cannot be relied upon because local people often group similar species under a single name or use different names to refer to the same tree species. Collect voucher specimens of all trees from which seeds are collected. If there are any subsequent doubts about the tree species in the nursery or those planted in field trials, the voucher specimen of the seed tree can be re-examined to confirm or change the species name. Botanical taxonomists frequently revise plant classifications and change species names, so having a voucher specimen with a species number attached can reduce confusion.

Always collect a voucher specimen that can be used to confirm species identification.

Construct a simple drying box in which light bulbs are used to dry specimens gently.



Use a cutter mounted on a pole to obtain a sample of foliage, fruits and/or flowers. Trim the specimen without losing essential features (e.g. leaf arrangement, infructescence branching etc.) until it fits well in a standard-sized plant press. In the nursery, construct a simple drying box that uses light bulbs to provide gentle heat to dry the specimens. Write a label for every specimen that includes 'S' and 'b' numbers, the local name(s), details of the tree's location, and descriptions of the bark and any features that may change with drying, particularly colours.

Mount the specimens on robust paper using standard herbarium techniques. If there is space and appropriate staff and facilities, start your own herbarium. Store mounted specimens in suitable cabinets and enter the information from the specimen labels into a database. Take precautions to prevent insects or fungi from attacking the specimens. For additional security, make several herbarium sheets for each specimen and lodge duplicates in recognised herbaria. Have the specimens examined and identified by a professional botanical taxonomist. For more detailed information on herbarium techniques, see '*The Herbarium Handbook*' published by the Royal Botanic Gardens, Kew, UK (www.kewbooks.com).

Phenology

Phenology is the study of the responses of living organisms to seasonal cycles in environmental conditions. In forestry, phenological studies are used to determine when to collect seeds and to learn how forests function (particularly in regard to tree reproduction and forest dynamics), so that the same functionality can be replicated in restored forest.

The flowering and fruiting of many tropical trees are usually related to seasonal variations in moisture (Borchert *et al*., 2004) and solar radiation energy (insolation) (Calle *et al*., 2010). Cycles in reproductive events are most marked in the seasonal tropics, but cycles of flowering and fruiting can be observed even in the less seasonal, equatorial forests. Not all tropical trees reproduce seasonally. Some flower and fruit twice or several times each year, whereas others exhibit 'masting', i.e. mass fruiting at intervals of several years.

Obtaining ripe seeds is the first big challenge in tree-planting projects, so it is worth the effort of carrying out phenology studies to determine optimal seed collection schedules so that the nursery is well stocked with all of the required species. Phenological studies can also be used to predict the length of seed dormancy, and which pre-sowing seed treatments are likely to be successful in breaking or prolonging dormancy. Furthermore, they enable the identification of 'keystone' tree species: those that flower and fruit at times when other food resources for animals are in short supply (Gilbert, 1980). Keystone tree species, such as fig trees (*Ficus* spp.), support whole communities of animal pollinators and seed dispersers upon which other tree species rely for their reproduction. They are obvious candidates for testing as framework tree species. Observations of pollination and seed dispersal mechanisms can also be made during phenological studies. Additional data on the leafing phenology of the trees are usually collected at the same time. These data can help to predict optimal planting sites for individual tree species; for example, deciduous species are more suited to drier habitats and evergreen species to wetter habitats.

Establishing phenology study

Phenology trails are set up as part of the target forest survey according to the procedure described in **Section 4.2**. Label at least five individuals of each tree species that characterise the target forest type. Collect voucher specimens (as described previously) from each labelled tree and get a botanist to identify them. Write a brief note, describing where each tree is located in relation to the trail (e.g. "10 m to the left"; "right 20 m by rocky overhang"). As you repeat the observations month by month, you will soon be able to remember where each individual tree is located.

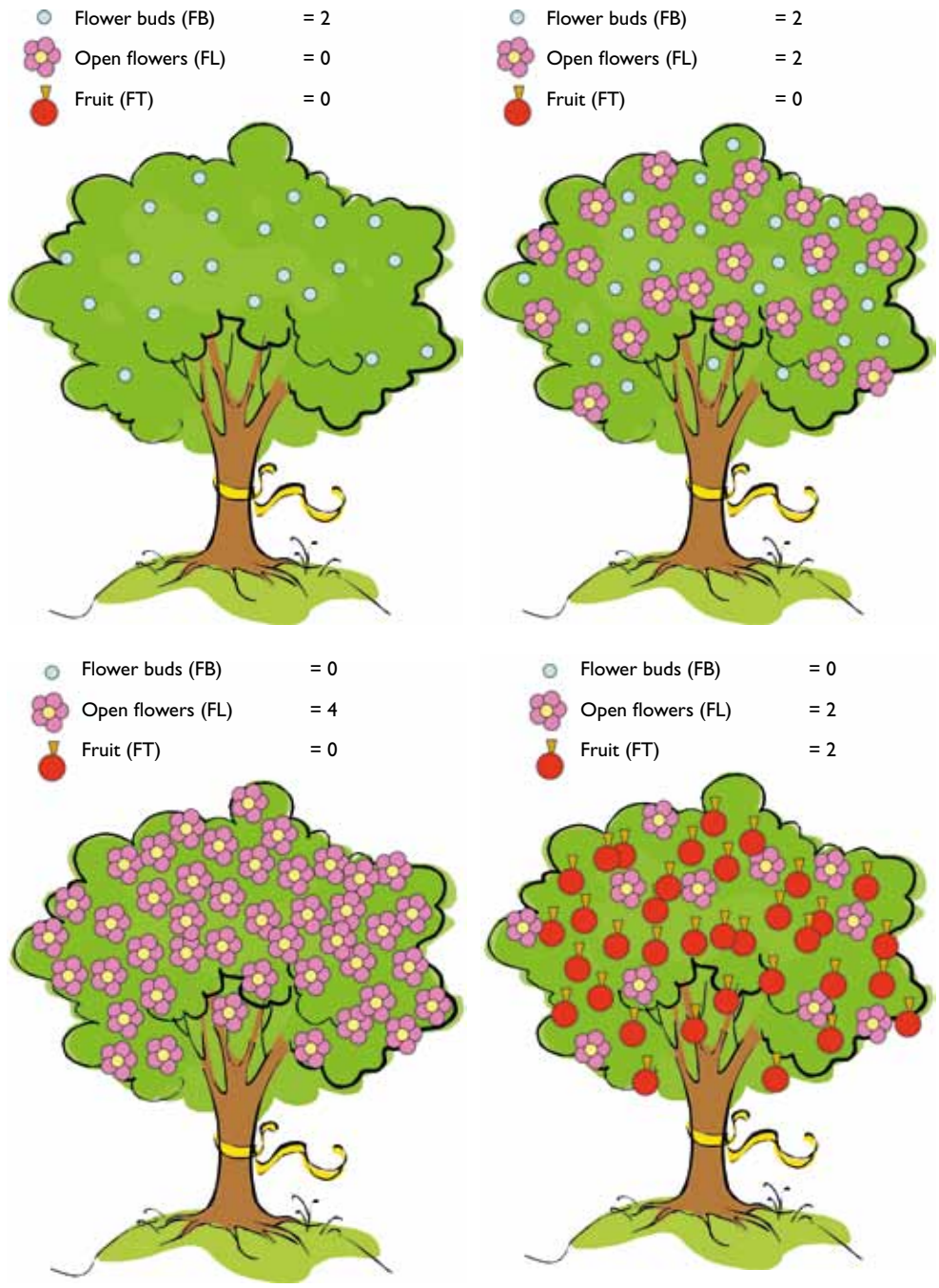
How often should data be collected?

The trees should be inspected at least once each month. Even with monthly observations, some tree-flowering events might be missed as some trees produce and drop their flowers within a month. Usually, such rapid-turnover flowering events can be inferred when the trees are subsequently observed in fruit. In such cases, the dataset can be adjusted during processing to add the 'estimated' time of a flowering event. If many flowering events are being missed, increase the frequency of data collection to twice each month.

Scoring system for phenology

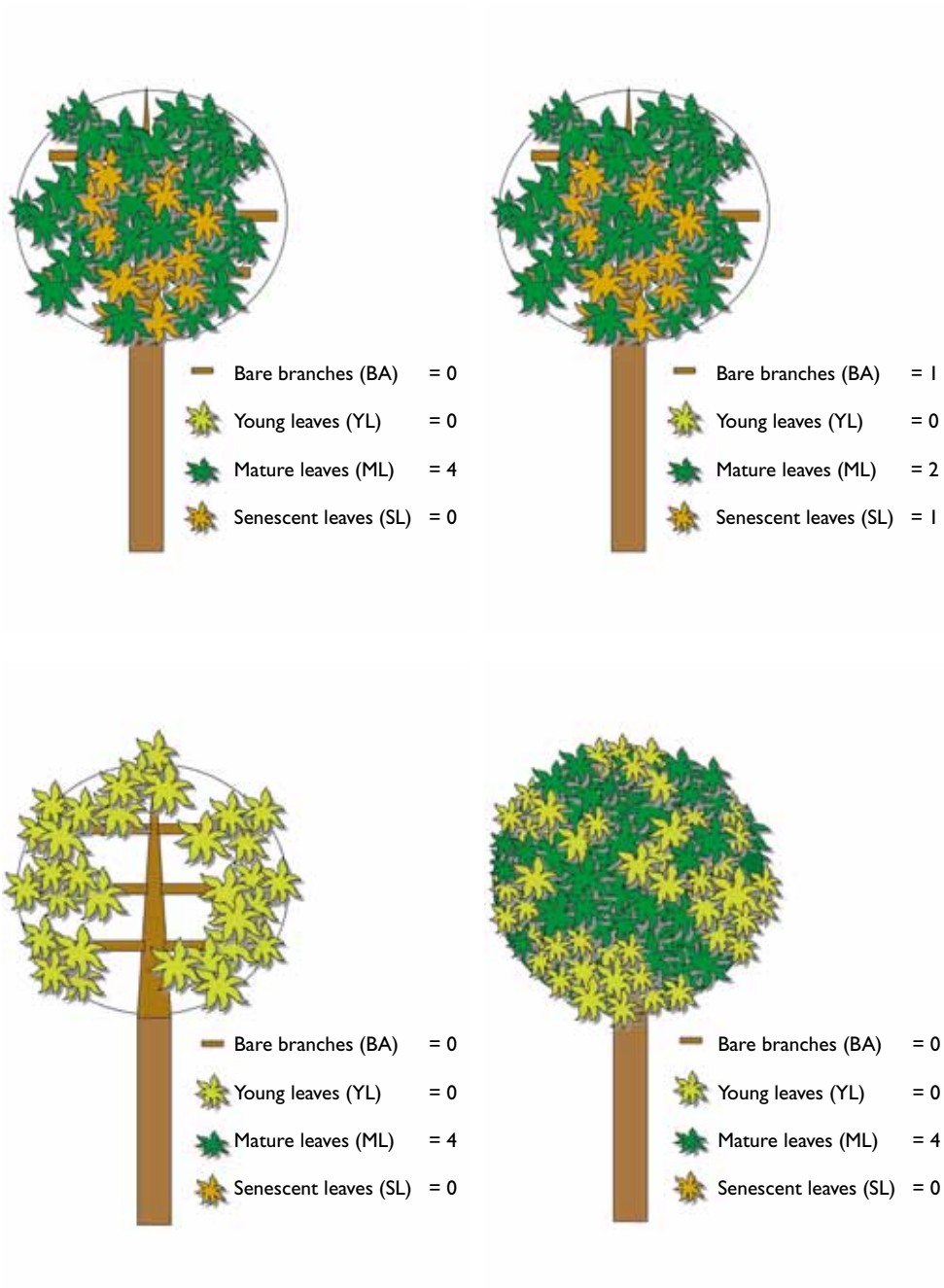
We recommend the 'crown density' method for recording tree phenology, which was originally devised by Koelmeyer (1959) and subsequently much modified by various authors. This semi-quantitative method uses a linear scale of 0–4, in which a score of 4 represents the maximum intensity of reproductive structures (flower buds (FB), open flowers (FL) and fruits (FR)) in the crown of a single tree. Scores of 3, 2 and 1 represent approximately ¾, ½ and ¼ of the maximum intensity, respectively. The 'maximum intensity' of a flowering or fruiting event varies among species, and judgments of it are bound to be subjective at first but they improve with experience.

The same approach can be used to score leafing. For individual tree crowns, estimate scores of between 0 to 4 for i) bare branches, ii) young leaves, iii) mature leaves and iv) senescent leaves. The sum of these four scores should always equal 4 (which represents the entire tree crown). Scores for flowers + fruits are always less than 4, except when flowering or fruiting is occurring at the maximum intensity typical for the species being observed.



Examples of phenology scores for flowers. (Designed by Khwankhao Sinhaseni.)

The crown density method is a compromise between very time-consuming absolute counts of flowers and fruit (or estimates of their biomass using litter-fall traps) and the very quick qualitative method of recording simple presence or absence. It is quick and it allows quantitative analytical techniques to be applied to the data. At the beginning of a study, however, it is important to train all data collectors to be consistent in their scoring, thereby minimising the subjectivity of the technique.



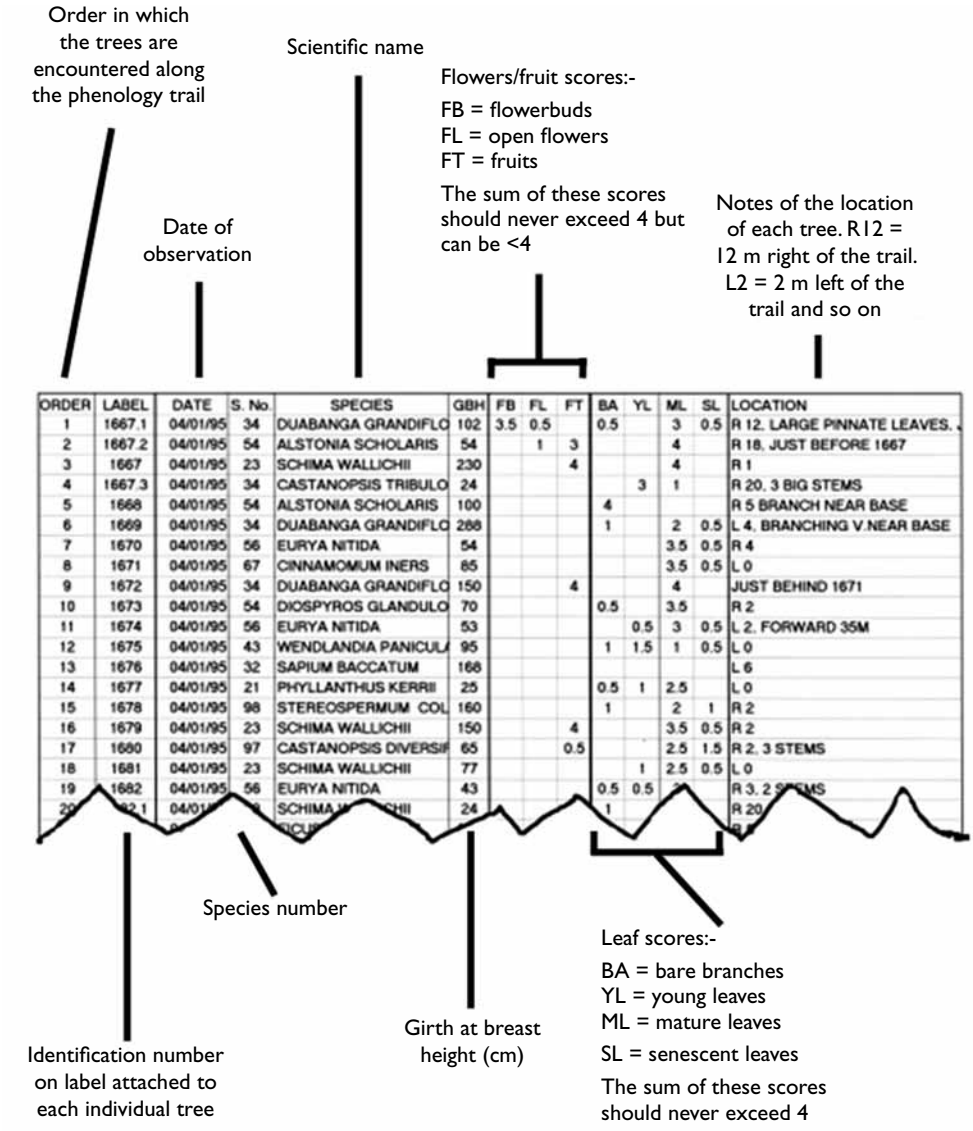
Examples of phenology scores for leaves. (Designed by Khwankhao Sinhaseni.)

Presenting and analysing phenology data

Microsoft Excel spreadsheets are ideal for storing and manipulating phenology data. Once the study trees have been selected and labelled, prepare a data sheet as shown below. List the trees in the order in which they are encountered along the phenology trail. In the field, carry the previous month's data sheets with you, as well as blank sheets for recording the current month's data.

Month by month, accumulate all of the data into a single spreadsheet. Always enter new data at the bottom of the spreadsheet (rather than to the right). After each data-collection session, paste a copy of the blank data record sheet at the bottom of the spreadsheet and then add the newly collected data.

To analyse the data, use the tools within Excel to sort the data first by 'SPECIES', then by 'LABEL', and finally by 'DATE'. This arranges the data in chronological order, for each individual tree of each species (see overleaf).

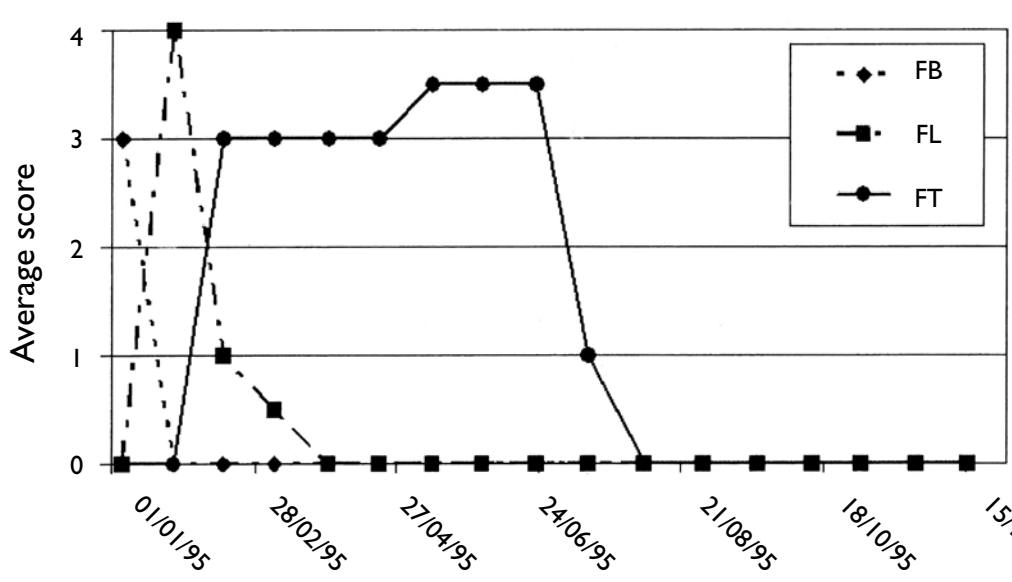


ORDER	LABEL	DATE	S. No.	SPECIES	GBH	FB	FL	FT	BA	YL	ML	SL	LOCATION
1	1667.1	04/01/95	34	DUABANGA GRANDIFLO	102	3.5	0.5		0.5		3	0.5	R 12. LARGE PINNATE LEAVES.
2	1667.2	04/01/95	54	ALSTONIA SCHOLARIS	54		1	3			4		R 18. JUST BEFORE 1667
3	1667	04/01/95	23	SCHIMA WALLICHII	230			4			4		R 1
4	1667.3	04/01/95	34	CASTANOPSIS TRIBULO	24					3	1		R 20. 3 BIG STEMS
5	1668	04/01/95	54	ALSTONIA SCHOLARIS	100				4				R 5 BRANCH NEAR BASE
6	1669	04/01/95	34	DUABANGA GRANDIFLO	288				1		2	0.5	L 4. BRANCHING V.NEAR BASE
7	1670	04/01/95	56	EURYA NITIDA	54						3.5	0.5	R 4
8	1671	04/01/95	67	CINNAMOMUM INERS	85						3.5	0.5	L 0
9	1672	04/01/95	34	DUABANGA GRANDIFLO	150			4			4		JUST BEHIND 1671
10	1673	04/01/95	54	DIOSPYROS GLANDULO	70				0.5		3.5		R 2
11	1674	04/01/95	56	EURYA NITIDA	53					0.5	3	0.5	L 2. FORWARD 35M
12	1675	04/01/95	43	WENDLANDIA PANICULA	95				1	1.5	1	0.5	L 0
13	1676	04/01/95	32	SAPIUM BACCATUM	168								L 6
14	1677	04/01/95	21	PHYLLANTHUS KERRII	25				0.5	1	2.5		L 0
15	1678	04/01/95	98	STEREOSPERMUM COL	160				1		2	1	R 2
16	1679	04/01/95	23	SCHIMA WALLICHII	150			4			3.5	0.5	R 2
17	1680	04/01/95	97	CASTANOPSIS DIVERSIF	65			0.5			2.5	1.5	R 2. 3 STEMS
18	1681	04/01/95	23	SCHIMA WALLICHII	77					1	2.5	0.5	L 0
19	1682	04/01/95	56	EURYA NITIDA	43				0.5	0.5			R 3. 2 STEMS
20	1682.1	04/01/95		SCHIMA WALLICHII	24				1				R 20

Then use the MS Excel graph wizard to construct a visual phenological profile like that shown opposite. Start by making a profile for each individual tree of each species. This will give you some idea of the variability in phenological behaviour within each species population and will enable you to assess the synchrony of phenological events. Only then, calculate mean score values across all of the individuals within each species

ORDER	LABEL	DATE	S. No.	SPECIES	GBH	FB	FL	FT	BA	YL	ML	SL	LOCATION -
272	296	05/01/95	34	ACROCARPUS FRAXINIF	222	3	0	0	1.5		1.5	1	L 4, OPP.297
272	296	26/01/95	34	ACROCARPUS FRAXINIF	222	0	4	0	3	1			L 4, OPP.297
272	296	15/02/95	34	ACROCARPUS FRAXINIF	222	0	1	3	1.5	2.5			L 4, OPP.297
272	296	08/03/95	34	ACROCARPUS FRAXINIF	222	0	0.5	3			4		L 4, OPP.297
272	296	30/03/95	34	ACROCARPUS FRAXINIF	222	0	0	3			4		L 4, OPP.297
272	296	20/04/95	34	ACROCARPUS FRAXINIF	222	0	0	3			4		L 4, OPP.297
272	296	12/05/95	34	ACROCARPUS FRAXINIF	222	0	0	3.5			4		L 4, OPP.297
272	296	01/06/95	34	ACROCARPUS FRAXINIF	222	0	0	3.5			4		L 4, OPP.297
272	296	23/06/95	34	ACROCARPUS FRAXINIF	222	0	0	3.5			4		L 4, OPP.297
272	296	14/07/95	34	ACROCARPUS FRAXINIF	222	0	0	1			4		L 4, OPP.297
272	296	06/08/95	34	ACROCARPUS FRAXINIF	222	0	0	0			4		L 4, OPP.297
272	296	30/08/95	34	ACROCARPUS FRAXINIF	222	0	0	0			4		L 4, OPP.297
272	296	21/09/95	34	ACROCARPUS FRAXINIF	222	0	0	0			4		L 4, OPP.297
272	296	13/10/95	34	ACROCARPUS FRAXINIF	222	0	0	0			4		L 4, OPP.297
272	296	02/11/95	34	ACROCARPUS FRAXINIF	222	0	0	0			4		L 4, OPP.297
272	296	25/11/95	34	ACROCARPUS FRAXINIF	222	0	0	0			4		L 4, OPP.297
272	296	16/12/95	34	ACROCARPUS FRAXINIF	222	0	0	0			4		L 4, OPP.297
329	464	05/01/95	34	ACROCARPUS FRAXINIF	575						4		EG 10/5
329	464	26/01/95	34	ACROCARPUS FRAXINIF	575	3	0	0	2.5		1.5		EG 10/5
329	464	15/02/95	34	ACROCARPUS FRAXINIF	575	3.5	0.5	0	3.5	0.5			EG 10/5
329	464	08/03/95	34	ACROCARPUS FRAXINIF	575	0	0	2	1.5	2	0.5		EG 10/5
329	464	30/03/95	34	ACROCARPUS FRAXINIF	575	0	0	0.5		3	1		EG 10/5
[illegible]	[illegible]	20/04/95	[illegible]	ACRO[illegible]PUS FRA[illegible]	575	0	[illegible]	0	[illegible]	[illegible]	[illegible]	[illegible]	EG 10/5
[illegible]	[illegible]	12/05/95	[illegible]	AC[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

population and construct an 'average' profile for each species. When analyzing flower or fruit data, the most important point to look for is the period during which the fruit scores decline for each species. This indicates the optimal seed collection month for that year, when natural seed dispersal is occurring. For example, the graph below shows that the optimum seed collection time for *Acrocarpus fraxinifolius* is from late June to early July, when maximum seed dispersal occurs. The fruit/seed maturation period is from February to June.



Phenology profile graph for *Acrocarpus fraxinifolius*.

FB = flower buds

FL = open flowers

FT = fruits

After phenology has been studied for several years, various useful indices of seed production can be calculated by extracting data from the spreadsheets (Elliott *et al*., 1994).

- **Duration** – the mean length of flowering-fruiting episodes (in weeks or months) for each individual tree and averaged across all trees sampled in a species.
- **Frequency** – the total number of flowering-fruiting episodes recorded for each individual divided by the number of years the study has run: then averaged across all individuals of the same species.
- **Intensity** – mean of the maximum flower or fruit scores (for each flowering-fruiting episode) recorded for each individual tree: then averaged for all flowering-fruiting individuals in the species sample.
- **Prevalence** – number of individual trees that flowered and fruited in each year, expressed as a percentage of the total number of individual trees in each species sample, averaged across the total duration of the study (in years).
- **Fruit set index** – for each flowering-fruiting episode, the maximum fruit score observed expressed as a percentage of the maximum flower score: averaged for all flowering-fruiting episodes for all individuals in the species sample.

Germination trials

Phenology studies provide ideal opportunities to collect seeds for germination trials, but remember that seeds can be collected from any trees bearing ripe fruits, even if they are not included in the phenology studies. Collect fruits when they are fully ripe but just before they are dispersed or consumed by animals. Label each seed tree with a unique number and fill in a seed collection data sheet. If a GPS is available, record the location of each seed tree.

Don't risk your life to collect a few seeds. If you need to climb trees, wear a safety harness.

Date collected: 20/03/2005 **Species No.:** 071 **Batch No.:** 1

SEED COLLECTION DATA SHEET

Family: *Rosaceae* **Botanic name:** *Cerasus cerasoides* (Buch.-Ham. ex. D. Don) S.Y. Sokolov

Common name: Nang Praya Sua Klong

Location: Doi Suthep-Pui National Park, roadside by Cinchona plantation

GPS location: 18 48 23.37 N; 98 54 44.76 E **Altitude:** 1,040 m

Forest type: primary evergreen forest, disturbed roadside area, granite bedrock

Collected from: ☒ ground ☒ tree

Tree label no.: 71.1 **Tree girth:** 88 cm **Tree height:** 6 m

Collector: S. Kopachon **Date seed sown:** 20/03/2005

Notes: Bulbuls were eating the fruit

☒ Voucher collected?

HERBARIUM, BIOLOGY DEPARTMENT, CHIANG MAI UNIVERSITY
FOREST RESTORATION RESEARCH UNIT, VOUCHER
NOTE: all dates are day/month/year

FAMILY: *Rosaceae*

BOTANICAL NAME: *Cerasus cerasoides* (Buch.-Ham. ex D. Don) S.Y. Sokolov

PROVINCE: Chiang Mai **DATE:** 20/03/2005

DISTRICT: Suthep **ELEVATION:** 1,040 m

LOCATION: Doi Suthep-Pui National Park, roadside by *Cinchona* plantation

HABITAT: Primary evergreen forest, disturbed roadside area, granite bedrock

NOTE: Height 6 m: DBH 28 cm
Bark lenticellate, peeling, dark brown
Fruit 14 mm × 6 mm, pericarps juicy, bright red
Seed stoney pyrene, about 7–10 diameter, light brown, contains 1 seed
Leaf blades green above, light green underneath

COLLECTED BY: S. Kopachon **NUMBER:** S071 **DUPLICATES:** 5

Germination trials can answer two basic questions: i) how many seeds germinate (percent germination) and ii) how quickly or slowly do the seeds germinate? Both of these parameters can be used and even manipulated when planning the growth of sufficient numbers of tree saplings for a specific planting time.

In seasonal tropical forests, the seeds of most tree species tend to germinate at the beginning of the rainy season (Garwood, 1983; FORRU, 2006). Seeds that are produced shortly before the rainy season usually have a short dormancy period; whereas those that are produced earlier have a longer dormancy. For the former, the saplings will be too small to plant in the first planting season, so it may be necessary to delay germination by storing seed as described in **Section 6.2** in order to prevent the saplings from outgrowing their containers before the second planting season. Conversely, it might be necessary to break the dormancy and accelerate the germination of seeds that are produced well before the ideal planting season so as to produce a crop of saplings that are ready to plant in less than 1 year. Failure to break the dormancy of such seeds could mean that plants must be kept in the nursery for 18 months or longer.

The objective of a germination trial is not to test the germination that would occur in nature, but to determine germination rates and periods under nursery conditions. Hence, seeds should be prepared using the standard protocol (see **Section 6.2**): fruit flesh should be removed, seeds should be air-dried and non-viable seeds identified by a flotation test removed.

Testing treatments for overcoming dormancy

To accelerate and maximise germination, seed treatments must overcome any dormancy mechanisms that are present (see **Box 6.2**, p. 168). The most common dormancy mechanisms involve the seeds coverings; treatments that perforate those coverings (scarification) are often effective as they allow water and oxygen to diffuse into the embryo. Use sand paper to roughen the entire seed surface or nail clippers to make small individual holes in the end of the seed opposite to that at which the embryo is located. Try cracking large pyrenes, which are covered by a hard, stony or woody endocarp, open gently in a vice or tapping them with a hammer. Scraping off a soft aril, if present, nearly always increases germination.

Try cracking large hard seeds gently in a vice.

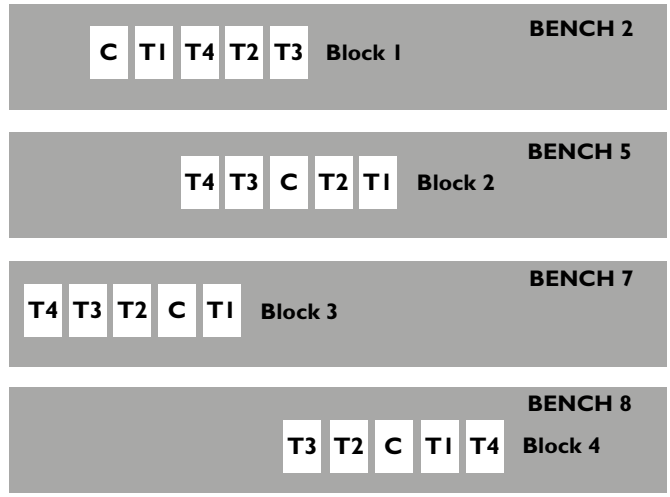
Acid can also be tested as a scarifying agent to break down impermeable seed coats. Soak the seeds in concentrated sulphuric acid for a few minutes to several hours (depending on the size of the seed and the thickness of its coat). You will need to experiment with the time required. This treatment is usually effective with legume tree seeds. Acids are obviously dangerous substances and must be handled with caution, following manufacturer's safety guidelines. If physical dormancy is suspected (i.e. if embryo development is restricted by a hard but permeable seed coat), acid may penetrate rapidly and kill the embryo, so acid treatment is not recommended for such seeds. Freezing and heat treatments (particularly burning) are also not recommended for tropical tree species. If dormancy is caused by chemical inhibitors, experiment with soaking the seeds in water for various lengths of time to dissolve out the inhibitory chemicals. Another option that is worth investigating is to collect seeds at different times of the year, from the same or different individual trees of the same species. Such experiments can be used to determine the optimum seed collection time.

Try to design treatments that change only one factor, even though this can be difficult to achieve in practice. For example, putting seeds into hot water has two simultaneous effects, i.e. soaking and heating.

Experimental design for germination trials

Use a randomised complete block design (RCBD) as described in **Appendix** (**A2.1**) to test for treatment effects. Place a control germination tray containing seeds that have been prepared in a standard way and several treatment trays, each one containing seeds that have been subjected to a different pre-sowing treatment, adjacent to each other on a nursery bench as a 'block'. Replicate the blocks several times on different benches and represent each treatment equally in every block (i.e. with the same number of seeds subjected to each of the treatments and in the control tray).

Allocate the positions of the control and the treatment replicates randomly within each block. The typical design shown here has four treatments (T1–T4) and a control (C), replicated in four blocks. Using a minimum of 25 seeds per replicate, this design requires 125 seeds per block or 500 seeds in total. If you do not have enough seeds, then reduce the number of treatments tested, but try to keep the number of replicates above three. If you have enough seeds, then increase the number of seeds per replicate to 50–100 (which would require 1000–2000 seeds, respectively, for the whole experiment).



Fill modular germination trays with the regular germination medium used in the nursery. Then, sow a single seed into each module. Do not bury the seeds too deeply, otherwise it will be difficult to observe when each seed germinates. Clearly label the trays with the species number and treatment applied, and if necessary cover the trays with wire mesh to prevent animals from interfering with the experiments.

Setting up one block of an RCBD germination experiment in Cambodia.

Collecting data in germination trials

Prepare a seed germination data sheet like that illustrated here. Inspect all seed germination trays at least once per week. During periods of very rapid germination, more frequent data collection might be necessary. For each seed that has germinated (see the definition in **Box 6.2**), use a correction fluid pen ('Liquid Paper') to place a waterproof white dot on the rim of the module, always in the same orientation (e.g. always on the top edge of the module). Count the total number of white dots and record the result on the data sheet. White dots indicate all those cells in which a seed has germinated, even if the seedling subsequently dies and disappears. Therefore, counting the white dots provides a better assessment of actual germination than counting the number of visible seedlings.

Early seedling mortality (i.e. death occurring after germination but before the seedlings grow large enough for pricking out) is also a useful parameter when calculating the number of trees that can be generated from a given number of seeds collected. To record early seedling mortality, count the number modules with white dots that contain no visible seedling or an obviously dead one. For additional insurance, draw diagrams of each modular tray, with one square representing each module. Then record in each square the date on which germination or seedling death was first observed.

Species Number: 133 **Batch Number: 10**

SEED GERMINATION DATA COLLECTION SHEET

Species name: *Afzelia xylocarpa* (Kurz) Craib **Family: Leguminosae**

Date seeds collected: 20/8/2010 **Date seeds sown: 24/11/2010** **Seeds sown per replicate: 24**

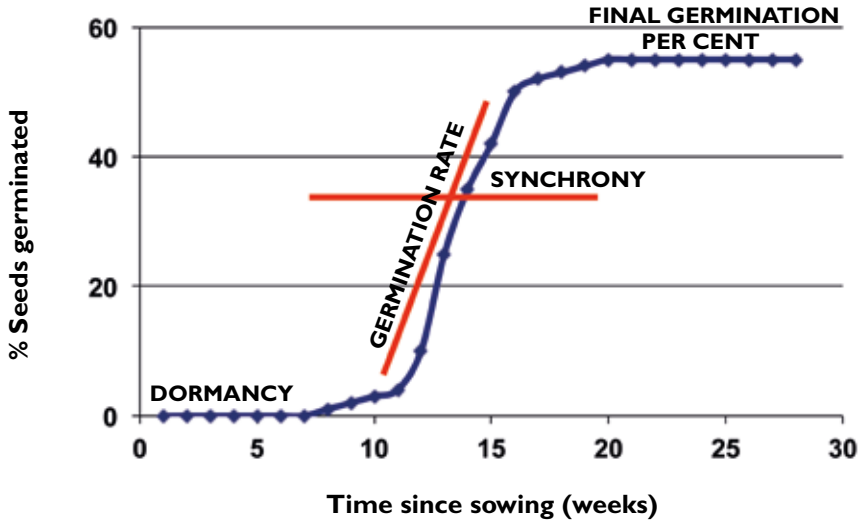
Description of standard seed preparation procedures applied to all seeds:

	TREATMENT DESCRIPTIONS
T1	Control
T2	Scarification
T3	Soaking in water for 1 night

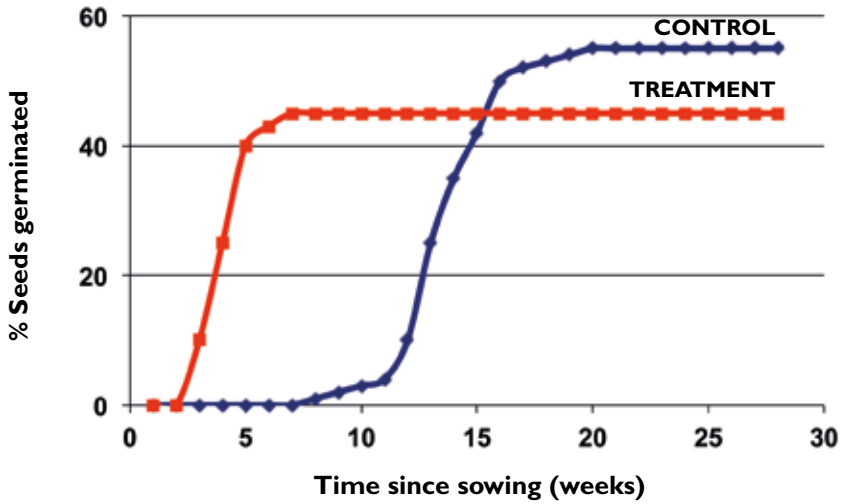
	BLOCK 1						BLOCK 2						BLOCK 3							
	T1R1		T2R1		T3R1		T1R2		T2R2		T3R2		T1R3		T2R3		T3R3			
Date	G	GD	G	GD	G	GD	G	GD	G	GD	G	GD	G	GD	G	GD	G	GD	Total germinated	Total died
1/12/2010	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8/12/2010	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15/12/2010	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3	0
22/12/2010	0	0	5	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	6	0
29/12/2010	0	0	6	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	7	0
5/1/2011	0	0	9	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	10	0
12/1/2011	0	0	9	0	0	0	0	0	3	0	0	0	0	0	5	0	0	0	17	0
19/1/2011	0	0	12	0	0	0	0	0	5	0	0	0	0	0	6	0	0	0	23	0
26/1/2011	0	0	17	1	0	0	0	0	7	0	0	0	0	0	7	0	0	0	31	1
2/2/2011	0	0	17	1	0	0	0	0	7	0	0	0	0	0	7	0	0	0	31	1
9/2/2011	0	0	19	1	0	0	0	0	8	0	0	0	0	0	9	0	0	0	36	1
16/2/2011	0	0	22	1	0	0	0	0	12	1	0	0	0	0	9	0	0	0	43	2
23/2/2011	0	0	22	2	0	0	0	0	15	1	0	0	0	0	11	0	0	0	48	3
2/3/2011	0	0	22	2	0	0	0	0	17	1	0	0	0	0	15	1	0	0	54	4
9/3/2011	0	0	22	2	0	0	0	0	17	1	0	0	0	0	19	1	0	0	58	4

Germination curves

One of the simplest and clearest ways to represent the results of germination trials is a germination curve, with time elapsed since sowing on the horizontal axis and cumulative number (or percentage) of seeds germinated (combined across replicates) on the vertical axis. The germination curve combines into a single graphic all germination parameters, including length of dormancy period, rate and synchronicity of germination, and final percent germination.



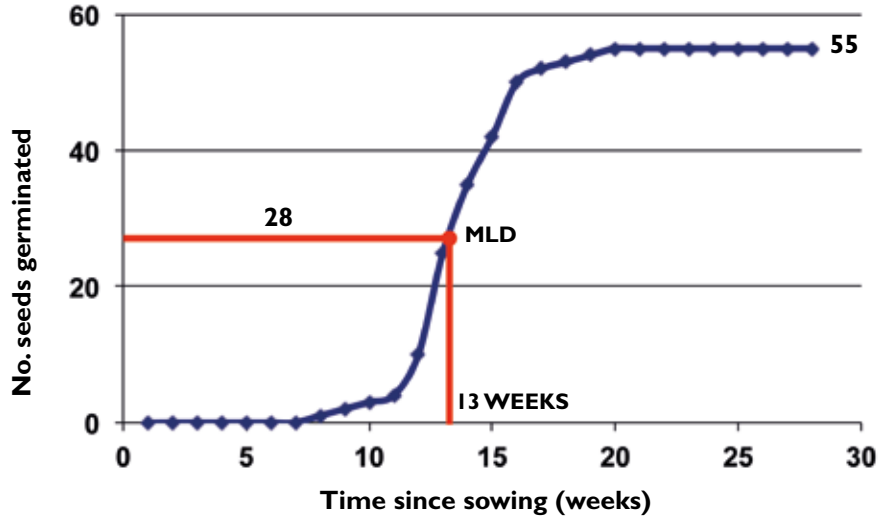
Germination curves can inform decision making without the need for complex statistical tests. In the example illustrated, the pre-sowing seed treatment accelerates germination but reduces the number of seeds that germinate. Getting the seeds to germinate faster may mean the difference between achieving a crop of saplings that are ready to plant by the first rainy season after seed collection and having to maintain saplings in the nursery until the second rainy season after seed collection. So even though the treatment reduces germination, it could have beneficial results.



Measuring dormancy

Dormancy length is defined as the number of days between sowing a seed and emergence of the radicle (the embryonic root or the plumule if the radicle cannot be seen). In any batch of seeds, this period of time varies among the seeds. One way to express the dormancy of a batch of seeds is to add together the number of days that each individual seed is dormant and then divide the total by the number of seeds that germinate. This is 'mean dormancy'. Within any batch of seeds, however, a few seeds usually take an exceptionally long time to germinate. This increases the mean dormancy disproportionately and can produce misleading results. For example, if 9 seeds germinate 50 days after sowing and one seed germinates 300 days after sowing, the mean dormancy is ((9×50)+300)/10) = 75 days. Even though germination was complete for 90% of the seeds by day 50, a single outlying seed, increased the recorded mean dormancy by 50%.

Median length of dormancy (MLD) overcomes this problem by defining dormancy as the length of time between sowing and the germination of half the seeds that eventually germinate. In the above example, MLD would be the time between sowing and germination of the 5th seed, i.e. 50 days.



Comparing germination treatments

For each treatment and for the control, sum the final number of seeds that germinate from all replicate blocks and divide the result by the number of blocks to calculate the mean value and then repeat the calculation for the MLD values. Then use an analysis of variance (ANOVA) (see **Appendix A2.2**) to test for significant differences among the means (i.e. among the treatments and control). If the ANOVA shows significant differences, then perform pair-wise comparisons between each treatment mean and the control mean to determine which treatments increase or decrease germination and/or dormancy (see **Appendix A2.3**).

Experimenting with seed storage

If you want to experiment with seed storage, first try to confirm from the literature, or by a pilot study, whether the species you want to work with has orthodox, intermediate or recalcitrant seeds (see **Section 6.2** and http://data.kew.org/sid/search.html). Seed storage is useful for those tree species with orthodox seed whose saplings would otherwise grow rapidly and reach a plantable size well before the optimal planting time. Tending such plants for longer than is necessary wastes nursery space and resources. Furthermore, pruning them becomes an added chore when the plants start to outgrow their containers, and some species do not respond well to pruning.

For such tree species, use records of previously germinated seedlings to calculate how many months are required to grow saplings to a plantable size. Count back that number of months from the optimal planting date to obtain the optimal seed-sowing date. Next, count forward from the fruiting month to the optimal sowing date to arrive at the duration of seed storage necessary to optimise nursery production. Carry out germination trials with some seeds immediately after collection, to determine their original viability (this is the 'control'). Then, store the rest of the seeds for the calculated length of time required. Sample the seeds at intervals to monitor any changes in viability. If there are enough seeds, experiment with different storage conditions (e.g. dry the seeds to different moisture contents or vary the storage temperature). Then, perform germination tests to determine if viability declines when the seeds are stored for the required length of time.

For direct seeding, carry out a germination trial on a sample of seeds immediately after collection. Then store the rest of the seeds for the required length of time (from seed collection to optimal direct seeding date). Remove the seeds from storage and sow samples in the nursery and in the field. Compare germination between these two groups and with the seed sample tested at collection time.

For species that fail to fruit every year, experiment with storing seeds for 1 year or longer to determine if seeds that are collected in fruiting years can be stored to grow seedlings in years when fruits are not produced. Similar experiments are useful for distributing seeds to other locations or if seeds are collected elsewhere to supplement a planting program (see **Box 6.1**, p.159).

When carrying out seed storage experiments, pre-sowing treatments can also be tested, but for a valid comparison, apply the same treatments to both the control batch (sown immediately after collection) and the stored batches.

Seedling growth and survival

Monitoring the performance of tree species in nurseries enables calculation of the time needed to grow trees of each selected species to a plantable size by the planting-out date. It also allows assessment of the susceptibility of each species to pests and diseases and detection of other health problems; thus it also provides a mechanism for quality control.

Comparing species and treatments

Tree species that grow well in nurseries usually perform well in the field. So one of the most useful nursery experiments is to compare survival and growth among species. Adopt a standard production method for all species and use a RCB experimental design (see **Appendix A2.1**) to compare performance among species. In this case, there are no 'control' and 'treatment' replicates. A 'block' consists of one replicate (no less than 15 containers) of each species.

Subsequently, additional experiments can be carried out to develop more efficient production methods for selected high-performing species. These should test different techniques to manipulate growth rates in order to grow saplings that reach a suitable size in time for hardening-off and planting-out. So many factors affect plant growth; the number of potential treatments is bewildering. The best plan is to start with the simplest and most obvious treatments, such as different container types, media composition and fertiliser regimes and test others (e.g. pruning, inoculation with mycorrhizal fungi) later if necessary.

The benefits of each treatment must be weighed against its costs and feasibility. So it is important to record the cost of applying each treatment. The main question being addressed is whether or not improving the quality of the planting stock in the nursery ultimately results in increased survival and growth of trees planted in the field. So, it is also useful to label trees that have been subjected to different nursery treatments and to continue to monitor them after they have been planted out in the field.

Factors that might influence seedling survival and growth

Container type

Experiments should be performed to test which container type is the most cost-effective for the species being grown. Start with a standard container type, such as plastic bags, and carry out simple experiments with different bag sizes to determine the effects of container volume on the size and quality of trees produced by planting-out time. Then, compare plastic bags with other container types that exert more control over root form (with or without air-pruning), such as rigid plastic cells or tubes (see **Section 6.4**).

Media and fertiliser regime

Start with a standard potting medium (see **Section 6.4**) and then experiment with varying its composition by using different forms of organic matter (e.g. coconut husk, rice husk or peanut husk) or by adding nutrient-rich materials such as cattle dung. For slow-growing species, try accelerating growth by experimenting with different fertiliser treatments (fertiliser type, dosage and frequency of application).

Pruning

If trees start to out-grow their containers before planting-out time, experiment with shoot-pruning treatments. Tree species vary in their responses to shoot pruning. Some are killed by pruning whereas others branch, producing a denser crown that enables them to shade out weeds more rapidly after planting out. Compare different shoot pruning intensities, timing and frequencies. In addition to growth and mortality data, also record plant form during pruning experiments.

Saplings that have a dense, fibrous root system are better able to supply their shoots with water. Therefore, a high root:shoot ratio improves the chances of survival after planting out. Large, woody roots are most resistant to desiccation, but they must have

a dense network of young, fine roots for efficient water absorption. Experiment with different root pruning schedules. At the end of such experiments, sacrifice a few plants for the recording of root form and root:shoot ratio.

Mycorrhizal fungi

Most tropical tree species develop symbiotic relationships with fungi that infect their roots to form mycorrhiza. Such relationships enable trees to absorb nutrients and water more efficiently than the tree's own root system can (see **Section 6.5**). If forest soil is included in the potting medium, most saplings become naturally infected with mycorrhizal fungi (Nandakwang *et al*., 2008). So first, survey saplings that are growing in the nursery to confirm the presence of mycorrhiza and assess the frequency of root infection.

For arbuscular mycorrhiza, i) wash a sample of fine roots; ii) treat them with a clearing solution (10% (w/v) KOH at 121°C for 15 minutes) to render the roots transparent; iii) apply 0.05% trypan blue in lactic acid:glycerol:water (1:1:1 v/v) to stain the fungal cells, and finally, iv) examine the roots under a dissecting microscope to estimate the percentage that are infected. Follow the safety precautions recommended for each of the chemicals.

For ectomycorrhiza, estimate the percentage of fine roots that have characteristic swollen ends to the root tips, then observe the roots under a microscope for the presence of fungal hyphae. Mycorrhizal fungus species are identified by examining their spores under a compound microscope. This requires specialist help (for general techniques for the study of mycorrhizae, see Brundrett *et al*., 1996).

If the tree roots of any species are not colonised by mycorrhizal fungi, or colonised only very sparsely, then consider experiments to assess the effect of artificial inoculation. Commercial preparations containing mixtures of common mycorrhizal fungi spores may be available for testing (but be aware that they may not contain the particular fungus species or strains required by the tree species being grown). Alternatively, it is possible to collect fungal spores from around the roots of forest trees and then culture them in pots on domestic crop plants such as sorghum. Such home-made inoculae might be more specific for the trees being grown, but producing them is time-consuming and requires specialised techniques. The success of inoculation is often reduced if the plants are given fertiliser. So, try experiments that test various combinations of fertiliser treatments with the application of mycorrhizal inoculum. First, determine if artificial inoculation can increase infection rates (and ultimately tree performance) above those achieved naturally by including forest soil in the potting medium. Compare the performance of saplings grown in standard medium (which includes forest soil) with those subjected to supplementary sources of inoculum at various doses. Mycorrhizal fungi can easily be spread from one container to another by water, either by splashing or drainage. So, raise the containers off the ground on a wire grid and separate treatment replicates with plastic shielding to prevent splashing.

Designing experiments to test sapling performance

As with germination experiments, use a randomised complete block design (RCBD; see **Appendix A2.1**) and analyse the results using a two-way ANOVA, followed by paired comparisons (**Appendices A2.2** and **A2.3**). The example experimental design for germination trials can be used equally well for sapling performance experiments (substituting 'beds' for 'benches').

The number of treatments that can be applied and the number of replicates possible (i.e. the number of blocks) depends on the number of seedlings that survive after potting. Decide on the treatments that can be applied. Then, for each block, select a minimum of 15 plants (more is better) to constitute one 'replicate' for each treatment, and the same for the control. Make sure that all treatments (and the control) are represented by the same number of plants in all blocks. Place each block, consisting of one replicate of each treatment + control, in a different bed in the standing down area of the nursery. Within each block, position the treatment and control replicates randomly.

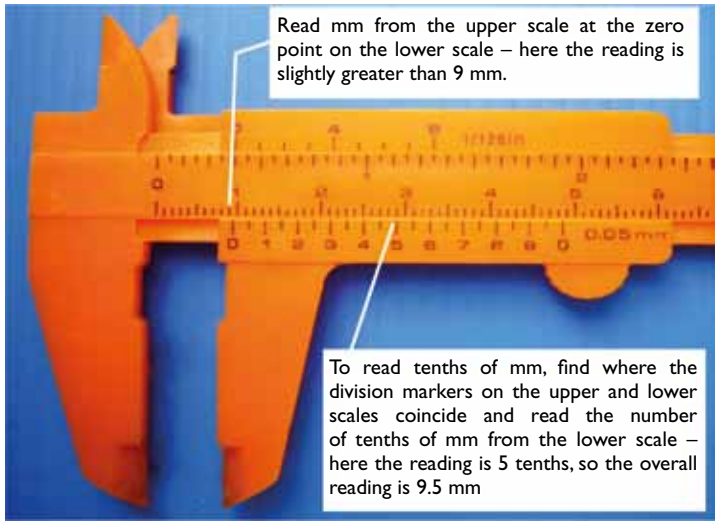
Seedling growth experiments in Cambodia: replicates are 15 seedlings in 23 cm × 6.5 cm plastic bags (3 rows of 5 plants), surrounded by a single guard row of 20 plants.

Select uniform plants for experiments; reject unusually tall or short plants and any showing signs of disease or malformations. Plants at the edge of a replicate may experience a different environment to those within it because treatments, such as watering or fertiliser application, may 'spill over' from one replicate to another. In addition, the plants at the edge of a block experience no competition from neighbours on one side and they may be affected by people brushing up against them. Reduce these 'edge effects' by surrounding each replicate with a 'guard row' of plants that are not assessed in the experiment. A simple experiment testing four treatments + a control in four blocks, would require a minimum of 15 × 5 = 75 uniform, healthy plants in each block, or 300 totally, plus extra plants to make the guard rows.

Assessment of growth

Collect data immediately after the experiment has been set up (as soon as possible after potting) and at intervals of approximately 45 days thereafter. The final data collection session should be just before the trees are removed from the nursery for planting out (even if this occurs earlier than 45 days after the previous data collection session).

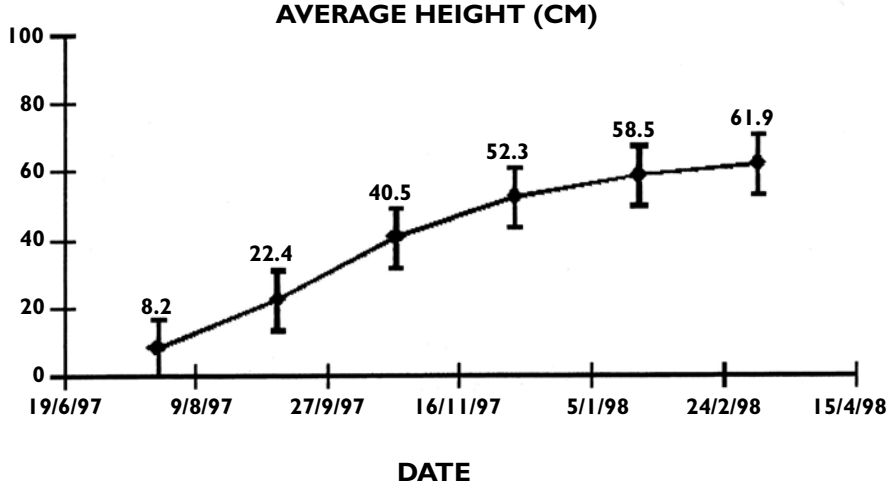
Measure the height of each sapling (from root collar (i.e. the point at which the shoot meets the root) to apical meristem) with a ruler. Measure RCD (i.e. diameter at the 'root collar') at the widest point with Vernier-scale callipers (available from most stationery stores). At the zero mark on the lower sliding scale, read number of millimetres diameter from the upper scale. For the decimal point, look for the point at which the division marks on the lower scale are exactly aligned with the division marks on the upper scale. Then, read the decimal point off the lower scale. The Vernier scale in the example



illustrated here reads 9.5 mm. Because RCD is a small value, it must be measured with high accuracy. For best results, measure RCD twice by turning the callipers at right angles and then use the average reading.

Use a simple scoring system to record plant survival and health (0 = dead; 1 = severe damage or disease; 2 = some damage or disease but otherwise healthy; 3 = good health). Also, record descriptions of any pests and diseases observed, as well as any signs of nutrient deficiency. Note when leaf shedding, bud break or branching occurs and record any unusual climatic events that might affect the experiment.

Determine root:shoot ratio (dry mass) by sacrificing a few plants at the end of the experiment. At the same time, photograph the structure of the root system. Remove sample plants from their containers and wash out the medium, taking care not to break the fine roots. Separate the shoot from the roots at the root collar. Dry them in an oven at 80–100°C. Weigh the dried shoot and dried root systems and calculate root dry weight divided by shoot dry weight for each plant sample.



Seedling growth data for a pioneer tree species. Trees reach a size suitable for planting out by January, six months ahead of the optimal planting time. Therefore, seed storage to delay germination is recommended to prevent waste of nursery space and to avoid the need to prune the saplings.

Species: *Cerasus cerasoides* **S. No.: S71B1**

Pricked out: June 6th 1997 **BLOCK: 1** **TREATMENT: NONE (CONTROL)**

HEIGHT DATA (CM)

		SEEDLING NUMBER															
DATE	**DAYS**	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	AVG
7/6/97	1	5.0	4.0	3.5	2.0	4.0	3.0	4.0	3.0	3.5	3.0	5.0	4.0	3.0	4.0	4.5	3.7
25/7/97	49	11.0	12.0	8.0	3.0	8.0	5.5	7.5	5.5	6.5	8.5	12.0	9.0	8.5	9.0	9.5	8.2
8/9/97	94	29.0	38.0	23.0	33.0	x	16.0	19.0	17.0	13.0	14.0	35.0	20.0	25.0	16.0	16.0	22.4
23/10/97	139	67.0	67.0	44.0	34.0	x	32.0	35.0	25.0	32.0	29.0	66.0	27.0	50.0	28.0	31.0	40.5
7/12/97	184	70.0	70.0	55.0	34.0	x	52.0	61.0	36.0	48.0	47.0	71.0	38.0	58.0	40.0	52.0	52.3
23/1/98	231	73.0	70.0	57.0	34.0	x	64.0	67.0	41.0	52.5	53.0	80.0	46.0	72.0	43.0	66.0	58.5
9/3/98	276	73.0	70.0	60.0	34.0	x	64.0	67.0	49.0	58.0	54.0	81.0	55.0	73.0	53.0	75.0	61.9

ROOT COLLAR DIAMETER DATA (MM)

		SEEDLING NUMBER															
DATE	**DAYS**	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	AVG
7/6/97	1	0.5	0.7	0.4	0.8	0.4	0.5	0.6	0.7	0.6	0.7	0.7	0.6	1.0	0.6	0.7	0.6
25/7/97	49	1.4	2.2	1.3	1.1	1.3	1.0	1.5	1.6	1.3	1.2	1.4	1.1	2.1	1.3	1.4	1.4
8/9/97	94	2.8	3.2	2.7	1.4	x	1.5	1.6	3.3	2.7	2.5	2.4	2.5	2.2	2.3	1.4	2.3
23/10/97	139	4.2	4.0	3.0	1.7	x	1.8	2.1	3.3	2.7	2.7	3.6	2.5	3.0	2.3	1.6	2.8
7/12/97	184	4.4	4.0	3.0	2.5	x	2.9	2.9	3.3	2.7	3.0	3.7	3.0	3.0	2.3	3.0	3.1
23/1/98	231	4.4	4.0	4.2	2.5	x	4.5	4.5	3.3	3.2	3.5	4.2	3.0	4.0	2.6	4.5	3.7
9/3/98	276	5.2	6.0	4.2	2.6	x	5.0	5.5	3.6	4.0	4.3	4.6	3.5	4.5	3.0	5.0	4.4

HEALTH DATA (0–3)

		SEEDLING NUMBER															
DATE	**DAYS**	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	AVG
7/6/97	1	2.5	2.5	2.5	1.5	2.0	1.5	3.0	3.0	2.5	3.0	3.0	2.5	2.0	3.0	3.0	2.5
25/7/97	49	3.0	3.0	3.0	2.0	3.0	2.5	3.0	2.5	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.9
8/9/97	94	3.0	3.0	3.0	2.0	x	2.5	3.0	3.0	2.5	2.5	3.0	3.0	3.0	3.0	2.5	2.8
23/10/97	139	3.0	2.5	3.0	2.5	x	3.0	3.0	3.0	3.0	3.0	3.0	3.0	1.5	3.0	3.0	2.8
7/12/97	184	3.0	3.0	3.0	3.0	x	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
23/1/98	231	3.0	3.0	3.0	3.0	x	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
9/3/98	276	3.0	3.0	3.0	3.0	x	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0

Calculations from growth data

Use a standard data collection sheet for each replicate in each block. After each data collection session, calculate mean values (and standard deviations) for each of the parameters measured.

Also calculate relative growth rates (RGR), thereby removing the effects of differences in the original sizes of seedlings or saplings immediately after potting on subsequent growth. This makes it possible to assess treatment effects despite differences in the initial sizes of the plants at the beginning of the experiment. RGR is defined as the ratio of the growth of a plant to its mean size over the period of measurement, according to the equation below:

$$\frac{(\ln FS - \ln IS) \times 36{,}500}{\text{No. days between measurements}}$$

where ln FS = natural logarithm of final sapling size (either sapling height or RCD) and ln IS = natural logarithm of initial sapling size. The units are per cent per year.

Analysing survival data

For each replicate, count the number of saplings that survive until planting-out time. Then calculate the mean value and the standard deviation for each treatment; repeat for the control. Apply ANOVA (see **Appendix A2.2**) to determine if there are significant differences in mean survival among the treatments. If so, then use paired comparisons (see **Appendix A2.3**) between each treatment mean and the control mean to identify which treatments significantly increase survival. The same approach can be used to make comparisons among species.

Analysing growth data

Represent sapling growth graphically by constructing a growth curve that can be updated after each data collection session. Plot time elapsed since pricking out (horizontal axis) against mean sapling height (or mean RCD), averaged across blocks, for each treatment (vertical axis). By extrapolation, such curves can be used to estimate roughly how long it will take saplings growing in the nursery to reach the optimum planting size.

Just before optimum planting-out time, calculate the mean sapling height and RCD for each replicate and average these mean values across all blocks to arrive at treatment means. Carry out an ANOVA (see **Appendix A2.2**) to determine if there are significant differences among treatment means and, if so, use paired comparisons (see **Appendix A2.3**) to determine which treatments result in saplings that are significantly larger than control saplings at planting time. RCD and RGR (for both height and RCD) can be analysed in the same way.

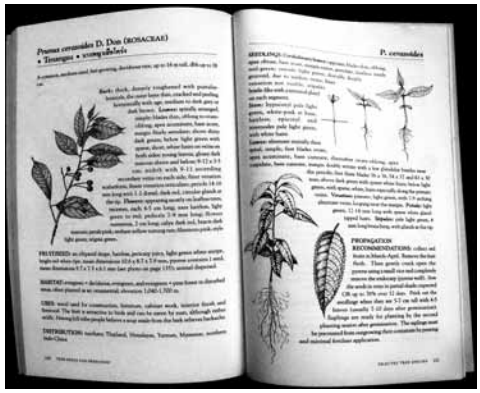
What are the targets to aim for?

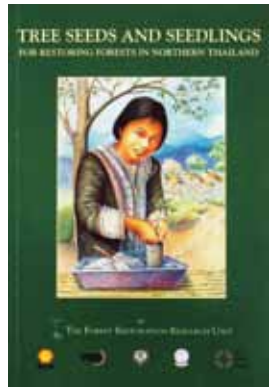
Adopt, as standard, any treatments that significantly contribute towards achieving the following targets by optimum planting-out time:

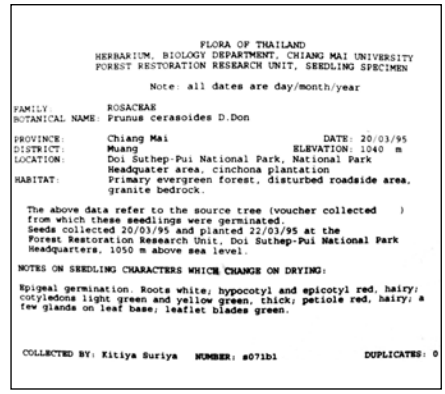
- >80% survival of saplings since pricking out;
- mean sapling heights >30 cm for fast-growing pioneer species (20 cm for *Ficus* spp.) and >50 cm for slow-growing climax tree species;
- sturdy stems, supporting mature, sun-adapted, leaves (not pale, expanding leaves) ('sturdiness quotient' can be calculated as height (cm)/RCD (mm) of <10);
- a root:shoot ratio of between 1:1 and 1:2; with an actively growing, densely branching root system that is not spiralling at the base of the container;
- no signs of pests, diseases or nutrient deficiency.

Tree seedling morphology and taxonomy

Surveys of natural forest regeneration require the identification of tree seedlings and very young saplings, but this is notoriously difficult. Plant species descriptions in floras are based primarily on reproductive structures. The morphology (particularly leaf shape) of seedlings often differs markedly from that of mature tree foliage and seedling specimens are hardly ever included in herbarium collections. Resources for the identification of tropical forest tree seedlings are almost non-existent (but see FORRU, 2000). Therefore, nurseries that are producing seedlings and saplings of known ages, from seeds collected from properly identified parent trees, provide an immensely valuable resource for studying tree seedling morphology and taxonomy.







FLORA OF THAILAND
HERBARIUM, BIOLOGY DEPARTMENT, CHIANG MAI UNIVERSITY
FOREST RESTORATION RESEARCH UNIT, SEEDLING SPECIMEN

Note: all dates are day/month/year

FAMILY: ROSACEAE
BOTANICAL NAME: Prunus cerasoides D.Don

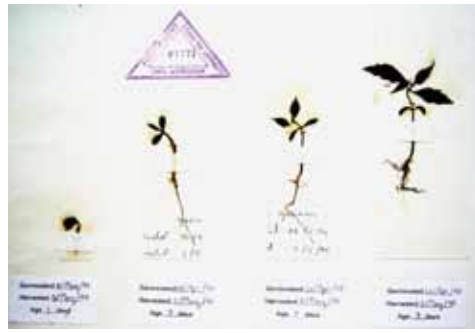
PROVINCE: Chiang Mai DATE: 20/03/95
DISTRICT: Muang ELEVATION: 1040 m
LOCATION: Doi Suthep-Pui National Park, National Park Headquater area, cinchona plantation
HABITAT: Primary evergreen forest, disturbed roadside area, granite bedrock.

The above data refer to the source tree (voucher collected) from which these seedlings were germinated.
Seeds collected 20/03/95 and planted 22/03/95 at the Forest Restoration Research Unit, Doi Suthep-Pui National Park Headquarters, 1050 m above sea level.

NOTES ON SEEDLING CHARACTERS WHICH CHANGE ON DRYING:

Epigeal germination. Roots white; hypocotyl and epicotyl red, hairy; cotyledons light green and yellow green, thick; petiole red, hairy; a few glands on leaf base; leaflet blades green.

COLLECTED BY: Kitiya Suriya NUMBER: s071b1 DUPLICATES: 0



The seedlings of tropical forest trees remain largely unstudied. A tree nursery provides a unique opportunity to collect seedling specimens of known species and ages and to publish their descriptions.

Try to collect at least three specimens of seedlings or saplings at all stages of development for every species grown. Prepare them as herbarium specimens in the usual way, mounting several specimens in chronological order on a single herbarium sheet. On the herbarium label, record the age in days of each seedling or sapling specimen, and include details of the parent tree from which seeds were collected. Engage an artist to produce line drawings of the seedlings. Publish the drawings and descriptions of seedlings in an identification handbook.

Experiments with wildlings

Producing planting stock from wildings (see **Box 6.4**, p. 174) is advantageous i) when seeds are not available; ii) when seed germination and/or seedling survival and growth are problematic or slow; or iii) when the production of planting stock must be accelerated.

Experiments with wildings should address three simple questions: i) can high-quality planting stock be produced from wildings more rapidly and cost-effectively than by germinating seeds, ii) can the growing of wildings in nurseries be manipulated to achieve optimum-sized plants by planting out time; and iii) do wildlings perform as well as, or better than, plants germinated from seed?

All of the seedling treatments described above can be applied to determine optimum conditions for growing-on wildlings in nurseries to a plantable size. However, two additional treatments are specific for wildlings: i) size when collected and ii) shoot-pruning at collection time.

Small seedlings are more delicate than larger saplings and are more easily damaged during transplantation. On the other hand, larger plants are more difficult to dig up without leaving some roots behind and can consequently suffer from transplantation shock. Group the wildlings collected into three size classes (short, medium and tall).

These then become three 'treatments' in a RCBD experiment (there is no control). Collect growth and survival data, as described above, and compare mean survival and RGR among the initial size classes.

Digging up plants inevitably damages their root system, but the shoot system remains intact, and so a reduced root system must supply water to an undiminished shoot. This imbalance can cause wildings to wilt and possibly die. Pruning shoots can bring the root:shoot ratio back into balance. Apply shoot pruning treatments of varying intensities at collection time (e.g. no-pruning (control) and pruning back ⅓ or ½ of the shoot length or of the leaves). Collect growth and survival data as described above, and compare mean survival and RGR among the pruning treatments.

Continue to monitor the performance of planting stock from wildlings after planting out (e.g. survival and growth rates) and then compare results with those from trees produced by germinating seeds.

FOREST RESTORATION RESEARCH UNIT

S. 146 B4

FORRU SEEDLING PRODUCTION DATA SHEET

1. COLLECTION

SPECIES: *Nyssa javonica* (Bl.) Wang — FAMILY: Cornaceae
LINKCODE: NYSSJAVA — VOUCHER NO.: 89
COLLECTION DATE: 11-Aug-06, ground — QUANTITY: 3,000 SEEDS

2. SEED GERMINATION

PRETREATMENT: seeds were soaked in water 1 night, after that sun dry 2 days
QUANTITY SOWN: 2,500 SEEDS
MEDIA/CONTAINER: Forest soil only, 8 baskets
SOWING DATE: 14-Aug-06
NUMBER GERMINATED: 2,059 SEEDS

OBSERVATION

1st germ. 26-Aug-06 to 11-Sep-06
Damping off diseases were destroyed about 12% of all germinated seedlings

3. PRICKING OUT

DATE PRICKING OUT: 3-Oct-06 — QUANTITY: 1,505 SEEDLINGS
MEDIA/CONTAINER: Forest soil: Cocounut husk: Peanut husk (2:1:1) in plastic bag
NURSERY CARE:

NURSERY CARE	1	2	3	4	5
FERTILIZER	13/11/06	12/2/07	13/3/07		
PRUNING (NO)					
WEEDING	13/11/06	13/12/06	13/1/07	13/2/07	13/3/07
PEST/DISEASES CONTROL	13/1/07 Leaf eating insects				

OBSERVATION

2–3 months after pricking out, red fungus and leaf blight occurred, but all seedlings look healthy

4. HARDENING AND DESPATCH

DATE HARDENING STARTED: 17-May-07 — DATE DESPATCHED: 19-Jun-07
NUMBER OF GOOD QUALITY PLANTS: 1,200 SEEDLINGS
WHERE PLANTED: MAE SA MAI, WWA PLOT

OBSERVATION

500 seedlings were planted on 30/6/07 at Ban Mae Sa Mai

Use a simple data sheet to collate all information about a batch of seeds as it passes through the nursery production process, from seed collection to delivery of saplings to the restoration site.

Production schedules — the ultimate aim of nursery research

Growing a wide range of forest tree species is difficult to manage. Different species fruit in different months and have widely different rates of germination and seedling growth; yet all species must be ready for planting by the optimal planting time. Species production schedules make this daunting managerial task easier.

In seasonally dry tropical climates, the window of opportunity for tree planting is narrow, sometimes just a few weeks at the beginning of the rainy season. In less-seasonal climates, there may be more latitude in the timing of tree planting. In either case, species production schedules are an excellent tool to ensure that the required species of trees are ready for planting when required.

What is a production schedule?

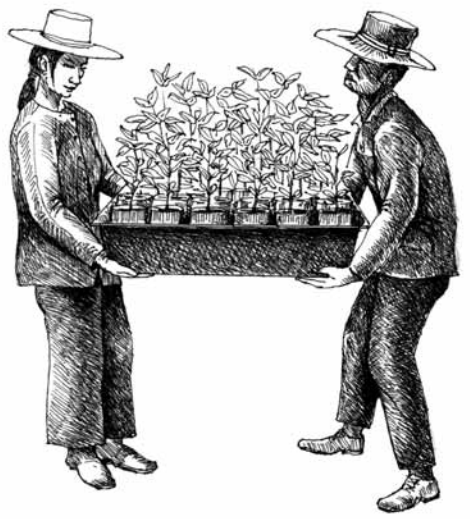
For each tree species being grown, the production schedule is a concise description of the procedures necessary to produce planting stock of optimum size and quality from seed, wildlings or cuttings by the optimum planting-out time. It can be represented as an annotated time-line diagram that shows: i) when each operation should be performed and ii) which treatments should be applied to manipulate seed germination and seedling or sapling growth.

Information needed to prepare a production schedule

The production schedule combines all available knowledge about the reproductive ecology and cultivation of a species. It is the ultimate interpretation of the results from all the experimental procedures described above, including:

- optimum seed collection date;
- germination time or natural length of seed dormancy;
- how seed dormancy might be manipulated by pre-sowing treatments or seed storage;
- length of time required from seed sowing to pricking out;
- length of standing-down time required to grow saplings to a plantable size;
- how plant growth and standing-down time can be manipulated with fertiliser application and other treatments.

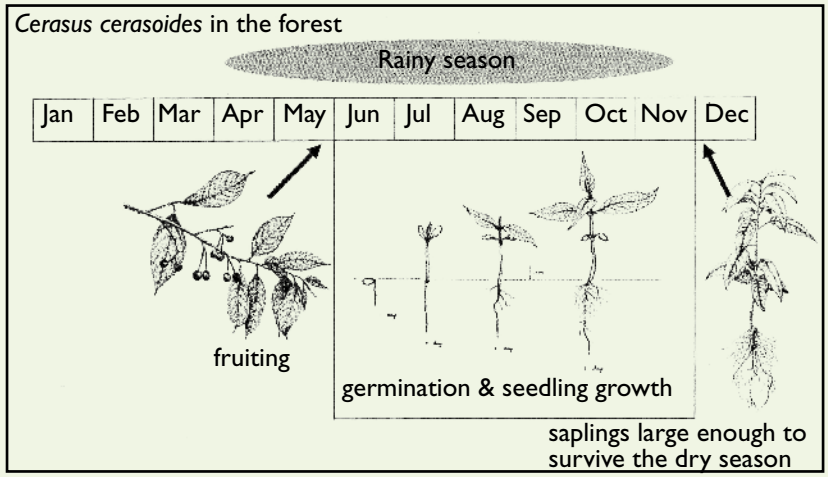
The finished product.



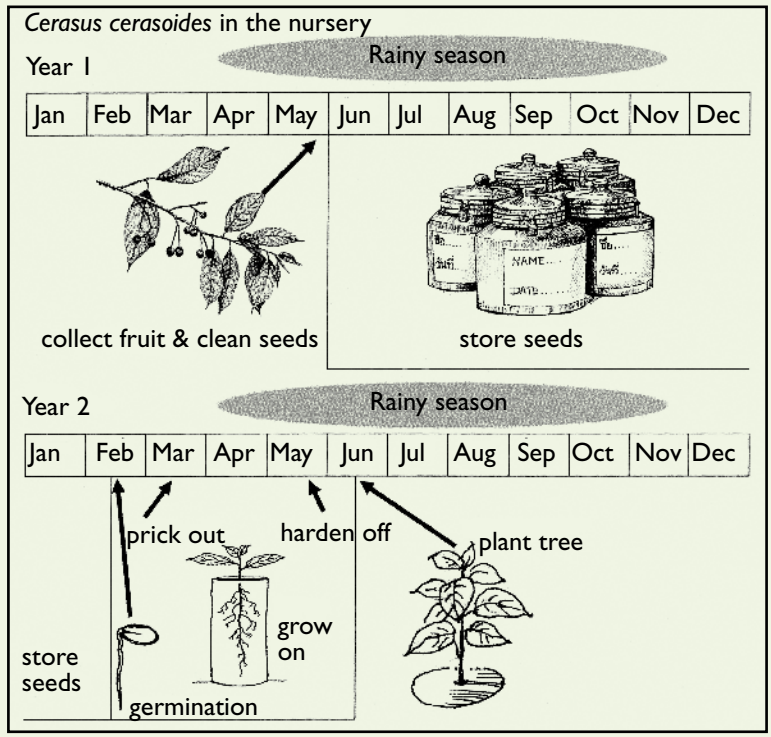
All of this information will become available from nursery data sheets if the procedures detailed above are followed. The production schedule is very much a working document. Draft the first version once the first batch of plants has been grown to a plantable size. This enables the identification of areas requiring further research and of appropriate treatments to be tested in subsequent experiments. As the results of experiments on each subsequent batch of plants become available, the production schedule will be gradually modified and optimised.

Box 6.6. Example production schedule (for *Cerasus cerasoides*).

In its natural habitat, this fast-growing pioneer tree fruits in April or May. Its seeds have short dormancy and the seedlings grow rapidly during the rainy season. By December, their roots have penetrated deep enough into the soil that they are able to supply the shoot with moisture during the harsh conditions of the dry season. In the nursery, saplings that have reached a plantable size by December would have to be kept for a further 6 months before the next planting season (the following June) and would outgrow their containers.



In the nursery, the production schedule therefore involves storing the sun-dried pyrenes at 5°C until January, when they are germinated. The plants then grow to the optimum size just in time for hardening off and planting out in June. Development of this production schedule involved research on phenology, seed germination, seedling growth and seed storage.



CASE STUDY 4 Doi Mae Salong: 'Treasure Tree Clubs'

Country: Thailand

Forest Type: Evergreen forest in seasonally dry tropical forestlands.

Ownership: The 'Treasure Tree Clubs' project was part of a 1,500 ha forest restoration program run as a partnership between Plant a Tree Today (PATT), the International Union for the Conservation of Nature (IUCN) and Chiang Mai University's Forest Restoration Research Unit (FORRU-CMU), working to assist Thailand's Supreme Command Office (SCO).

Management and community use: A mixture of carefully chosen cash crops and indigenous framework tree species was planted with the duel aims of alleviating poverty through sustainable agro-forestry and restoring a degraded watershed.

Level of degradation: Cleared of all but fragments of forest for agriculture.

Collecting sufficient seeds of enough tree species to restore diverse tropical forests is one of the most difficult challenges facing project managers, but it also provides an opportunity to engage entire communities in forest restoration right from the start. If many hands make light work, then ... "many eyes spot more seeds"!

At Doi Mae Salong in northern Thailand, Chiang Mai University's Forest Restoration Research Unit (FORRU-CMU) and the IUCN engaged eight village schools to start their own native tree nurseries. As part of IUCN's 'Landscapes and Livelihoods Strategy'[1] initiatives (sponsored by PATT[2]), the 'Treasure Tree Clubs' provided training to both school teachers and their pupils, increased awareness of the value of native forest trees, and provided incentives for children to collect seeds for the nurseries.

Labelling a 'treasure tree': their seeds are the treasure and children were rewarded for collecting them.

In return for collecting seeds, 'Treasure Tree Club' members accumulated stickers on their membership cards, which they could redeem for rewards.

[1] www.forestlandscaperestoration.org/media/uploads/File/doi_mae_salong/watershed_forest_article_6.pdf
[2] www.pattfoundation.org/what-we-do/reforestation/complete-project-list/doi-mae-salong.php

Nursery activities became part of the school curriculum, providing students with plant-growing skills that could be applied to both horticulture and forestry.

First, surviving forest trees within walking distance of the schools were identified and marked with treasure tree symbols (a diamond to imply high value with a tree seedling growing out from it) along with the local name of the tree species and the known fruiting months.

Children were issued with 'Treasure Tree Club' member cards. Any member who brought a bag of seeds from any labelled tree to the teachers in charge of the school tree nurseries received a sticker for their card. Stickers could also be gained for joining in simple tasks in the nursery, such as potting seedlings.

Tree nursery activities were included in the weekly agriculture classes and the children also applied their newly acquired arboricultural skills to growing fruit trees. For every five stickers gained, the member received a reward.

Top-performing schools won trophies and all schools were awarded large packs of environmental education materials.

The nurseries were used to grow framework tree species for a 1,500 ha forest restoration program in the area. Saplings were sold to the program and the income used to buy materials and equipment for the schools. Thus, both individual children and the community as a whole benefited from the project. Furthermore, an element of friendly competition among the schools was introduced. Schools were judged on the basis of the species and number of tree saplings produced, as well their quality. The school children were quizzed on the tree nursery procedures they had learnt and to show that they could recognise local framework tree species. The judging process also served as the project's formal monitoring procedure. The results of the competition were revealed at a 'gala' project event, at which the top performing schools received trophies. Over one year, the project generated a total of nearly 10,000 trees of 24 species for the Supreme Command's forest restoration program, earning the schools a total of US$ 918 from tree sales.