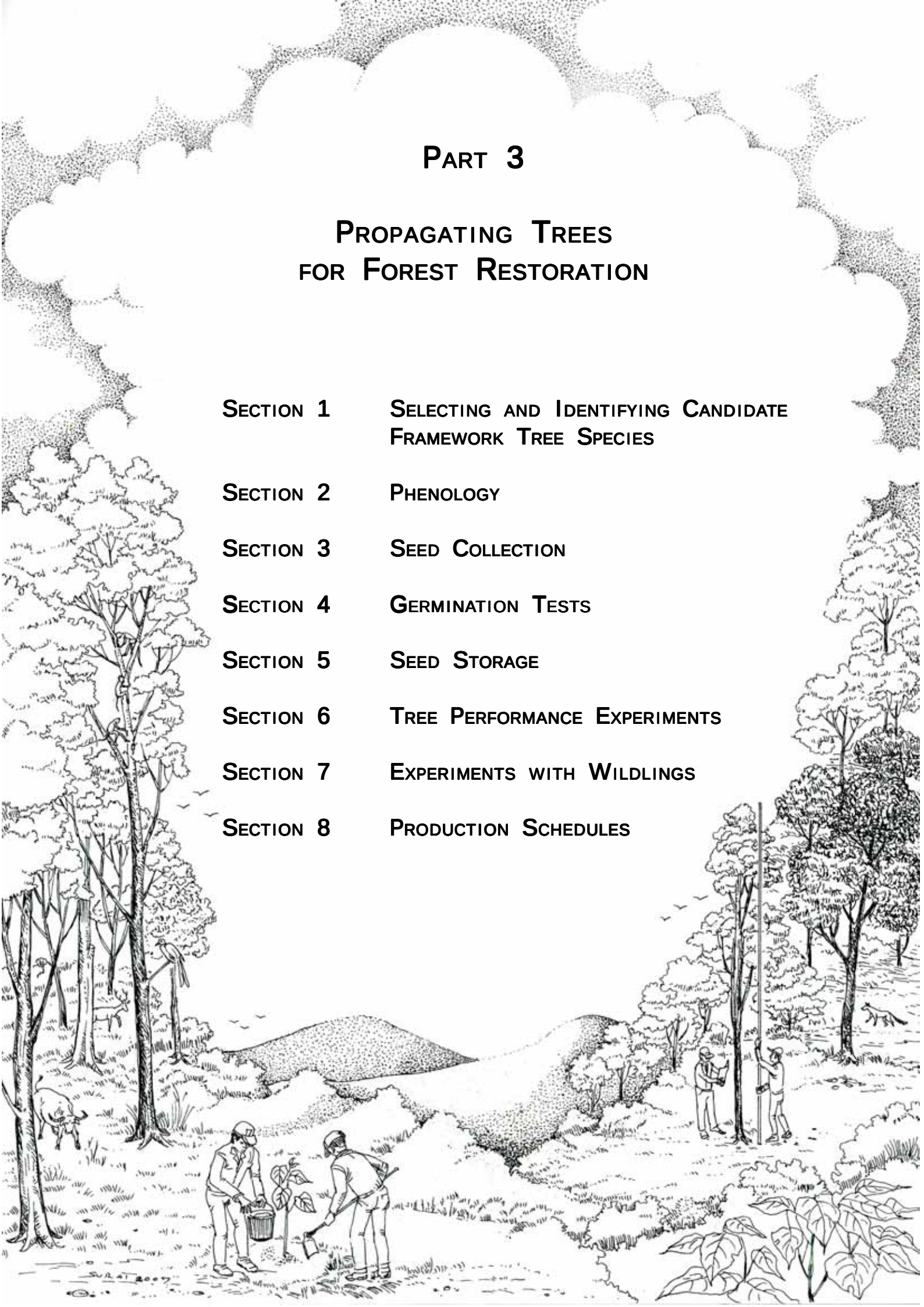
Part 3

Propagating Trees for Forest Restoration



Voucher specimens (above) of all phenology and seed trees should be kept at the FORRU with selected duplicates sent to larger herbaria (Section 1).

Seeds come in all shapes and sizes (above). Understanding seed structure can help determine which treatments may be appropriate to break dormancy.

Right: Germination experiments (Section 4) are carried out in modular plastic trays at FORRU-CMU.

Below left: Seed storage experiments (Section 5) at a FORRU in Tengchong District, Yunnan, China.

Below: Measuring the performance of trees growing in the nursery (Section 6) helps to formulate species production schedules (Section 8).

PROPAGATING TREES FOR FOREST RESTORATION

The main aim of research in a FORRU tree nursery is to discover how to produce saplings of framework tree species, which are vigorous, disease free and large enough for planting (30-60 cm tall) by the optimum planting time (i.e. the beginning for the rainy season). Research should aim to develop techniques to grow high quality saplings as rapidly and cost effectively as possible, using minimum time, labour and materials in the nursery. This can be achieved by conducting controlled experiments to test treatments that either accelerate or slow down seed germination and/or seedling growth. Alternatively, when constraints to growing trees from seed cannot be overcome, experiments can be devised to test the feasibility of using wildlings as planting stock (Section 7). However, before nursery research can begin, decisions must be made on which tree species to study and how to obtain seeds (or wildlings).

SECTION 1 - SELECTING AND IDENTIFYING CANDIDATE FRAMEWORK TREE SPECIES

Tropical forest ecosystems are renowned for the very high species richness of their tree communities. Most forests are home to several hundred tree species. Not all of them can be studied at once and not all of them are suitable for forest restoration projects. So, when starting a FORRU, it makes sense to prioritize species according to their likely potential to act as framework tree species (according to the characteristics listed in Part 1 Section 2) and then to concentrate research activities on such species, at least initially.

How are candidate framework species selected?

A candidate framework species should be non-domesticated (unless it is also a member of the indigenous flora); formerly present in the target forest type* and suited to the elevation of the planting site. This information can usually be found in floras.

Data on tree growth rates are more difficult to find, but, for South-east Asia, try the PROSEA (Plant Resources of South-east Asia) handbooks on timber trees (Soerianegara *et al*., 1994; Lemmens *et al*., 1995 and Sosef *et al*., 1998). Monitoring of early seedling growth in a nursery, can also indicate potential field performance. In most cases, species that perform well in nurseries are worth testing in the field.

Likely attractiveness to wildlife can be determined by looking for tree species with edible, fleshy fruits; those with nectar-rich flowers or those favoured by bats and birds as perching, roosting or nesting sites. Although some of this information can be derived from flower and fruit descriptions in floras, it is important to complement it by observing animal activity in forest trees during phenology studies (see Section 3). Such field studies also provide

*"Target forest type" = original forest type on the site before deforestation occurred. Usually this means the climax forest type, but it may mean a form of secondary forest, where such forest is of high conservation value.

an opportunity to observe tree crown structure and consequently to judge how effectively each tree species might shade out weeds.

Studies of the botanical knowledge of local people (ethno-botany) can provide insight into the potential of some trees to act as framework species. When carrying out such studies, it is important to work with communities that have a long history of living close to forest and deforested areas, especially those that practice swidden (slash and burn) agriculture. Farmers from such communities usually know which tree species colonize fallow fields and grow fast. However, the results of such studies must be critically scrutinized. Local people sometimes provide information, which they think will please the researcher, rather than that based on actual experience. Superstition and cultural beliefs can also distort objective assessment of a tree species' properties. Consequently, ethno-botanical information is reliable only if it is provided independently, by members of several different communities, with different cultural backgrounds. To design effective ethno-botanical surveys, please refer to Martin (1995).

Table 3.1 - Summary of information sources to determine which species may be suitable for testing as "candidate" framework species.

Framework characteristic	Literature	Nursery research	Field observations	Ethnobotany
Indigenous and suited to local conditions	Species distribution information in floras and other botanical literature		Survey tree species in nearest patch of intact forest.	Unreliable: villagers often fail to distinguish between native and exotic species
High survival and growth rates in harsh deforested landscapes	FORRU-CMU literature; PROSEA handbooks	Assess survival and growth of seedlings growing in nurseries.	Little opportunity before trials, but assess survival and growth of any trees establishing naturally in fallow fields.	Ask local people which tree species survive well and grow rapidly in fallow fields
Dense broad crown shades out weeds.	Few texts cover tree crown structure, particularly of young saplings		Observe crown structure of trees in the forest (and fallow fields) and weed cover beneath them.	
Attractive to wildlife	Fleshy fruits or nectar-rich flowers of some species in taxonomic descriptions		Observe fruit type and animals eating fruits or flowers in forest.	Villagers often know which tree species attract mammals and birds
Resilience after fire	No information for most species		Survey tree survival in areas accidentally burnt	Villagers often know which tree species recover after burning
Easy to propagate	No information for most species	Germination experiments and sapling monitoring	Density of wildlings may provide some indication of seed viability from some parent trees	

Recognizing and identifying trees

It is essential that all tree species included in the research program are scientifically named. Local names may be noted, but they cannot be relied upon. Local people often make mistakes when naming trees. They group similar species under a single name or use different names to refer to the same tree species.

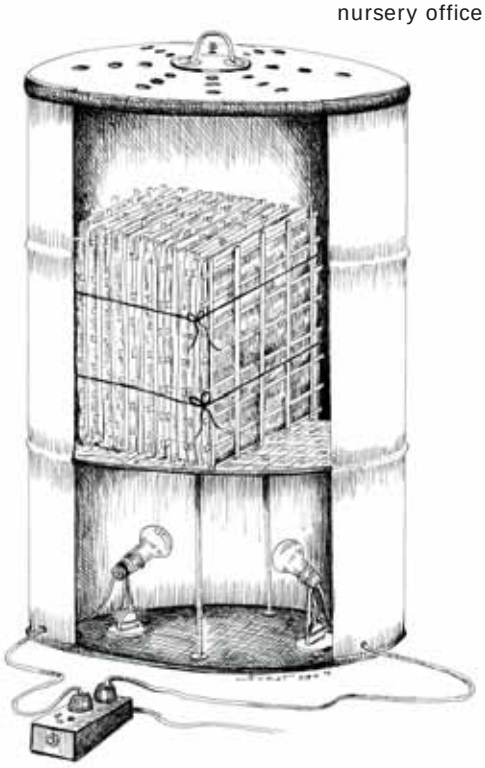
At the start of a research program, the scientific names of all species will not be known, so it is useful to assign a species number to every tree species studied. Local staff may find species numbers easier to remember than scientific names and, with a little experience, the numbers will be used more consistently than local names.

For each species studied, collect specimens of all trees from which seeds are collected, and all those selected for phenology studies. These specimens are called “vouchers”. Their purpose is to enable the scientific name of every tree included in the study to be verified. If there are any subsequent doubts about the identification of seedlings grown in a nursery or of trees planted in field trials, the voucher specimen of the seed tree can be re-examined for confirmation. Species name changes are frequent in botanical taxonomy, so having a voucher specimen, with a species number attached, can reduce confusion.

Use a cutter mounted on a pole to obtain a sample of foliage and 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. At the research nursery, construct a simple drying box with light bulbs to provide gentle heat, to dry the specimens. Write a label for every specimen, which includes species number, seed batch number (if relevant) and local name, as well as details of the tree’s location and descriptions of the bark and any features that may change with drying, particularly colours. An example of a voucher specimen label is illustrated in Section 3.

Mount specimens on robust paper, using standard herbarium techniques. If there is space and appropriate staff and facilities within the FORRU host institute, start your own herbarium. Store mounted specimens in suitable cabinets and enter information from the specimen labels into a database. Take precautions against insects or fungi attacking the specimens. For additional security, make several herbarium sheets of each specimen and lodge duplicates in other recognized 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, U.K. (www.kewbooks.com).



An oil drum and a couple of light bulbs makes a suitable “oven” for drying plant specimens in the FORRU nursery office.

Essential Equipment for Phenology Studies and Seed Collection

You never know when you might come across a fruiting tree or a new tree species to add to the phenology circuit. So, pack a backpack with the equipment shown here and carry it with you whenever you walk in the forest. Label trees with numbered metal tags. Measure their girth at breast height (GBH) with the tape measure and record their location with a GPS and the elevation with an altimeter. Use the secateurs to trim voucher specimens and place them in a plant press. Always carry plenty of spare data record sheets and herbarium labels. A first aid kit is useful to alleviate minor cuts and insect bites.

SECTION 2 – PHENOLOGY

What is phenology?

Phenology is the study of the responses of living organisms to seasonal cycles in environmental conditions. In seasonally dry tropical forests, contrasts between the hot dry season and the cooler rainy season are particularly marked. Seasonal cycles in moisture availability and temperature strongly influence both the growth and reproduction of plants, including trees.

Why study phenology of trees?

Studies of tree phenology are essential for forest restoration research programs, to determine when fruit and seeds develop, ripen and are dispersed. They can be used to determine the effort required for seed collection throughout the year and optimal seed collection times for individual tree species. They 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.

During phenology studies, observations of pollination and seed dispersal mechanisms can also be made. Furthermore, phenological studies enable the identification of "keystone" tree species; those which flower or 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; animals upon which other tree species rely for their reproduction. Keystone species are obvious candidates for testing as framework tree species. Additional data on the leafing phenology of the trees is usually collected at the same time. This can help to predict optimal planting sites for individual tree species. In short, phenology studies are a great way to learn how forest ecosystems function and how to reproduce such function in forest restoration projects.

Where should forest phenology studies be located?

Use existing trails for phenology studies (circular ones are ideal) in an undisturbed area of the target forest type; as close as possible to where forest restoration is planned. Elevation, topography and aspect of the phenology trail should also be as similar as possible to those of proposed planting sites.

How should phenology studies be established?

If you are starting a forest restoration research programme in a new area, you will not know which tree species will eventually be tested as candidate framework tree species and you may not be able to identify the trees. Select at least 5 individuals of each distinguishable tree species and assign a species number to each species. Collect voucher specimens as described previously, from each tree and have them identified by a botanist.

To each tree, attach a metal label with a unique identification number for each individual. Prepare labels with running numbers

PHENOLOGY DATA RECORDING SHEET

Order in which the trees are encountered along the phenology trail

Scientific name

Flower/fruit scores:-

FB = flower buds

FL = open flowers

FT = fruits

The sum of these scores should never exceed 4 but can be <4

Date of observation

Notes of the location of each tree. R12 = 12 m right of the trail. L2 = 2 m left of the trail and so on.

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	2.1	04/01/		SCHIMA CHII	24				1				R 20
				FICUS									R 5

Species number

Identification number on label attached to each individual tree

Girth at breast height (cm)

Leaf scores:-

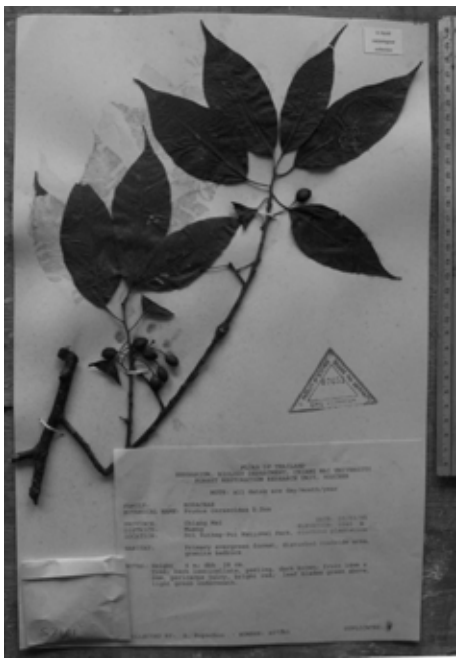
BA = bare branches

YL = young leaves

ML = mature leaves

SL = senescent leaves

The sum of these scores should always equal 4



Make sure all tree specimens along the phenology trail are represented by properly labeled voucher specimens in the herabrium.

stamped into them and nail each label at breast height onto the trunk of each tree included in the phenology study. Use rust resistant nails, at least 5 cm long, and hammer them only half way into the tree trunks to allow room for tree growth. Nails and labels must survive for several years. Make sure there is a vantage point for each selected tree from where you can scan the whole of the tree crown. Measure the girth at breast height and write a brief note, describing where the tree is located in relation to the trail (e.g. “10 m to the left”; “right 20 m by rocky overhang” etc.). 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?

At least once per month. Even with monthly observations, some tree flowering events may be missed, since some trees produce and drop their flowers within a month. Usually, such rapid turnover flowering events can be inferred, when trees are subsequently observed in fruit. In such cases, the data set can be adjusted during processing to add a flowering event. If many flowering events are being missed, start collecting data twice per month.

A semi-quantitative scoring system for monitoring tree phenology

For recording tree phenology we recommend the “crown density” method, originally devised by Koelmeyer (1959) and much modified by various authors since. This semi-quantitative method uses a linear scale of 0-4 with 4 representing the maximum intensity of reproductive structures (flower buds (FB), open flowers (FL) and fruits (FR)) in the crown of a single tree. Values of 3, 2 and 1 represent approximately three quarters, half and one quarter of the maximum intensity respectively. The “maximum intensity” of flowering/ fruiting events 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 between 0 to 4 for i) bare branches, ii) young leaves, iii) mature leaves and iv) senescent leaves (these are termed “phytophases”). 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/fruiting is occurring at the maximum intensity, typical of that species.

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 rapid and it allows quantitative analytical techniques to be applied to the data. However, at the beginning of a study, it is important, to train all data collectors to be consistent in their scoring, to minimize the subjectivity of the technique.

SORTED PHENOLOGY DATA

Sort phenology data by species (column 5), tree identification label (column 2) and date (column 3). This results in a chronological phenology history for each tree of each species running from top to bottom.

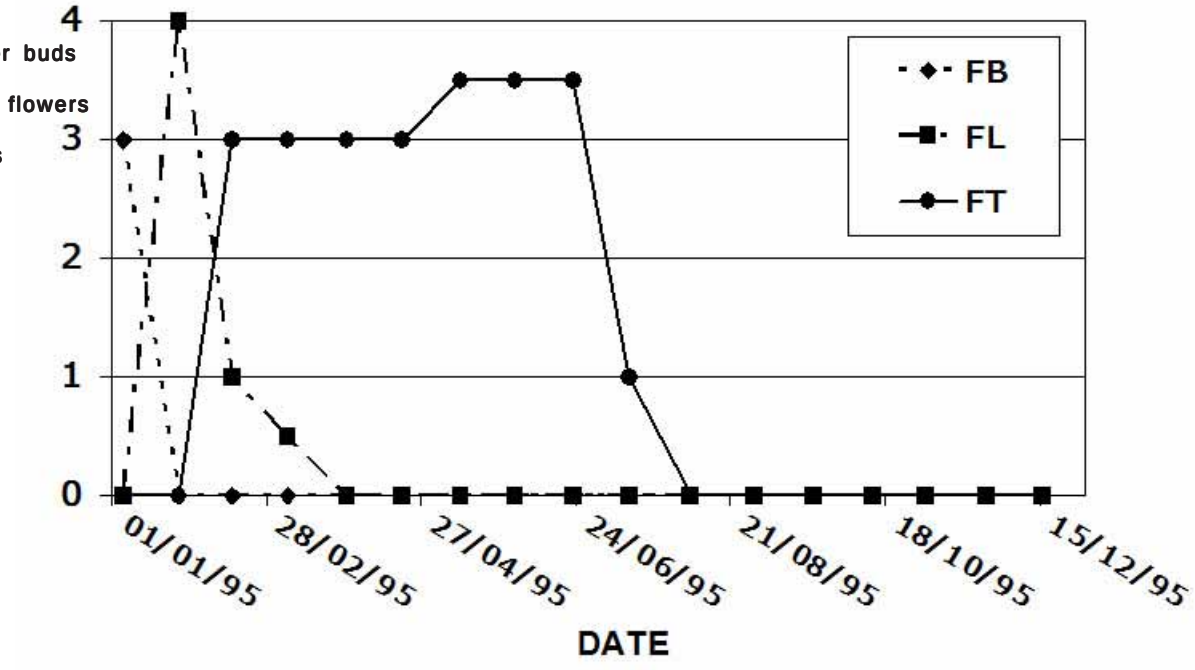
ORDER	LABEL	DATE	S. No.	SPECIES	GBH	FB	FL	FT	BA	YL	ML	SL	LOCATION
272	296	05/01/95	34	ACROCARPUS FRAXINIF	222	3	0	0	1.5		1.5	1	L 4, OPP.297
272	296	26/01/95	34	ACROCARPUS FRAXINIF	222	0	4	0	3	1			L 4, OPP.297
272	296	15/02/95	34	ACROCARPUS FRAXINIF	222	0	1	3	1.5	2.5			L 4, OPP.297
272	296	08/03/95	34	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]		20/04/95	[illegible]	ACR[illegible]RPUS FRA[illegible]	575	0		0			4		EG 10/5
		12/05		AC[illegible]	575								

Then use the MS Excel graph wizard to construct a visual phenological profile as shown below. Start by making a profile for each individual tree of each species. This will give you some idea of the variability of phenological behaviour within each species population and will enable you to assess the synchrony of phenological events and calculate several of the indices defined opposite. Only after that should you calculate mean score values across all individuals for each species population and construct an "average" profile for each species.

FB = flower buds

FL = open flowers

FT = fruits



The graph above 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.

How should phenology data be presented and analyzed?

Microsoft Excel spreadsheets are ideal for storing and manipulating phenology data. Once the study trees have been selected and labeled, prepare a data sheet, as shown on page 44. 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 data into a single spreadsheet. **Do not** store each month's data on separate spreadsheets. 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 enter the newly collected data.

To analyse the data, first select the entire spreadsheet (by clicking on the grey, blank rectangle between the column headings and row numbers in the top left hand corner of the spreadsheet). Next click on "Data" in the top menu bar and select "Sort". In the dialogue box, sort first by "SPECIES", then by "LABEL" and finally by "DATE". This arranges the data in chronological order, for each individual tree of each species. The graph wizard can easily be used to create graphical phenological profiles of each tree. When analyzing flower/fruit data, the most important point to look for is the period during which fruit scores decline for each species. This indicates the optimal seed collection month, when natural seed dispersal is occurring.

After the study has continued for several years, various useful indices of seed production may 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 in a species sample.
- **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/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/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.
- **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.

For *Acrocarpus fraxinifolius,* seed dispersal occurs very rapidly after a maturation period of several months. Without a phenology study, FORRU staff could easily miss the chance to collect seeds of this species.

Date collected: (วันที่เก็บ) / / **Species no.** **Batch no.**

SEED COLLECTION DATA SHEET

(แผ่นข้อมูลการเก็บเมล็ด)

Family: (วงศ์) **Botanical name: (ชื่อวิทยาศาสตร์)**

Common name: (ชื่อสามัญ)

Location: (สถานที่) **Altitude: (ความสูงจากระดับน้ำทะเล)**

Forest type: (ประเภทป่า)

Collected from: (วิธีการเก็บ) ☐ **ground (พื้น)** ☐ **tree (บนต้น)**

Tree label no.: (หมายเลขต้นไม้) **Tree girth: (เส้นรอบวง)** **Tree height: (ความสูง)**

Collector: (ผู้เก็บ) **Date seeds sown (วันที่ปลูก** / /

Notes: (หมายเหตุ).....

.....

☐ **Voucher collected? (เก็บตัวอย่างกิ่ง ใบ และผล)**

Notes for herbarium label (บันทึกสำหรับป้ายตัวอย่างแห้ง)

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

NOTE: all dates are day/month/year

FAMILY:
BOTANICAL NAME:

PROVINCE: DATE: / /
DISTRICT: ELEVATION: m
LOCATION:

HABITAT:

NOTE: Height m; dbh cm

Bark
Fruit
Seed
Leaf

COLLECTED BY: NUMBER: DUPLICATES:

SECTION 3 – SEED COLLECTION

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.

When should seeds be collected?

Phenology studies provide ideal opportunities for seed collection at optimal times, but seeds may be collected from any trees bearing ripe fruits, even if they are not included in phenology studies. Collect fruits when they are fully ripe but just before they are dispersed or consumed by animals.

In most tropical forest ecosystems, many tree species fruit in every month of the year, so at least one seed collection trip is needed every month. In seasonally dry tropical climates, fruiting usually peaks at the end of the dry season and at the end of the rainy season, whereas fewer species fruit in the early rainy season, so fewer seed collection trips may be needed then.

How should seeds be collected?

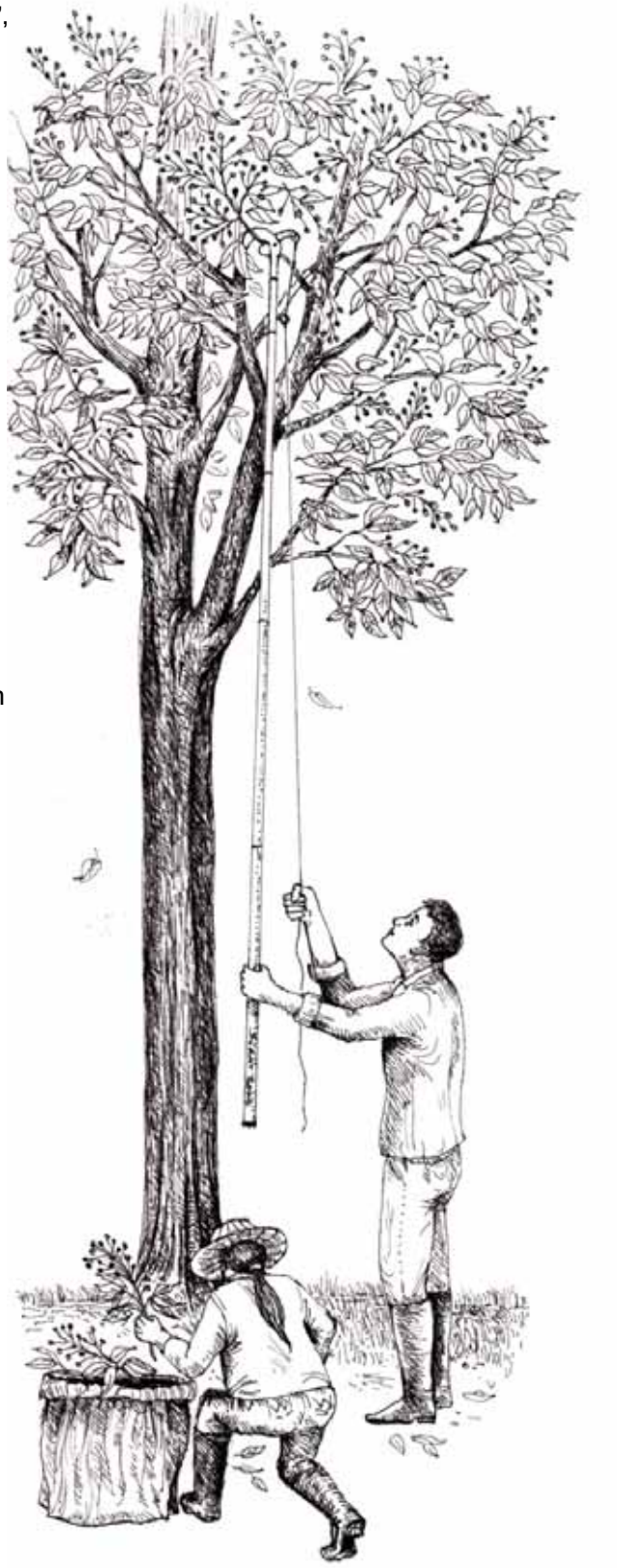
Label each seed tree with a unique number and fill in a seed collection data sheet (example opposite). If a GPS is available, record the location of each seed tree.

If possible, cut fruits from tree branches rather than picking them up from the ground. If fruits are within reach, use a cutter mounted on the end of a long pole to cut down fruit-bearing branches. To reach higher fruits, use a safety harness to climb the tree, but never do this alone.

For very tall trees, collecting fruits from the forest floor may be the only option. If so, make sure 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 any fruits or seeds with signs of fungal infection, teeth marks from animals or small holes made by seed-boring insects.

Carry collected fruits in cloth bags (not plastic bags), until they are processed in the nursery. Seed collection trips require planning and liaison with the people responsible for

Seed collection from all but the tallest trees can usually be accomplished by using a cutter mounted on a sectioned metal pole. The sections are connected by screw thread sockets.



treating and sowing the seeds, because the seeds are vulnerable to desiccation and/or fungal attack, if they are not processed quickly. Sow seeds as soon as possible after collection. Do not leave them in the sun, where they may dry out and do not leave them in damp places, where they may rot or germinate prematurely.

From how many trees?

Genetic variability is essential to enable species to survive in changeable environments. Maintaining it is one of the most important considerations in any tree planting programme for biodiversity conservation. It is therefore crucial that planted trees are not all closely related and genetic diversity is maintained. The best way to prevent this is to collect seeds from as many parent trees as is practicable (preferably 25-50), situated as close as possible to the planting site. Equal numbers of seeds from each seed tree should be mixed together (known as bulking) prior to sowing, to ensure equal representation of all the seed trees. If seeds are collected from just one, or a few trees, the planted trees derived from them may inbreed with each other in the planted plots, reducing genetic variability in subsequent generations. Cross-pollination with unrelated trees can restore genetic diversity, but only where unrelated trees grow near to planted sites.

How many seeds should be collected?

The number of seeds collected depends on the number of seedlings required, seed germination percentage and seedling survival rates. Keeping accurate records will help determine the numbers required in future collections.

Box 3.1 - Defining Dormancy

Dormancy is defined as a period during which viable seeds delay germination, despite having conditions (moisture, light, temperature etc.) that are normally favourable for the later stages of germination and seedling establishment. It prevents seeds from germinating when seedlings are unlikely to survive.

Dormancy can originate in the embryo or in the tissues that surround it (endosperm, testa or pericarp). The latter can i) restrict the transport of water or oxygen into the seed; ii) mechanically restrict embryo expansion or iii) contain chemicals that inhibit germination (most commonly abscisic acid).

Dormancy originating in the embryo can be due to i) a need for further embryonic development (after-ripening); ii) chemical inhibition of metabolism; iv) failure to mobilize food reserves or v) insufficient plant growth hormones. In most plant species, dormancy results from a combination of several such mechanisms.

Germination is defined as emergence of an embryonic root through the seed coverings. In germination trials, this can be difficult to observe for buried seeds, so emergence of the embryonic shoot (plumule) can also be used to indicate germination.

SECTION 4 - GERMINATION TESTS

Germination tests can answer two basic questions: i) how many seeds germinate (per cent germination) and ii) how quickly or slowly do they germinate (length of dormancy). Both of these parameters can be manipulated to grow tree saplings large enough by the optimal planting time (i.e. 4-6 weeks into the rainy season).

In seasonally dry tropical forests, seeds of most tree species tend to germinate at the beginning of the rainy season. Seeds produced shortly before the rainy season usually have short dormancy; whereas those produced earlier have longer dormancy. For the former, saplings are too small to plant in the first planting season. It may be necessary to delay germination (by seed storage, see Section 5) to prevent them from outgrowing their containers before the second planting season. For the latter, breaking dormancy and accelerating germination could produce a crop of saplings ready to plant in less than 1 year, whereas failure to break dormancy may mean that plants must be kept in the nursery for 18 months or longer.

How should seeds be prepared before germination tests?

Remove any fruit flesh to deter insect attack or fungal infection and air dry the seeds. Put larger seeds in a bucket of water and remove the rotten ones, which float. The objective of a germination trial is not to test the germination that would occur in nature, but to determine germination under nursery conditions for tree production.

What treatments should be tested?

To accelerate and maximize germination, treatments should aim to overcome the dormancy mechanisms described in Box 3.1. The most common dormancy mechanisms involve the seed's coverings, so treatments to perforate those coverings (scarification) are often effective, as they allow water and oxygen to diffuse into the embryo. Scraping off a soft aril, if present, nearly always increases germination. Use sand paper to roughen the entire seed surface or nail clippers to make small individual holes, in the opposite end of the seed to where the embryo is located. For large pyrenes, which are covered by a hard, stony or woody endocarp, try cracking them open gently in a vice or tapping them with a hammer. Acid can also be tested as a scarifying agent to break down impermeable seed coats. Soak seeds in concentrated sulphuric acid for a few minutes to several hours (depending on seed size and seed coat thickness). You will need to experiment with the time required. This treatment is effective with legume tree seeds. If mechanical dormancy is suspected (i.e. embryo development is restricted by a hard, but *permeable* seed coat), acid may rapidly penetrate and kill the embryo, so acid treatment is not recommended for such species. Freezing and heat treatments (particularly burning) are also not recommended for tropical tree species.

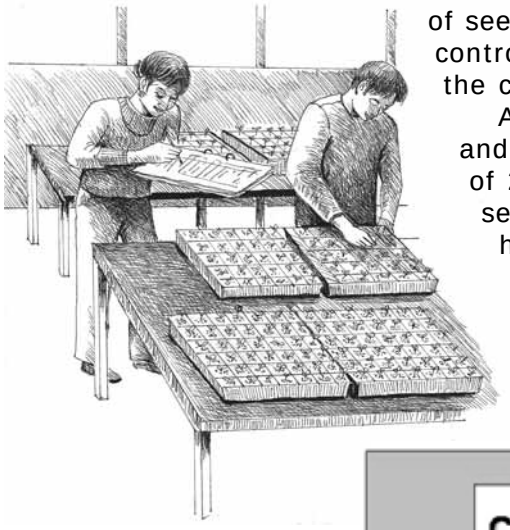
If dormancy is caused by chemical inhibitors, experiment with soaking the seeds in water for various lengths of time to dissolve out the inhibitory chemicals. Another option, worth investigating,

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.

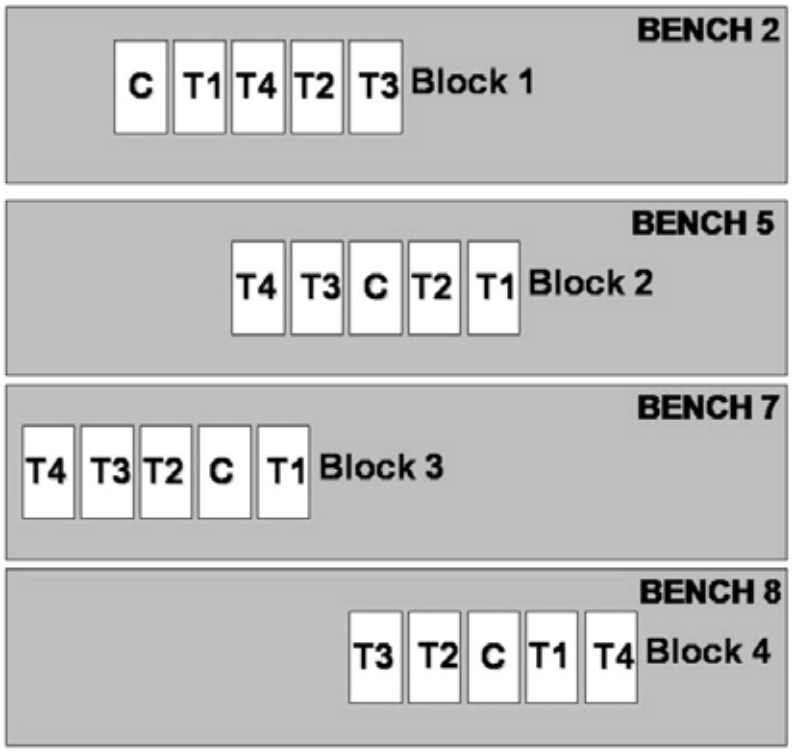
Experimental Design

To test the effects of the treatments selected, use a randomized complete block design (RCBD) as described in the Appendix, Section 1. Place a control germination tray (with seeds prepared in a standard way) and several treatment trays (each one containing seeds 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. the same number of seeds subjected to each of the treatments and in the control tray). Within each block, allocate the positions of the control and the treatment replicates randomly.



Collect germination data weekly. Mark, with a white spot, each module where a seed has germinated.

A typical design is shown below with 4 treatments and a control, replicated in 4 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 3. If you have enough seeds, then increase the number of seeds per replicate to 50-100 (which would require 1000-2000 seeds respectively).



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 is difficult to observe when each seed germinates. Protect the seed germination area with wire mesh to prevent animals from interfering with the experiments.

How often should data be collected?

Prepare a seed germination data sheet, like the one below. Inspect all seed germination trays at least once per week. During periods of very rapid germination, more frequent data collection may be necessary. For each seed that has germinated (see definition in Box 3.1), 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 the true assessment of actual germination; not counting the number of visible seedlings.

Species Number: Batch Number:

SEED GERMINATION DATA COLLECTION SHEET

Species Name: Family:
Date Seeds Collected: Date Seeds Sown: No. Seeds Sown Per Replicate

Description of standard seed preparation procedures applied to all seeds:

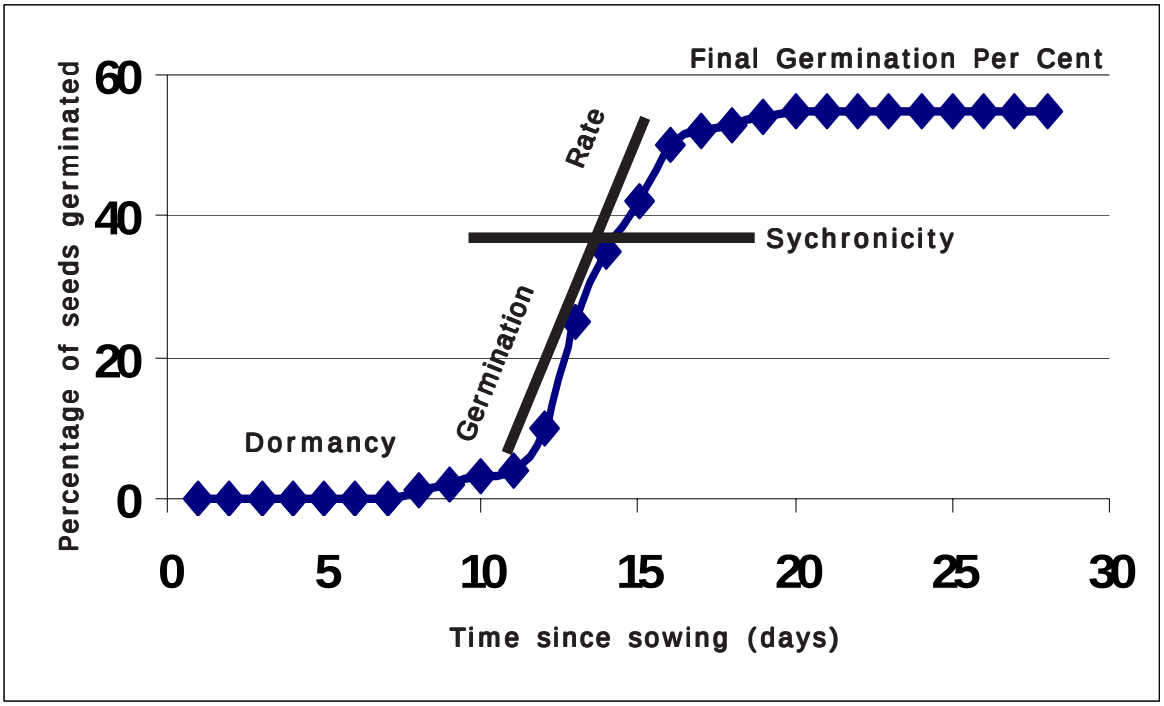
	TREATMENT DESCRIPTIONS
T1	
T2	
ETC.	

	BLOCK 1						BLOCK 2						ETC.>>>							
	Control		T1R1		T2R1		Control		T1R2		T2R2		Control		T1R3		T2R3			
DATE	G	GD	G	GD	G	GD	G	GD	G	GD	G	GD	G	GD	G	GD	G	GD	TOTAL GERMINATED	TOTAL DIED

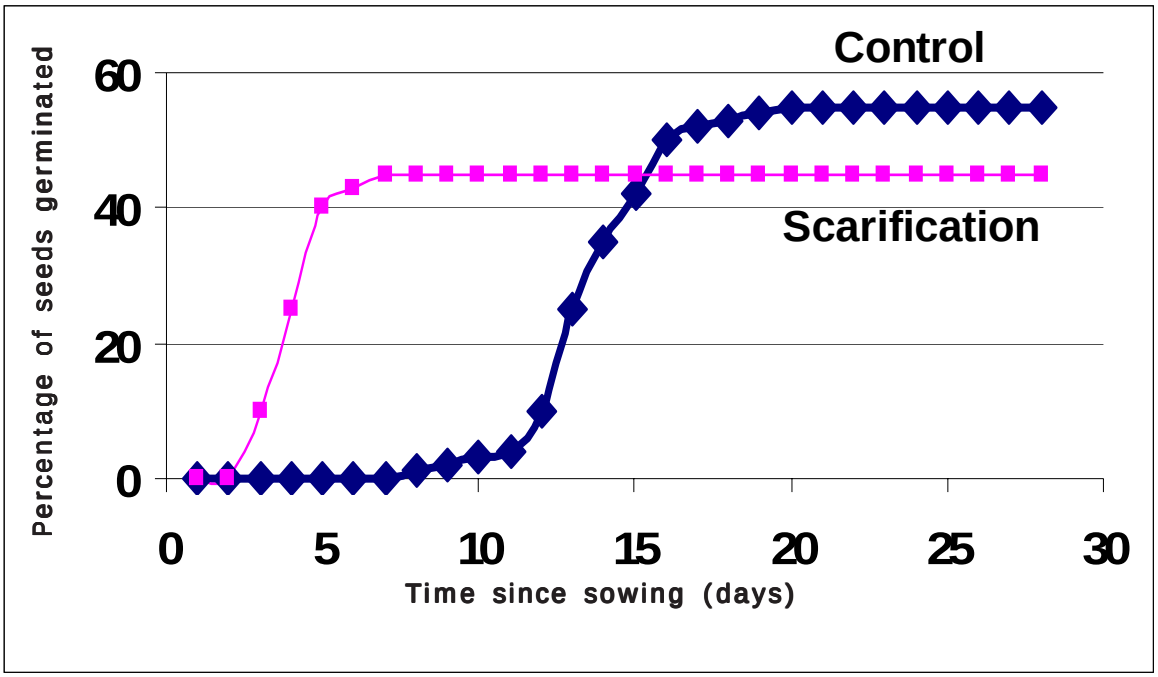
G = cumulative total number of seeds germinated since sowing until the observation date (each cell where germination has occurred is marked with a white spot, so this number is the number of white spots)
GD = cumulative number of seedlings that died before pricking out (number of modules with white spots but no visible seedling).
R = replicate; T = treatment

GERMINATION CURVES

A standard germination curve, displays all germination parameters in an easily understandable graphical format.



Decisions can be made easily even without the need for complex statistical tests. In the example below, scarification accelerates germination but reduces the number of seeds that germinate, compared with the standard germination procedure (the control). Getting the seeds to germinate faster may mean the difference between achieving a crop of saplings ready to plant by the first rainy season after seed collection or having to maintain saplings in the nursery until the second rainy season after seed collection. So even though scarification reduces germination, it may be the most beneficial treatment.



Recording early seedling mortality (death occurring after germination, but before the seedlings grow large enough for pricking out) is also a useful parameter to help to calculate 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, but containing 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 was first observed or seedling death was first observed.

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 all germination parameters into a single graphic, including length of dormancy period, rate and synchronicity of germination, as well as final percent germination.

How is dormancy measured?

Dormancy is defined as the number of days between sowing a seed and emergence of the radicle (the embryonic root) (or plumule if the radicle cannot be seen). In any batch of seeds, this period of time varies among the seeds. One way to express dormancy for a batch of seeds is to sum the length of dormancy for each individual seed and then divide the total by the number of seeds which germinate. This is “mean dormancy”. However, within any batch of seeds, there are often a few seeds that take an unusually long time to germinate. This increases the mean dormancy disproportionately and can produce misleading results. For example, if 9 seeds germinate 10 days after sowing and one seed germinates 100 days after sowing, the mean dormancy is ((9x10)+100)/10)=19 days. Even though germination was complete for 90% of the seeds by day 10, a single outlying seed, nearly doubled the recorded mean dormancy.

Median length of dormancy (MLD) overcomes this problem by defining dormancy as the length of time between sowing and germination of half the seeds which eventually germinate. In the above example, MLD would be the time between sowing and germination of the 5th seed, i.e. 10 days.

Treatment comparisons

For each treatment and for the control, calculate mean values of the number of seeds that germinate and MLD. Then use an ANOVA (Appendix, Section 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 (Appendix, Section 3).

SECTION 5 - SEED STORAGE

Although it is usually best to germinate seeds as soon as possible after collection, seed storage can be useful to streamline tree production, share seeds among nurseries and accumulate seeds for direct seeding (Part 4, Section 5). For most non-commercial forest tree species, seed storage has never been studied, so experiments are necessary to determine optimum storage conditions. The experimental treatments applied will depend on whether the seeds are orthodox or recalcitrant.

What are orthodox and recalcitrant seeds?

Seeds are classified as orthodox or recalcitrant, depending on their physiological storage potential.

Orthodox seeds are easy to store for many months or even years. They can tolerate drying to low moisture contents (2-5%) and chilling to low temperatures (usually a few degrees above freezing), without a significant reduction in viability.

Recalcitrant seeds are much more sensitive to drying and chilling. They may tolerate storage for only a few days or weeks. They have high moisture contents (usually >30%), and are very sensitive to dessication. Some have no dormancy at all and are relatively short-lived. Most cannot be dried to moisture contents lower than 60-70% and they cannot be chilled. Therefore, storing recalcitrant seeds is very difficult.

There is also a sub-group of species known as 'intermediate', which can be dried to low moisture contents approaching those of orthodox seed, but they are sensitive to chilling when dried.

So, 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 is orthodox, intermediate, or recalcitrant.

How can orthodox seeds be stored?

Store them under conditions that reduce seed metabolism and prevent the entry, or growth, of pests and pathogens. Clean the seeds and then sun-dry them for several days, to at least 5-10% moisture content, but preferably lower. To ensure the seeds are dry enough, weigh a sub-sample of the sun-dried seeds; put them in an oven at 120-150 C for an hour and then reweigh them. The value ...

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

...should be < 10%.

Throw away the sub-sample of seeds used to confirm dryness. Then, put the rest of the seeds into airtight containers. Fill the containers to the top, to minimize the volume of air (and moisture) inside. Efficient sealing of containers is crucial, to prevent entry of moisture or fungal spores. If containers are likely to be opened frequently, store seeds in small sealed packets within larger containers, to minimize exposure of remaining seeds to air and moisture. Putting a small sachet of silica gel in containers helps maintain

dryness. Storing the containers at ambient temperatures should be sufficient to maintain viability for 12 - 24 months. Longer storage periods may require low temperatures, but this can be expensive, and it is not usually necessary for most forest restoration projects.

Can recalcitrant and intermediate seed be stored?

Storage tolerance of recalcitrant and intermediate seeds varies enormously. Some species have no dormancy at all. Highly recalcitrant seeds may die when moisture content drops below 50-70%, whereas less sensitive ones may remain viable down to 12% moisture content. Chilling tolerance also varies. For storing recalcitrant seeds, keep storage duration to a minimum. When storage is unavoidable, prevent desiccation and microbial contamination and maintain an adequate air supply.

Why experiment with seed storage?

Storing seeds is useful for those tree species whose saplings grow rapidly to a plantable size well before the optimal planting time, since tending such plants for longer than is necessary wastes nursery space and resources. Furthermore, pruning becomes an added chore, when the plants start to outgrow their containers and some species may 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 optimize the nursery production schedule (Section 8). 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 (Part 4 Section 5), 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 seeds from storage and sow samples in the nursery and in the field. Compare germination between them and with the 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 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 – although, to maintain genetic diversity, it is recommended to use locally collected seeds whenever possible.

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

SECTION 6 – TREE PERFORMANCE EXPERIMENTS

Why monitor plant performance in nurseries?

Monitoring the performance of tree species in nurseries generates data to help with the selection of candidate framework trees species for field trials. It enables calculation of the time needed to grow trees, of each selected species, to a plantable size by planting-out date; one of the most important components of production schedules (Section 8). It also allows assessment of the susceptibility of each species to pests and diseases, detection of other health problems and is thus 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 simplest nursery experiments is to compare survival and growth among species. Adopt a standard production method for all species and use a RCB experimental design (Appendix - Section 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 on the selected high-performing species to develop more efficient production methods for them. These should test different techniques to manipulate growth rates in order to grow saplings to a suitable size, just 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 most simple and obvious treatments, such as different container types, media composition and fertilizer regimes and test others (pruning, inoculation with mycorrhizal fungi etc.) later if necessary.

The benefits of each treatment applied must be weighed against the costs and feasibility. So it is important to also 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 subjected to different nursery treatments and continue to monitor them, after they have been planted out in the field.

Start by testing different sized plastic bags..

Which treatments should be tested?

Container type - Growing trees in beds of soil and digging them up just before planting-out (“bare-rooted” planting stock) results in high post-planting mortality, so containerized saplings are recommended for forest restoration projects. Experiments should therefore be performed to test which container type is the most cost effective for the species being grown.

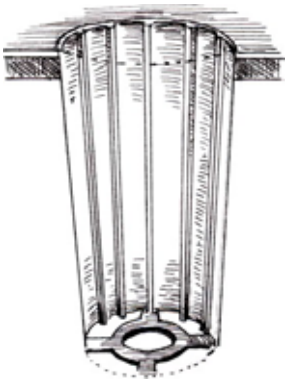
Start with a standard container type. Black plastic bags 6.5 x 22 cm are recommended, because they are cheap, widely available and usually effective. To start with, carry out simple experiments with different sizes

of plastic bags 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. Various kinds of rigid plastic cells or tubes (root trainers) are available, which exert more control over root form. They have vertical ridges inside the container, which direct root growth downwards and prevent root spiraling. They work best with "air-pruning". Root trainers are placed on raised benches of wire mesh, so that any roots that grow out from the containers gradually die as they become exposed to air. This encourages root branching inside the container and the formation of a compact root ball. Although initially more expensive than plastic bags, root trainers can be re-used many times.

Media and fertilizer regime - Start with a standard potting medium and then experiment with varying its composition. A **standard medium** consists of 50% forest soil (to provide mycorrhizal fungi and other microbes essential for tree growth), mixed with other locally available, inexpensive forms of organic matter, such as rice husk, coconut husk, peanut husk, compost etc. The latter "open out" the medium, which helps to supply water, oxygen and nutrients to plant roots and promote efficient drainage.

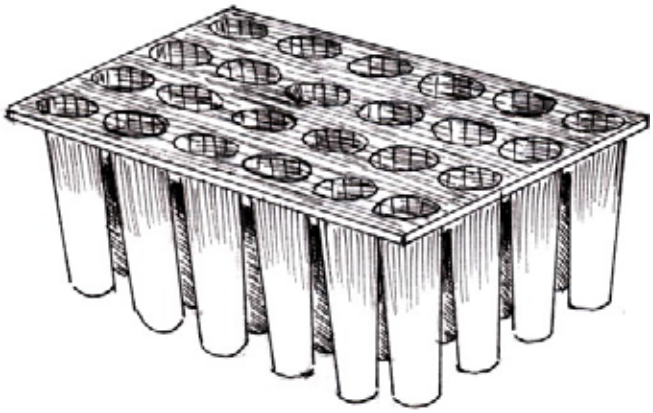
For slow-growing species, try accelerating growth by experimenting with different fertilizer treatments (fertilize type, dosage and frequency of application). Controlled release fertilizers (such as Osmocote or Nutricote), in which nutrients are held in resin pellets, are usually effective and convenient, since they are applied in small quantities every 3-6 months. Alternatively, experiment with a liquid feed, by dissolving a standard NPK fertilizer in water and applying with a watering can.



...then experiment with more elaborate containers such as rigid root-trainers with internal grooves to direct root growth downwards and prevent spiraling.

Pruning - Experiment with shoot pruning treatments if trees start to out-grow their containers before planting-out time or when it is desirable to encourage branching. Tree species vary in their responses to shoot pruning. For those with strong apical dominance, pruning may have little effect on tree form, but for those with weak apical dominance, it may result in a denser, branching crown; a desirable character of framework species. Saplings of some species can actually be killed by over-zealous pruning. Test different shoot pruning intensities, timing and frequencies. In addition to growth and mortality data, also record plant form during pruning experiments.

Saplings with 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. It is therefore important to know how long it takes to develop an efficient root system prior to planting-out. The option of using root trainers for air pruning roots was mentioned above. For trees grown in plastic bags, experiment with different root pruning



schedules. At the end of such experiments, sacrifice a few plants to record root form and root:shoot ratio.

Mycorrhizal fungi - Most tropical tree species develop symbiotic relationships with fungi, which infect their roots to form mycorrhizas. Such relationships enable young trees to out-compete weeds. The fungi form extensive networks of fine hyphae in soil, which transfer nutrients and water into the growing trees more efficiently than the tree's own root system can. Vesicular-arbuscular mycorrhizal fungi (VAM) grow inside non-woody root cells of about 95% of tropical tree species. Ectomycorrhizal fungi infect far fewer tree species. They form a sheath around fine roots and penetrate between the root cells.

If forest soil is included in the potting medium, most saplings become naturally infected with mycorrhizal fungi (Nanda-kwang et al., 2007). So first, survey saplings, growing in the nursery, to confirm the presence of mycorrhizas and assess the frequency of root infection.

For VAM: i) wash a sample of fine roots; ii) treat them with a clearing solution, 10% (w/v) KOH at 121 C for 15 min 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 ecto-mycorrhizas, estimate the percentage of fine roots with characteristic swollen ends to the root tips, then observe the roots under a microscope for the presence of fungal hyphae. Identifying species of mycorrhizal fungi is done by examining spores using a compound microscope. This requires specialist help (for general techniques to study mycorrhizas, see Brundrett et al. 1996).

If colonization of tree roots of any species by mycorrhizal fungi is low or is not occurring, then try experiments with artificial inoculation. Various commercial preparations, containing mixtures of common mycorrhizal fungi spores, are available for testing (but be aware that they may not contain the particular fungi 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 (Nandakwang et al., in press). Such home-made inoculae may be more specific for the trees being grown, but producing them is time-consuming and requires specialized techniques. Inoculation success is often reduced if plants are given fertilizer. So, try experiments that test various combinations of fertilizer treatments with application of mycorrhizal fungi inoculum.

First, determine if artificial inoculation can increase infection rates (and ultimately tree performance), compared with the natural infection rates achieved 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 containers off the ground on a wire grid and separate treatment replicates with plastic shielding to prevent splashing.

How should sapling performance experiments be designed?

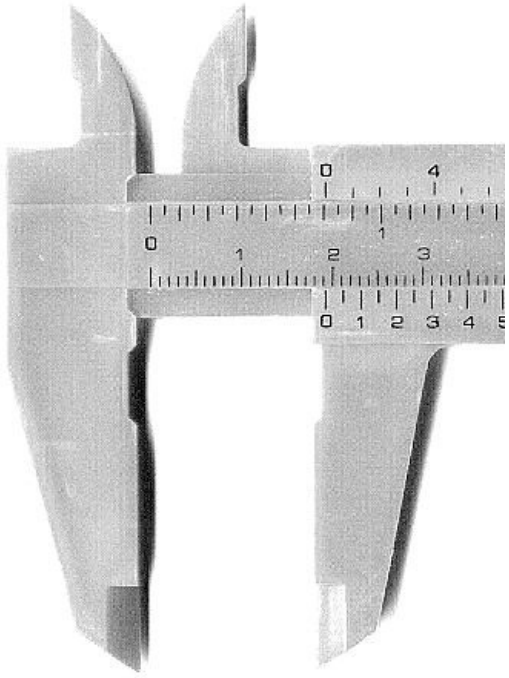
As with germination experiments, use a randomized complete block design (RCBD, Appendix Section 1) and analyze the results using a two-way ANOVA, followed by paired comparisons (Appendix Sections 2 and 3). The example experimental design for germination trials, illustrated on page 52, can be used equally 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 a 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 within the standing down area of the nursery. Position treatment and control replicates randomly within each block.

Select only 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. Treatments may "spill over" from one replicate to another, such as watering or fertilizer application. 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 included in the experiment.

A simple experiment testing 4 treatments + control in 4 blocks (rdf=12) would require a minimum of (15x5) 75 uniform, healthy plants, per block or 300 totally, plus extra plants to make the guard rows.



MEASURING ROOT COLLAR DIAMETER (RCD)

Measure RCD with callipers with a Vernier scale, available in most stationary stores. Measure RCD at the widest point. 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. For example the Vernier scale left reads 19.3 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.

EXAMPLE SAPLING PERFORMANCE DATA SHEET

Record sapling performace data as shown below. Make a separate sheet for each replicate in each block.

Species: Prunus cerasoides **S.no.: S71B1**

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

HEIGHT DATA (CM)

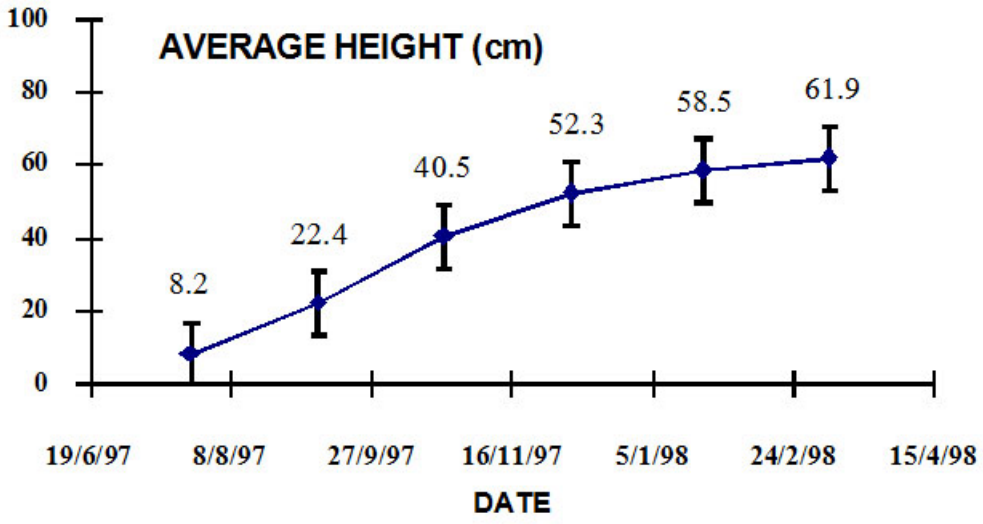
		SEEDLING NUMBER															
DATE	**DAYS**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**AVG**
7/6/97	1	5.0	4.0	3.5	2.0	4.0	3.0	4.0	3.0	3.5	3.0	5.0	4.0	3.0	4.0	4.5	**3.7**
25/7/97	49	11.0	12.0	8.0	3.0	8.0	5.5	7.5	5.5	6.5	8.5	12.0	9.0	8.5	9.0	9.5	**8.2**
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**



What data should be collected and how often?

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 to apical meristem) with a ruler and root collar diameter (RCD) with Vernier calipers (see page 61).

Use a simple scoring system to record plant survival and health (0=dead, 1=severe damage or disease; 2=some damage/disease but otherwise healthy; 3=perfect or almost perfect 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 may 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 root system structure. 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 sampled.

How should data be recorded?

Use a standard data collection sheet (shown opposite) for each replicate in each block. Calculate mean values (and standard deviation) for each of the parameters measured and relative growth rates (see below) after each data collection session.

How can survival data be analysed?

For each replicate, count the number of saplings that survive until planting out time. Then calculate the mean values for each treatment and standard deviation; repeat for the control. Apply ANOVA (Appendix Section 2) to determine if there are significant differences among treatments in mean survival. If so, then use paired comparisons (Appendix Section 3) between each treatment mean and the control mean, to identify which treatments significantly increase survival.

How should growth data be analysed?

Represent sapling growth graphically by constructing a growth curve, which can be updated after each data collection session (see opposite). Plot time elapsed since pricking out (horizontal axis) vs. mean sapling height (or mean RCD), averaged across blocks, for each treatment (vertical axis). Ideally, the curve should show rapid early growth, followed by slower growth prior to planting out. By extrapolation, such curves can be used to roughly estimate how long saplings must be kept in the nursery to grow to the optimum planting size.

Just before optimum planting-out time, calculate 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 (Appendix, Section 2) to determine if there are significant differences among treatment means and if so, use paired comparisons (Appendix Section 3) to determine which treatments result in significantly larger saplings at planting time, compared with the control.

Calculation of relative growth rate removes the effects of differences in the original sizes of seedlings/saplings, immediately after potting, on subsequent growth, i.e. it can be used to compare plants that were larger at the beginning of the experiment with those that were smaller. It is defined as the ratio of 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.

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 (i.e. 4-6 weeks into the rainy season):-

- >80% survival of sapling since pricking out.
- Mean sapling heights >30 cm for fast-growing pioneer species and >50 cm for slow-growing climax tree species.
- Sturdy stems, supporting mature, sun-adapted, leaves (not pale, expanding leaves) (“sturdiness quotient”, height (cm)/RCD (mm) <10).
- Root:shoot ratio of between 1:1 and 1:2; with actively growing, vertical tap root (no root spiraling) and dense mass of branching fine roots.
- No signs of pests, diseases or nutrient deficiency.

These saplings of *Carallia brachiata*, grown at a FORRU in Krabi Province, S. Thailand, have achieved most of the targets listed above. They were planted out to restore lowland tropical rain forest; the habitat of the endangered bird species, Gurney’s Pitta.

Tree seedling morphology and taxonomy

Surveys of natural forest regeneration require identification of tree seedlings and very young saplings, but this is notoriously difficult. Floras base plant species descriptions 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 identification of tropical forest tree seedlings are therefore almost non-existent (but see FORRU, 2000). Therefore, nurseries, producing seedlings and saplings of known ages, from seeds collected from properly identified parent trees, represent an immensely valuable resource for studying tree seedling morphology and taxonomy.

Try to collect at least 3 specimens of seedlings/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/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.

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

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

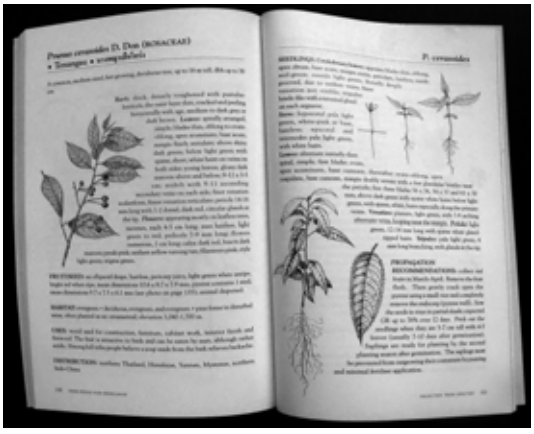
PROVINCE: Chiang Mai
DISTRICT: Muang
LOCATION: Doi Suthep-Pui National Park, National Park Headquater area, cinchona plantation
HABITAT: Primary evergreen forest, disturbed roadside area, granite bedrock.
DATE: 20/03/95
ELEVATION: 1040 m

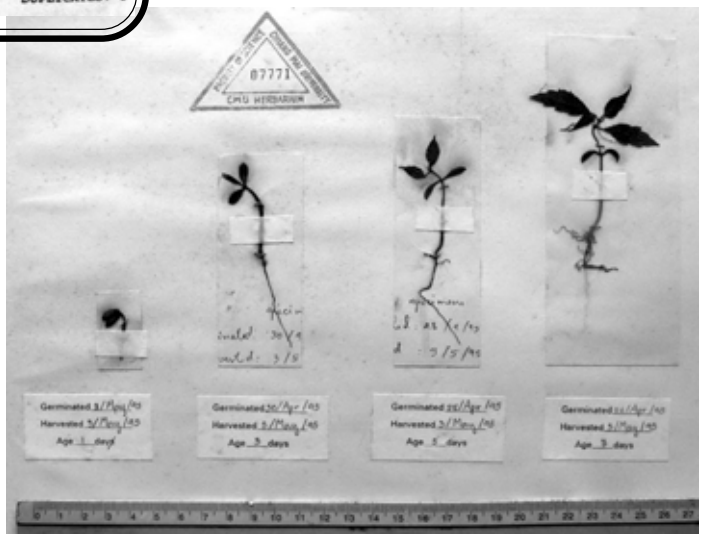
The above data refer to the source tree (voucher collected) from which these seedlings were germinated.
Seeds collected 20/03/95 and planted 22/03/95 at the Forest Restoration Research Unit, Doi Suthep-Pui National Park Headquarters, 1050 m above sea level.

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





Seedling specimens of *Prunus cerasoides*, with herbarium label top left.

SECTION 7 – EXPERIMENTS WITH WILDLINGS

What are wildlings?

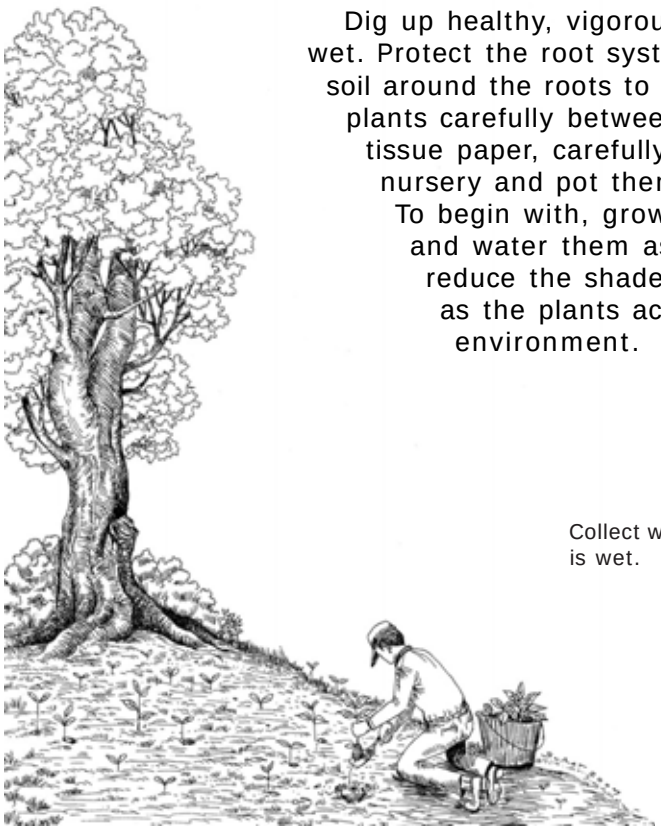
Wildlings are seedlings or saplings growing naturally in native forest. They are usually most abundant within a few metres of the seed trees, from which they originated, although they can be collected from wherever they are found. As with seed collection, capture as much genetic diversity as possible by collecting wildlings over a wide area to obtain plants originating from several parent trees. In general, very few wildlings survive to maturity in the forest, so removing some of them to grow in nurseries has a negligible impact on forest dynamics.

What are the advantages of producing planting stock from wildlings?

Producing planting stock from wildings is advantageous when i) seeds are not available; ii) seed germination and/or seedling survival and growth are problematic or slow or when iii) planting stock production must be accelerated. Producing planting stock from wildlings can usually be done more rapidly than from seed since, seed collection, storage and pre-sowing treatments and germination trials are all rendered unnecessary. Furthermore, wildlings are usually already infected with mycorrhizal fungi by the time they are collected from the forest.

How should wildlings be collected?

Dig up healthy, vigorous plants, when the soil is wet. Protect the root system by retaining plenty of soil around the roots to make a “root ball”. Pack the plants carefully between layers of wet cloth or tissue paper, carefully transport them to the nursery and pot them up as soon as they arrive. To begin with, grow them under dense shade, and water them as needed, but gradually reduce the shade and frequency of watering as the plants acclimatize to the nursery environment.



Collect wildlings when the soil is wet.

What experiments should be carried out on wildlings?

Experiments with wildings should address 3 simple questions: i) can producing planting stock from wildings be achieved 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 the treatments described in Section 6 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 wildlings collected into 3 size classes (short, medium and tall). These then become 3 “treatments” in a RCBD experiment (there is no control). Collect data, as described in Section 6, and compare mean survival and RGR among the initial size classes.

Digging up plants inevitably damages the root system, but the shoot system remains intact i.e. a reduced root system must supply water to an undiminished shoot. This can cause willdings to wilt and possibly die. Pruning back shoots can bring the root:shoot ratio back into balance. Apply shoot pruning treatments with varying intensities at collection time (e.g. no-pruning (control) and pruning back one third or one half shoot length or leaves). Collect data as described in Section 6 and compare mean survival and RGR among the pruning treatments.

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

Labeling plants in the nursery at all stages of development, not only makes nursery management easier, but it also allows the nursery to be used as an educational facilty.

FOREST RESTORATION RESEARCH UNIT

S. 146 B4

FORRU SEEDLING PRODUCTION DATA SHEET

1. COLLECTION

SPECIES Nyssa javanica (Bl.) Wang. FAMILY NYSSACEAE

LINKCODE NYSSJAVA VOUCHER NO. 89

COLLECTION DATE 11-Aug-06, ground QUANTITY 3000 SEEDS

2. SEED GERMINATION

PRETREATMENT Seed were soaked in water 1 night, after that sun dry 2 days

QUANTITY SOWN 2500 SEEDS

MEDIA/CONTAINER Forest Soil only, 8 baskets

SOWING DATE 14-Aug-06

NUMBER GERMINATED 2059 SEEDS

OBSERVATIONS

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 : Coconut Husk : Peanut Husk (2:1:1) in plastic bag

NURSERY CARE

NURSERY CARE	1	2	3	4	5
FERTLIZER	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			

OBSERVATIONS

2-3 months after pricking out, Red fungus and Leaf blight were occurred, but all seedlings look healthly

4. HARDENING AND DISPATCH

DATE HARDENING STARTED 17-May-07 DATE DISPATCHED 19-Jun-07

NUMBER OF GOOD QUALITY PLANTS 1200 SEEDLINGS

WHERE PLANTED MAE SA MAI, WWF plot

OBSERVATIONS

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

SECTION 8 - PRODUCTION SCHEDULES

Growing a wide range of forest tree species is difficult to manage. Different species fruit in different months and have different germination requirements and growth rates. Yet, all must be ready for planting by the optimal planting out time (4-6 weeks into the rainy season). Species production schedules makes this daunting managerial task easier.

What is a production schedule?

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

What information is 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 in this Part, including:-

- Optimum seed collection date (Sections 2 & 3);
- Natural length of seed dormancy (Section 4);
- How seed dormancy might be manipulated with pre-sowing treatments or seed storage (Sections 4 & 5);
- Length of time required from seed sowing to pricking out (Section 4);
- Length of standing-down time required to grow saplings To a plantable size (Section 6);
- How plant growth and standing-down time can be manipulated with fertilizer application and other treatments (Section 6).

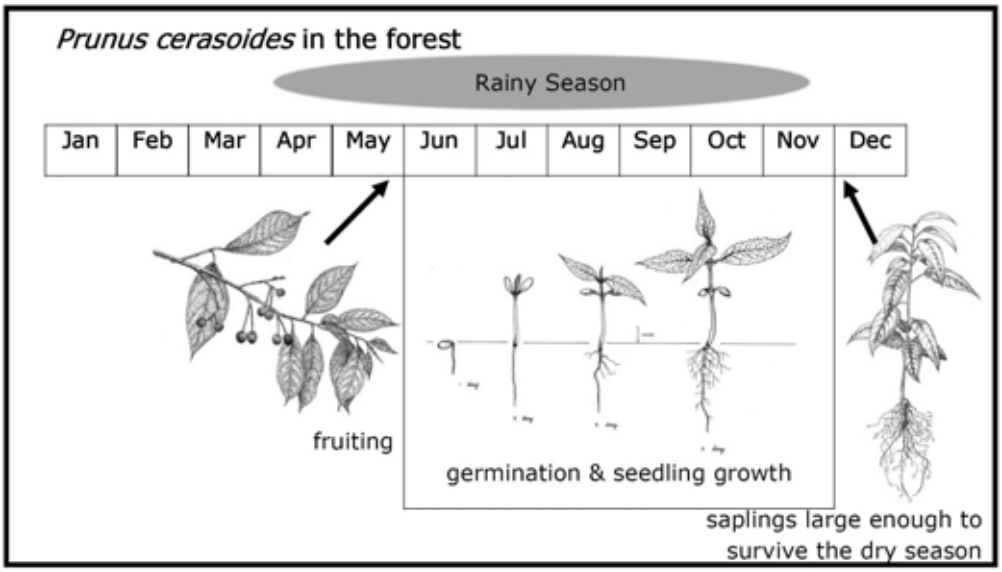
The ultimate result of a FORRU nursery research program is production of healthy trees of optimal size by the optimal planting time.

All this information should be available from the nursery data sheets, if the procedures detailed in this Part are rigorously 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 identification of areas requiring further research and appro-priate treatments to test in subsequent experi-ments. As the results of those experiments from each subsequent batch of plants, become available, the production schedule can be gradually modified and optimized.

EXAMPLE PRODUCTION SCHEDULE

In its natural habitat, the fast-growing pioneer tree, *Prunus cerasoides*, fruits in April-May. Its seeds have short dormancy and the seedlings grow rapidly during the rainy season, so that by December their roots have penetrated deep enough into the soil to supply the shoot with moisture during the harsh conditions of the dry season. In the nursery, saplings which 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 out-grow their containers.



In the nursery, the production schedule, therefore, involves storing the sun-dried pyrenes at 5 degrees centigrade until January, when they are germinated. Plants 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.