

Vegetative Tree Propagation in Agroforestry

Training guidelines and references



ICRAF

Vegetative Tree Propagation in Agroforestry

Training Guidelines and References



Edited by Hannah Jaenicke and Jan Beniest

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Contents

vii	Acknowledgements
viii	Introduction
ix	About the training course

Unit 1 Introduction to vegetative tree propagation

1	Training guidelines
3	Concepts and principles
3	Introduction
5	Reasons for vegetative propagation
6	Plant hormones and growth regulators
8	Tissue maturity
8	Tree domestication and vegetative propagation
10	References
11	Clone selection and collection
11	Introduction
11	Collection principles
13	Collection guidelines
14	An example
15	References

Unit 2 Tree nurseries

17	Training guidelines
19	Nursery management and seedling production
19	Introduction
20	Good nursery practices
29	Nursery experiments
29	Troubleshooting
30	References
31	Phytosanitation
31	Introduction
31	Phytosanitary problems

36	Phytopsanitary measures
38	Curative measures
39	References
40	Nursery management - Practical
40	Objectives
40	Prerequisites
40	Assignments

Unit 3 Cuttings

43	Training guidelines
45	Cuttings principles and techniques
45	Introduction
45	Rooting process
46	Factors affecting the rooting process
48	Preparing cuttings
49	Propagation facilities
50	Post propagation care
50	References
51	Cuttings practical
51	Objectives
51	Prerequisites
51	Assignments

Unit 4 Grafting

55	Training guidelines
57	Grafting principles and techniques
57	Introduction
57	Definitions
58	Reasons for grafting and budding
60	Physiology
65	Grafting and budding techniques
69	References
70	Grafting practical
70	Objective
70	Prerequisites
71	Assignments

Unit 5 Layering

75	Training guidelines
77	Layering principles and techniques
77	Introduction
77	Air layering or marcotting
79	Simple layering
79	Stooling or mound layering
80	References
81	Layering practical
81	Objective
81	Prerequisites
82	Assignment

Unit 6 Micropropagation

83	Training guidelines
85	Principles and techniques
85	Introduction
85	Terminology
87	Physiology
87	Reasons for micropropagation
87	Requirements
88	Techniques
92	Conclusion
92	References

Unit 7 Propagation experiments

93	Training guidelines
95	Design and management of experiments
95	Introduction
96	Design basics
97	Application to propagation experiments
102	Common problems
103	Managing trials to give good results
104	References

107	<i>Annex 1 - Course brochure</i>
109	<i>Annex 2 - Application form</i>
113	<i>Annex 3 - Course programme</i>
115	<i>Annex 4 - Evaluation</i>
129	Index



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Introduction

One of the biggest problems associated with agroforestry technologies is the multiplication on a large scale of agroforestry trees and shrubs.

The common way by which plants regenerate naturally is propagation by seed. For research and rapid improvement of undomesticated species, however, vegetative propagation methods offer several advantages. For example, in wild populations, a large variation in important product characteristics (e.g. fruit quality, bole straightness, biomass) may be expressed. Furthermore, individuals may be recognized within a population that produce a higher quality of the desired product(s) or services. It would therefore be advantageous to propagate these individuals vegetatively to 'capture' the genetic variation expressed, which may otherwise get lost or diluted during sexual propagation.

Vegetative propagation methods have been developed and used for centuries. Especially in temperate regions, vegetative propagation has been an important approach in the domestication of fruit species and particular methods have been developed for different species. Tropical fruit species have been subjected to vegetative propagation in a number of cases that have found a lucrative export market, e.g. citrus, mango, avocado, and macadamia nut. Tropical timber species have also been cloned, mainly for plantations where uniform trees are needed.

Many indigenous trees with a potential high monetary or nutritional value are so far only used from natural stands. By integrating these high value trees into agroforestry systems, smallholder farmers in the tropics could greatly benefit. Vegetative propagation is seen as a possibility to select superior germplasm and bring this important resource into the farmers' fields.

As part of its programme on 'Domestication of Agroforestry Trees', ICRAF has a project on 'Propagation Systems for Agroforestry Trees', which aims to develop jointly, with users, options for appropriate propagation and management practices for agroforestry trees, to enhance the efficiency, level and stability of tree production. Its outputs are globally applicable or adaptable tree propagation and nursery management guidelines. It is in the context of this project that the Centre organized a short training course on vegetative propagation of agroforestry trees in collaboration with the International Programme for Arid Land Crops, which also conducts research and development activities in this area.

The first version of the course has been organized for the benefit of participants from Eastern and Southern Africa, but it is likely that in the future it will also be organized in other agro-ecological regions where ICRAF conducts agroforestry research in collaboration with national institutions. In addition to supporting this initial training course, these training materials are expected to facilitate the planning, organization and implementation of this type of course.



About the training course

These training materials have been developed in support of a one-week practical training course on vegetative propagation of agroforestry trees. The following paragraphs briefly highlight aspects of this training activity such as: target audience, training objectives, instructional methods, programme, resource persons and training materials. This will help future course organizers and resource persons to better plan, prepare and implement this training activity. Training materials presented in this document can be adapted to suit the need of individual courses. A course brochure with the necessary background information on the activity, accompanied by an invitation-to-attend letter, can be developed for the benefit of potential course candidates. An example is attached in annex 1.

Target audience

The target audience for this type of training course consists of field technicians and nursery managers active in tree propagation research or development. They are expected to have a minimum degree or diploma in any of the plant sciences (forestry, horticulture, plant biology, agricultural crops science) relevant to this type of work.

Additional selection criteria used to identify participants for the course can be considerations aimed at obtaining an equal gender representation, English language proficiency and commitment from the candidate's employing institution both in terms of participation and application of acquired knowledge and skills in future work. A sample application form has been attached in annex 2.

ix

Training objectives

The overall objective of the course is to improve the practical vegetative plant propagation skills of field technicians or nursery managers and to provide them with the necessary theoretical knowledge on the subject that should allow them to apply these skills in their future work.

In order to achieve this, the course is structured into specific units and each of these has its own learning objectives that contribute to the overall objective. Specific learning objectives are listed under the unit summaries at the beginning of each unit.

Programme

The training course covers a total of 7 units, most of them divided into one or several short theoretical presentations, demonstrations and practical work.

Unit 1 – Introduction

This unit briefly reminds course participants about the role of vegetative propagation in forestry and agriculture with an emphasis on its importance in agroforestry tree domestication research and development. The most common vegetative propagation techniques are listed and described, and some background information on their underlying physiological principles, including the role of phytohormones, is given. The unit also covers the important considerations that need to be made when selecting and collecting material for propagation.

Unit 2 – Tree nurseries

Plant propagation almost always requires a nursery. The unit covers the basics of nursery infrastructure in terms of soil substrates, equipment and materials, irrigation, etc. and further highlights important concepts and principles related to good nursery management. Since phytosanitation should be an important concern of any nursery manager, the last part of this unit deals with pests and diseases in the nursery and plant propagation context.

Unit 3 – Cuttings

Multiplication through the rooting of cuttings is probably the most common technique by which trees can be propagated vegetatively. The unit covers the different phases of the rooting process and explains its physiological background. Environmental conditions play an important role in this process and the unit includes information about simple structures that can be used to improve the success rate of this propagation technique.

X

Unit 4 – Grafting

Grafting and budding are more complicated vegetative propagation techniques. The unit deals with some important reasons to consider this technique in its different applications and highlights its underlying physiological principles as well as the conditions for its success.

Unit 5 – Layering

In a number of cases, agroforestry trees can be propagated through layering techniques. The unit covers the different methods for this and explains some of the underlying principles and conditions influencing its success.

Unit 6 – Micropropagation

Micropropagation covers a wide range of methods and techniques to vegetatively propagate relatively small parts of plant material in extremely controlled environments. Even though this is not a common vegetative propagation technique, course participants should be familiar with the overall concepts and principles, as to understand why this method can be

considered in the broader context of plant propagation and tree domestication.

Unit 7 – Propagation experiments

Since the whole subject of agroforestry tree propagation is partly seen in the context of ongoing research in this area, the last unit of the course covers both general and specific experimental design concepts and principles as applied to tree propagation experiments. As an example, the unit also covers a case study of tree propagation research.

The seven units are covered within a period of one week. Normally, the programme designed for this course should follow the chronological order of these units but for practical reasons they can be scheduled differently. A sample training course programme and timetable are presented as an annex to these training materials.

Instructional methods

This is a practical training course and thus *exercises and demonstrations* constitute the most important instructional methods used to teach the course. These practicals and demonstrations need to be well prepared in advance. Guidelines to do this are included in each unit. Several practical exercises will demand some follow-up later in the course and thus it will be useful to dedicate an entire morning or afternoon each day to exercises and demonstrations. Local weather conditions will often determine the best time to organize these.

x i



Various instructional methods can be used to teach the course: learning by doing, demonstration, field visit, classroom presentation.

Short *theoretical presentations* aim at reviewing basic concepts and principles and providing the necessary background information needed to understand the practicals and demonstrations. They should take less than 40% of the time and focus on what is needed to better understand the practical application of the various multiplication techniques.

Time permitting, *field visits* to nursery sites or micropropagation laboratory facilities can be included to illustrate a series of aspects related to vegetative propagation research and development, taught or practiced during the course.

Suggestions on instructional methods for each unit are included in the unit summaries.

Resource persons

As far as the technical content and delivery of the course is concerned, resource persons teaching this course are scientists or staff from training and education institutions actively involved in tree propagation work. Experienced senior technicians and nursery managers may also contribute. Nursery technicians or labourers will help out with the practical work and the demonstrations.

xii

Training staff will assist in course curriculum development, training materials preparation and general course organization, implementation, monitoring and evaluation.

Since the course targets practicing participants, they are also considered resource persons in the context of one or another topic taught during the course.

Training materials

This training manual has been developed for the following reasons:

- To support the various activities and presentations of the course.
- To provide *reference material* for participants when they are propagating agroforestry trees.
- To provide *guidelines and resource material* for participants or resource persons who wish to organize a similar training course in the future.

For each of the seven units of the training course, a 'fast track' summary is presented. This consists of *instructional objective(s)*, recommended *instructional methods*, *instructional materials and recommended reading* and a *summary* of the theoretical presentation(s).

Instructional objective(s):

Instructional objectives indicate what course participants are expected to have gained in knowledge or skills upon completion of each unit. They guide resource persons in the preparation and implementation of the unit and inform participants of what is expected from them during or after the unit.

Instructional methods:

The *instructional methods* suggest how the unit can best be delivered using different methods and activities.

Most often, a unit will be introduced through a short *theoretical presentation* supported by a *lecture note* or *handout* developed by the resource person presenting it.

Since the focus of this training course is on active participation, an important part of the time is reserved for *practical work* and *demonstrations*. Clear *guidelines* on the implementation and outcome of these activities must be formulated for the benefit of the participants.

Field visits will require the development of some *background information* on the experiments that will be visited. A detailed experimental protocol and signboards containing a minimum information set (title, treatments, experimental design etc.) needed to understand the experiment will be sufficient. In order for the field visit to involve greater participation, resource persons can develop a *list of questions* on certain aspects of the subject and let participants discover the answers during the visit.

Group or *individual exercises* will also require the formulation of clear *guidelines* as to the expected outcome of the exercise.

Instructional materials and recommended reading:

These sections of the training guidelines refer to the various materials and publications that will be used during the training workshop such as *textbook chapters*, *published articles*, *lecture notes* or *handouts*.

Lecture notes or *handouts* are produced in direct support of an introductory presentation to a unit. Lecture notes are more formal and will reflect the presentation in more detail than a lecture handout, which may only consist of an outline of the presentation or copies of the overhead transparencies used by a resource person.

Unit summary:

The last part of the training guidelines gives a summary of the theoretical presentation(s) made in support of a unit. This can be a lecture outline or a list of major points that will be discussed during the presentation. The purpose of the summary is to highlight the important information that needs to be included in a presentation. Different resource persons may develop the units in different ways, but there should be a level of consistency in content from course to course.

Evaluation and follow-up

The impact of any short training course such as this one, requires constant monitoring of the event, course evaluation by the participants, tests and a follow-up activity aimed at assisting course participants in the application of knowledge, skills and attitudes acquired during the course. Examples of evaluation forms and personal action plans are attached in annex 4.

Course evaluation

Evaluation forms are distributed at the beginning of the course so that participants can fill them out at the appropriate time. The completed forms are analyzed by course organizers who may also prepare a summary of the information for inclusion in a course report.

The purpose of the daily evaluation of theoretical sessions, field visits, demonstrations and exercises is to establish whether:

- Participants have acquired new knowledge
- The session is important for a trainee's daily work
- The duration of a session is appropriate or not
- The timing of a session is appropriate or not
- The session is well-presented
- The session is well supported by teaching materials
- Participants have any other comments regarding a session

The outcome of this daily evaluation is discussed with individual resource person(s) and aims at improving their training skills.

Final evaluation focuses on the training event as a whole, both in terms of contents and logistics, and deals with:

- Pre-course arrangements
- Duration and timing of the event
- Quality and usefulness of the units

- Appropriateness and effectiveness of course objectives
- Training materials
- Logistic aspects
- Interactions between participants and resource persons
- Overall evaluation: best and worst features of the course

Feedback to participants and resource persons can be captured in a course report and taken into account for the organization of the next training event.

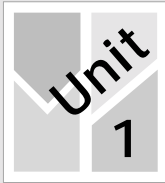
Knowledge and skill testing

Course organizers and resource persons will be interested to see if the course content has been well understood by the course participants and this can be best assessed through some form of a test at the beginning and at the end of a session or the course as a whole. This can be done informally through a question and answer session or more formally through a written test. The outcome of such tests can eventually be linked to the granting of a course certificate that serves as proof of attendance and qualification of a course attendee in one or several areas covered by the course.

Follow-up

In order to assess the impact of the course, course organizers need to conduct follow-up activities. This can be done through the development of a 'personal action plan' in which participants indicate how they intend to use the knowledge and skills acquired as the result of attending the course in their day-to-day work. This will allow course organizers to follow-up after a certain period of time in order to verify if these action plans have been implemented and with what results. Where possible, this action planning can be supported through the provision of small grants as to limit the effect of 'lack of resources' as the cause of non-implementation of the action plan.

Course organizers can also follow-up through a simple impact assessment questionnaire that participants complete six to twelve months upon completion of the training. Where possible, this can be complemented through visits to the place of work of the course participants in order to visually ascertain that participants do apply knowledge and skills, or change their attitudes, as the direct result of attending a training activity.



Introduction to vegetative tree propagation

Training guidelines

Instructional objectives

At the end of the introductory unit on vegetative propagation, participants will be able to:

- List and explain the reasons for vegetative propagation in agroforestry and give some examples of trees that can be successfully propagated this way.
- List and describe the most common vegetative propagation techniques.
- Explain some of the physiological principles behind vegetative propagation techniques, including the role of phytohormones.
- Describe the principles involved in selecting and collecting plant material for vegetative propagation.

Instructional methods

The unit consists of two 60-minute theoretical presentations, followed by discussion; one on the basic concepts and principles of vegetative propagation and one on the selection and collection of plant material for multiplication. These presentations can be supported by the usual audio-visual tools such as slides and overhead transparencies. Where possible, some visits can be organized to reinforce certain concepts or principles.

Instructional materials

Lecture notes and handouts support both theoretical presentations.

Unit summary

The first part of this unit gives an overview of the most common vegetative propagation techniques used to multiply agroforestry trees. The presentation lists

and explains the main reasons for considering vegetative, over sexual (through seed), propagation. Phases of development of vegetatively propagated materials are described and the role of plant hormones and growth regulators in this process is discussed. Finally, vegetative propagation is presented in the overall context of agroforestry tree domestication.

The second part of this unit deals with the principles involved in vegetative sampling during germplasm collection. The relative advantages and disadvantages of the approach are discussed, while general points of importance during collection, including the participation of local communities and the requirement for good documentation, are highlighted. The targeted vegetative collection of tree germplasm from nature may result in superior trees being made available more quickly to farmers, with earlier expression of desired products and uniformity of growth form. However, such collection may also lead to narrowing of the genetic base of cultivated material and can be both costly and time consuming. The relative merits of vegetative sampling of clones in the field will depend on the biology of the taxon and the situation in question - these must be evaluated on a case-by-case basis for any collection.

2

Recommended reading

The following publications may further enhance your understanding of this unit:

- Dawson I and Were J. 1997. Collecting germplasm from trees - some guidelines. *Agroforestry Today*, 9(2): 6-9.
- Hartmann HT, Kester DE, Davies FT and Geneve RL. 1997. *Plant propagation: Principles and practices*. 6th ed. New Jersey: Prentice Hall.



Concepts and principles

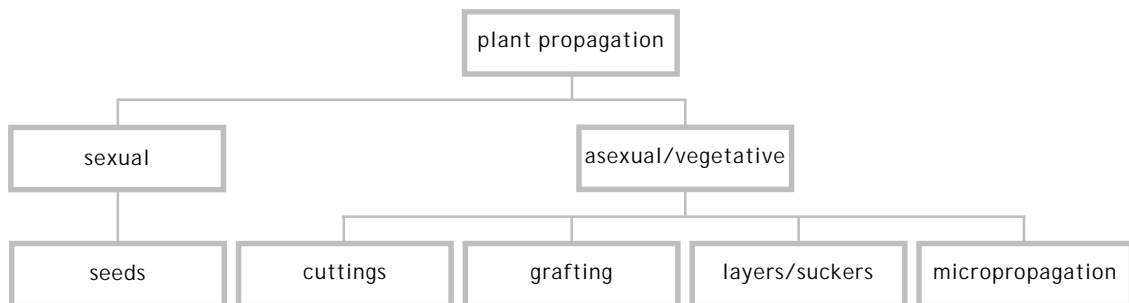
Ze'ev Wiesman—IPALAC and Hannah Jaenicke—ICRAF

Introduction

The concept of vegetative propagation is that an exact copy of the genome of a mother plant is made and continued in new individuals. This is possible because plants, – unlike animals or humans, – have meristematic, undifferentiated cells that can differentiate to the various organs necessary to form a whole new plant. A piece of plant shoot, root, or leaf, can therefore, grow to form a new plant that contains the exact genetic information of its source plant.

Whereas sexual reproduction by seeds provides opportunity for variation and evolutionary advancement, vegetative propagation aims at the identical reproduction of plants with desirable features such as high productivity, superior quality, or high tolerance to biotic and/or abiotic stresses, and as such, plays a very important role in continuing a preferred trait from one generation to the next. This method has been used for fruit tree species in the Mediterranean since biblical times, and continues to be of value in today's tree domestication efforts.

The most important vegetative propagation techniques for tree species are the propagation by stem or root cuttings, grafting and budding, and various methods and techniques of layering and micropropagation.



Cuttings

Cuttings are severed plant pieces with at least one node. Various plant organs can be used for cuttings: stem, root or leaf cuttings. Cuttings are usually placed into a suitable rooting substrate and kept under high humidity until roots and shoots have formed. Plant propagation by cuttings can yield a high multiplication rate and produces plants with their own root system.

Grafting

Grafting allows the combination of two or more plants. It is the technique of choice when a single genotype does not possess all the required characteristics, such as nematode resistance of a rooting system and/or high yield from the above ground parts (wood, leaves, fruits).



Layering

This is a technique of propagation similar to cuttings, with the advantage that the propagules are detached from the mother plant only after roots have formed. It is therefore a method that can provide rooting success with difficult-to-root species. Its multiplication rate is lower than with cuttings, but it can yield larger individual plants.

Micropropagation

Under this heading, all forms of tissue culture and micropropagation are combined. The characteristic of these techniques is that plants are developed from single cells or tissue, which are grown in aseptic culture media. Micropropagation allows a very high multiplication rate; from a single plant thousands of new 'daughter' plants can be produced. This technique initially requires high investment, in terms of equipment and training. Therefore micropropagation is usually only used for high value tree crops, which are deemed to be of commercial importance.

Other techniques

Trees and shrubs can also be multiplied using offsets or apomixis.

Offsets are lateral shoots that develop from the base of the stem of some plants. Offsets are of importance for agroforestry in propagating monocotyledons, such as palms. They are severed from the mother plant with roots attached and can be potted immediately or, if insufficient roots are present, they can be treated like a stem cutting and placed into a propagator. Cutting back the main stem, thus breaking the apical dominance of the mother plant, which is usually very strong, may trigger offset production.

Apomixis is a process common to certain plants in which the normal sexual process of zygote formation does not occur. Thus, the embryo develops from a haploid or diploid cell within the reproductive structures (nucellus, embryo sac or an egg that was produced without undergoing reductive division). The most common form of apomixis is the adventitious embryony in which embryos originate from cells of the nucellus or the embryo sac. These embryos are diploid and exact copies of the mother plant. Seeds thus developed often have more than one embryo ('polyembryony'), one having sexually developed, the others of apomictic origin. This form of apomixis occurs in some fruit species such as citrus and mango. It is suggested that it may occur quite often in undomesticated tropical tree species but this has yet to be proven.

The importance of the apomictic phenomenon lies in the production of cloned seeds. Plants germinated from apomictic seeds undergo the same development stages as sexually

produced seedlings. It depends therefore on the species, whether this is a horticulturally acceptable form of propagation. If juvenile and vigorous plants are needed, for example for timber production, this form of clonal production is desired. Apomictic seedling production is also interesting in terms of costs involved in the production of clonal plants, as seedling production in most cases, is cheaper than the production of vegetative propagules.

Reasons for vegetative propagation

The most important reasons for vegetative propagation are:

- maintaining superior genotypes
- problematic seed germination and storage
- shortening time to flower and fruit
- combining desirable characteristics of more than one genotype into a single plant
- controlling phases of development
- uniformity of plantations

Maintaining superior genotypes

Most tropical tree species are outbreeders, which means that through the recombination of genes during sexual reproduction, many important characteristics might disappear. If a superior individual tree has been identified by farmers or researchers, its genetic information can be 'fixed' through vegetative propagation, thus allowing the reproduction of the same superior individual in the next generation.

Problematic seed germination and storage

Some tree species produce seedless fruits (e.g. some citrus cultivars) and need to be propagated vegetatively, others bear fruit very scarcely or erratically. Many tropical tree species have recalcitrant seeds that require special and often cumbersome seed handling procedures. In these cases, vegetative propagation might be a suitable and cheaper alternative to seedling production.

Shortening time to flower and fruit

An important reason for vegetative propagation is the shortening of the reproductive cycle of a tree. This is particularly important when the flowers, fruits or seeds are the desired products. Most vegetative propagation is done with scions or cuttings from mature trees, which maintain the characteristics of maturity after grafting or rooting as will be explained in more detail below.

Combining more than one genotype in one plant

Grafting is a unique way of combining desired characteristics from two or more plants into a single one. Scions with particular fruit characteristics can be grafted onto rootstocks with other desired characteristics, such as nematode resistance. Another possibility is the grafting of more than one cultivar onto the same stem, for example, to extend the period of bearing by grafting early and late varieties on a single tree. The introduction of a pollinator branch into a female tree is a possibility for dioecious species.

Controlling phases of development

A plant undergoes several age phases that can be distinguished by their growth vigour and flowering. Juvenile plants are vigorous, have a strong apical dominance and regenerate easily by vegetative propagation. Mature plants are not vigorous, branch heavily, and they flower. They do not regenerate easily by vegetative propagation. Intermediate grades of maturity can also be defined. Vegetative propagation perpetuates the phase of maturity of the mother plant. This 'fixation' of the developmental phase of a tree can have economic benefits such as in the case of fruit trees that will flower soon after grafting, where the scion was taken from a mature tree, or of timber trees that will retain their juvenile vigour when rooted as a cutting from juvenile plant material. It is, however, also important to note that certain forms of vegetative propagation, notably root cuttings, always lead to juvenile plants, a feature which might be undesirable in certain cases.

Uniformity of plantations

For many commercially grown species, uniformity of growth form or fruiting season is important economically. Uniformity can also be important in agroforestry experimentation.

Plant hormones and growth regulators

Plant hormones play an important role in the development of callus and the differentiation into new roots or vascular tissues. They are chemical substances, which occur naturally in plants in very low concentrations. In addition to the naturally occurring (endogenous) hormones, there are several synthetic or natural substances that have similar effects. These, together with the plant hormones, are commonly combined under the term plant growth regulators (PGR). There are five main groups of plant hormones and growth regulators that can be distinguished by their dominant effect. These are auxins, gibberellins, cytokinins, abscisic acid and a gaseous growth regulator, ethylene.

Auxins

The auxins are a group of natural and synthetic chemicals that are derived from L-tryptophan. The endogenous auxin is indole acetic acid (IAA). It is produced in the leaf primordia, young leaves and developing seeds, and moves basipetal (from tip to base). It influences many plant activities, such as bending towards light, apical dominance (inhibition of lateral buds by a strong terminal growth), formation of abscission layers in fruits and leaves, and activation of cambial cell growth. This latter activity is the most important for vegetative propagation as it has a direct effect on root formation in cuttings and wound healing in graft union formation. There are a number of known synthetic auxins that have stronger effects than IAA and are used commercially in plant propagation, for example indole butyric acid (IBA), naphthyl acetic acid (NAA), and a well-known herbicide, 2,4-D.

Gibberellins

Gibberellins occur naturally in plants. They regulate shoot elongation through cell growth (as opposed to cell division). There is evidence that they interfere with root initiation. Experiments have shown that use of so-called 'antigibberellins', substances that inhibit the synthesis of gibberellins in plants, can enhance the rooting success in combination with exogenous auxin application. A well-know antigibberellin is paclobutrazol ('Cultar').

Cytokinins

Cytokinins occur naturally in plant endosperm. They regulate cell division and initiation of buds and shoots. Natural cytokinins include kinetin and zeatin, and there are a large number of known synthetic cytokinins. The balance of cytokinins and auxins is most important for plant propagation: high auxin/low cytokinin ratio favours adventitious root formation, whereas low auxin/high cytokinin ratio favours the formation of adventitious buds. Cuttings of species with a high natural cytokinin level are more difficult to root than those with a low natural level.

Absciscic acid

Absciscic acid (ABA) is a growth inhibitor responsible for the formation of abscission layers in buds and leaves. It also regulates stomatal closure and controls water and ion uptake by roots. It is a natural antagonist of cytokinins and may play a part in plant propagation, however its role is not yet clear.

Ethylene

Ethylene is a gas that is produced by ripening fruits and senescing plants. Under research

conditions, contradicting effects of ethylene on the formation of adventitious roots have been observed. It seems that endogenous ethylene is not directly involved in rooting of cuttings.

Tissue maturity

Although plants, like animals, exhibit embryonic, juvenile, adolescent and adult phases, there is a distinct difference in the way in which animals and plants grow and age. The main difference is that animal cells are more or less determinate; all cells within a body mature and age more or less together. Plants, on the other hand, develop consecutive layers of cells in the meristem of apical or lateral shoots. Thus, juvenile to mature development occurs in the meristem as the shoots grow. Paradoxically, the base of a tree, chronologically the oldest part, is the least mature in terms of 'ontogenetic' age. The crown however, chronologically youngest, is ontogenetically most mature. Lateral buds are often dormant due to strong apical dominance of the main growing tip, but retain the ontogenetic level of maturity that they had from the time they originated. Once they start to grow, they develop following the normal steps of ontogenetic development. Therefore, buds or epicormic shoots taken from the base of a tree lead to juvenile plants whereas buds or cuttings, taken from crown shoots, lead quickly to flowering plants.

When propagating from cuttings, juvenile plant material is needed as the formation of adventitious roots decreases with maturity. Coppice shoots developing on a stump of a felled tree show juvenile characteristics, such as vigour and easy rooting, and can be used for stem cuttings. As the resulting plants exhibit the same juvenile characteristics, this form of propagation is preferred for example in timber species, where vigour and low branching are desired characteristics.

On the other hand, crown material is desired when propagating fruit trees, as the aim is to reduce the time to maturity of the new plants. As rooting from cuttings is difficult from mature plant material, other techniques, such as layering or grafting are preferred for the vegetative propagation of fruit trees. Consecutive vegetative propagation of such derived trees leads to 'fixing' of the ontogenetic age, most prominently the fixing of the mature growth phase whereby juvenile characteristics disappear completely from the plant.

Tree domestication and vegetative propagation

Vegetative propagation, for a tree domestication researcher, can be a valuable tool in assessing the potential of certain tree individuals from selected populations (Tchoundjeu et al. 1997). For example, it can be used to define whether the superior fruit quality of a tree is genetic or a response to its environment or management. By planting members of the same clone on different sites, these traits, as well as the climatic and environmental resilience of the clone, can

be tested. In traditional plant breeding the average performance of a provenance, or family, is usually recorded and used for evaluations. In contrast, when looking for particularly suited clones, these individuals may be found within otherwise inferior populations. Once a superior individual has been identified, cultivars can be produced by continuous vegetative propagation. Whether improved material will be developed using vegetative propagation methods or traditional breeding techniques depends largely on the species concerned and the product it provides (Weber et al. 1997). Tree species that produce a highly valuable product, like fruit, and which may be outbreeders, usually justify the higher investment of a vegetative propagation programme.

Maintaining genetic diversity

It is commonly assumed that a vegetative propagation programme in conjunction with range-wide germplasm collections will preserve the genetic diversity existing in wild populations. This is true for populations otherwise threatened with extinction, or in which earlier selection has resulted in the dominance of a few families or provenances at the cost of others. However, vegetative propagation can also result in threatening the genetic diversity of a population if the selection from the broad natural diversity has been very narrow. If, for example, only one or few clones pass very strict selection criteria, and all others were rejected, the resulting planting of mono- or oligoclonal populations would be highly detrimental to the environmental and societal resilience of a species. Similarly although we know of several species that have been successfully introduced in the past from only a few seeds, this practice might be potentially dangerous as such a population is more prone to biotic or abiotic disasters such as insect attack or prolonged drought.

As for seed collections, where seed should be collected from at least 30 trees of a population (Dawson and Were 1997), care needs to be taken to maintain the broad genetic diversity present in any vegetative propagation programme. If selecting for a certain trait, several clones may be chosen that show a similar performance for that trait. For example, if maturity early in the season was the target for domestication of a species, this trait would be present in several individuals, although linked to a variety of other, more or less important traits, such as size of the tree or leaf colour. A population could therefore be maintained which had a common trait of early production, but would differ in size of tree and colour of leaves. Such clonal mixtures are common in forestry, where in some countries the law regulates the number of clones that must be contained in any one mixture (e.g. in Canada >50) (Zsuffa et al. 1993). For commercial fruit tree species, people have diverse enough tastes so that sufficient genetic diversity seems to be guaranteed. However, it is important to note that in the centres of origin of most of our exotic fruits, natural populations still exist that help to preserve the gene pool and that can provide 'new' genes for new cultivars to be produced.

Diseases

Another problem in vegetative propagation that is not to be underestimated is the spreading of diseases, especially viral diseases. Once a plant gets infected with a virus or virus-like organism, often through sap-sucking insects such as aphids, it can become systemic and spread within the plant, cross graft-unions and become a source for further infection through scion and budwood. The 'greening disease' is a well-known example in citrus. It has resulted in many a failure of ambitious production plans. The virus is naturally transmitted through the citrus psyllid (*Trioza erytreae*) but is largely spread by the budding operation from a diseased onto a non-diseased plant. In Kenya, for example, strict regulations exist governing the regions in which citrus nurseries may exist. These nurseries have a special certification, skilled staff and close monitoring to prevent any diseased material from being released (Will et al. 1997). Several sophisticated methods have been developed to eliminate pathogens from plants, such as thermotherapy, heat treatments and micrografting, or combinations thereof. These methods are usually only available in specialized laboratories.

History has shown that devastating disasters can occur through plant diseases spread in vegetatively propagated plants. An example is the *Phytophthora infestans* epidemic in 1845/46 that destroyed the potato harvest in Ireland, and resulted in a famine that led to a wave of emigration to the USA.

Vegetative propagation, in turn, can also be used to improve a species' resistance to pathogens, either by propagating resistant individuals, or by grafting valuable, though susceptible, scions onto resistant rootstocks.

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Clone selection and collection

Ian Dawson—ICRAF

Introduction

The presentation describes some of the principles involved in vegetative sampling during germplasm collection. The relative advantages and disadvantages of the approach compared to other methods of seed collection are discussed. General points of importance during collection, including the participation of local communities and the requirement for good documentation, are highlighted.

The targeted vegetative collection of tree germplasm creates the potential that superior trees are made available more quickly to farmers, with earlier expression of desired products and uniformity of growth form. However, such collection may also lead to a narrowing of the genetic base of cultivated material and can be both costly and time consuming. The relative merits of vegetative sampling of clones in the field will depend on the biology of the taxon and the situation in question - these must be evaluated on a case-by-case basis for any collection.

Collection principles

Germplasm may be collected for a number of reasons. These include:

- For immediate distribution to farmers or other users.
- For conservation purposes.
- For the selection of superior germplasm in tree domestication programmes.

Normally, germplasm is collected in the form of seed, although vegetative sampling is another option. A number of advantages are associated with vegetative sampling of germplasm compared to seed collection. These include the following:

- Collection of an exact genetic copy of the sampled tree is taken during vegetative collection. Hence, if selection for superior trees is possible during collection, the favourable genes and adaptive gene complexes of those individuals are maintained. Targeted vegetative collection can then lead to increased efficiency in the selection of superior quality material when compared to seed collection. This is because most trees are outbreeding species, which means that only 50% of the nuclear genome of seed is contributed by the mother tree. Collection of seed could result in the loss of favourable genes and adaptive gene complexes from the mother trees.

- Accelerated expression of important characteristics may be exhibited by vegetatively sampled material, depending on the method of collection and the age of the tree. For example, marcotted material of fruit trees normally produces earlier than if sampled as seed. This has advantages both for the evaluation of germplasm in tree improvement programmes and in the direct distribution of material to users. In the latter case, farmers probably receive benefits more quickly from collected material.
- Collection is possible when no seed is available. For some species, the appropriate time for seed collection is difficult to predict and varies greatly between years. Some species do not fruit in some years, or, if outbreeders, are unable to produce fruit at all, due to genetic isolation, as a result of population fragmentation. In these instances, the ability to collect vegetative material provides the only method of obtaining germplasm.

There are also a number of potential limitations (no neighbouring trees to carry out pollination) associated with vegetative sampling of germplasm. These may include the following:

- Phenotypic selection during collection could be ineffective. Normally, characters can only be selected for in the field, if they are of high heritability, because of the influence of a non-uniform environment on character expression. While some characters of interest may be of high heritability (possibly, fruit size and sweetness), other traits may not be (e.g. tree form). Little work has been carried out in determining the efficacy of phenotypic selection in the field.
- Vegetative collection at times suffers from practical difficulties. The techniques involved in collection may be difficult (possibly requiring considerable prior research for optimisation) and time consuming. Vegetative material is perishable – it must therefore be handled carefully in the field (Leon and Withers 1986) and cannot normally be stored for long periods of time. Sometimes material is bulky and difficult to process. Quarantine regulations may be stricter due to the increased potential for the transmission of viruses or other diseases, when compared to seed.
- Due to practical difficulties, vegetative collection tends to focus on a small number of trees from any given provenance. This is most likely to lead to a narrowing of the genetic base in collected material compared to the population from which it is collected. This is particularly true for trees, which normally show considerable variation within populations. Although reduced genetic variation can be an advantage in certain situations (e.g. when markets demand a product of uniform size and character), it may also lead to a reduced capacity of germplasm to adapt to varying environmental conditions (e.g. pest and disease attack, climate change) or user requirements (e.g. a change in user emphasis between the different products which a tree provides) (Simons et al. 1994). If subsequent propagation

is undertaken via seed, this can lead to inbreeding depression (loss of performance). One approach to avoid a narrowing of the genetic base is to collect, evaluate and distribute a greater number of clones from provenances. However, this practice has rarely been followed. Because of the potential narrowing of the genetic base of collected material, vegetative collection is not normally employed for conservation purposes, unless seed is unavailable or germplasm normally reproduces by vegetative means.

Whether or not vegetative collection is appropriate in a given situation will depend on the biology, use and desired level of improvement of the species in question – these factors must be assessed on a basis before collection begins. A targeted vegetative approach is most appropriate for those tree species that:

- produce high value products (e.g. some fruits)
- are outbreeders
- have a long maturation period before fruiting
- produce recalcitrant seed
- whose important characteristics are under strong genetic control (see example on p. 14).

Low value species used for service functions (e.g. for soil fertility improvement or fodder), with short generation times and prolific production of orthodox seed, are often better collected as seed.

Regardless of the approach used to collect germplasm, many tree species exhibit considerable phenotypic variation across their ecogeographical ranges. Hence, during collection for evaluation in genetic improvement programmes, it is important to sample material from a range of provenances across the geographical range of a species, as well as across ecological gradients (such as altitude or rainfall clines) that occur within the distribution of the taxon.

Collection guidelines

The selection and collection of germplasm is best carried out in a participative manner with potential end users. First, this involves a determination of those species which users are interested in growing. Second, suitable collection methods for those species must be defined through learning experiences or experimentation from users. Third, those characteristics of a species, which are important to users for which improvement would be desirable, must be determined. Collections should then be carried out directly with local communities. Targeted sampling should be based on the important characteristics that end users have defined. Participative collection increases awareness among farmers of the potential uses and benefits of planting a species. It also allows them to learn the techniques required for its vegetative

propagation. In this way, the potential for accelerated impact and adoption of technologies and germplasm may be greatly increased.

Particular points of importance in the collection process are:

- The need for a well-developed rationale describing the methodology and purpose of collection, before collection commences. This information can be usefully presented in the form of a summary table and should be written up in the form of a protocol before collection begins.
- The need for good documentation during and after collection, for future reference. More specifically, the particular selection characteristics of collected trees should be recorded, as well as the names of those individuals from the community whom were involved in selection.
- Because vegetative material is perishable, proper preparations for the immediate handling of material on return to base need to be made, before collection begins.
- After collection, a report detailing work carried out should be written.

Further information on how to carry out germplasm collection is available from Dawson and Were (1997) and FAO (1995).

An example

Irvingia gabonensis and *I. wombolu* (bush mango) are important fruit tree species in humid West Africa. The fruit is eaten fresh, and dried seed is used as a thickener for soup. Bush mango contributes significantly to the local economy of the region and during priority-setting exercises was identified as a key taxon for research, including activities such as germplasm collection and genetic evaluation. Both species can be vegetatively propagated through marcotting or air layering. Seed is recalcitrant and remains viable for only four weeks after collection.

Before germplasm collection, communities were surveyed in order to determine those tree characteristics of importance to users. These included fruit size and sweetness. Subsequently, two separate approaches were chosen for collection. Initially, fruit was collected from trees, which users determined to have superior characteristics in Nigeria, Gabon and Cameroon. In this approach, collected seed was then quickly transferred to field sites at various locations for establishment in on-station provenance trials and conservation stands.

Following this first round of collection, a range of collected trees was assessed with molecular genetic techniques to establish patterns of variation in both species, in order to determine optimum genetic management strategies. As part of this study, analysis indicated that considerable genetic variation exists even among seed sampled from a single tree (as is

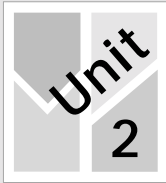
often the case for outbreeding species). Seed is therefore not 'true to type' and data therefore indicated that clonal sampling of germplasm would be a more effective approach for targeted tree collection.

In a second round of collection, targeted vegetative sampling was carried out in Nigeria and Cameroon. Users were asked to identify superior trees in various locations and marcots set with the assistance of communities. Between 5 and 10 marcots were set per tree. After a number of months, marcots were collected. A portion was then taken for on-station field trials to compare genetic differences between clones and regions, while a proportion was left with users to establish community nurseries.

Apart from clonal sampling, the advantages of vegetative collection of *Irvingia* are twofold. First, the period to fruiting is accelerated (two or three years to fruiting, compared to a minimum of seven years from seed) - hence germplasm can be more quickly evaluated and superior material subsequently delivered over a shorter time scale to users. Second, by directly involving communities in the collection of marcots, interest in managing and planting the species was stimulated. Marcotting is a relatively straightforward technique, which users may then use for the selection and propagation of their own favourite trees, without the further intervention of researchers. The disadvantage of the marcotting approach was the relatively low level of success at the establishment phase. Further research is therefore required before marcotting can be used as a routine method for collecting *Irvingia* germplasm

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Tree nurseries

Training guidelines

Instructional objectives

At the end of the unit on tree nurseries, participants will be able to:

- List and explain the most common problems affecting nursery management and propose solutions to address them.
- List and describe tools and materials needed to operate a nursery.
- Describe the characteristics of a good nursery substrate.
- Organize and plan nursery activities leading to the production of high quality agroforestry trees.
- List and describe some common pest and disease problems that may affect plants in tree nurseries and propose ways to control them.

Instructional methods

The unit consists of two 60-minute theoretical presentations; one on nursery management and one on phytosanitation at the nursery level, with audio-visual support. The nursery management presentation is followed by a series of practicals and demonstrations highlighting important nursery management operations with a focus on specific vegetative propagation techniques. The presentation on phytosanitation can be illustrated through a demonstration or practical exercise on pesticide use in a nursery.

Instructional materials

Lecture notes support both theoretical presentations. The materials needed for the nursery management practical and demonstrations are listed in the detailed description of the nursery practicals and demonstrations.

Unit summary

The production of high quality agroforestry trees requires a well-managed tree nursery. The ultimate goal of a good nursery manager should be the timely and cost-effective production of healthy, uniform plants with a strong fibrous root system.

Common problems in producing seedlings are: lack of a reliable source of water, delays in supplies, poor quality equipment, variability in seed sources, potting mixtures, nursery hygiene and phytosanitation, nursery skills and planning. Some of these are nursery management related, others can be addressed through improved infrastructure.

Nursery substrates are an important aspect of successful tree production. Good propagation substrates should be light, but hold the seedlings firmly in place, retain moisture, but well aerated and drained, free from pathogens and contain the necessary nutrients needed for plant growth without being saline.

Organic material such as manure, straw, plant residues, wood or sawdust, is an important addition in a good nursery substrate. Such material needs to be composted properly before mixing it with the other parts of the nursery soil as to become a nursery substrate. There are several methods to produce good quality compost.

There are a number of basic tools that are required in a nursery: pickaxe, hoe, shovel, flat-pronged fork, rake, string, sieve, watering can/hose, wheelbarrow, pruning knife, trowel, secateur, panga (machete), pointed wooden stick. This material should be properly used and maintained.

Good nursery management and organization requires proper planning. The most important aspects being timing and the estimation of nursery substrate and plants needed to produce the required number of trees. Nursery managers should interact more with their customers as to improve their management skills and the quality of the planting material they supply.

The usual range of plant pests and diseases such as fungi, bacteria, viruses, insects, mites, nematodes, weeds and others can affect seeds and seedlings in nursery beds. The lecture highlights some common problems in tree nurseries and proposes preventive and curative measures to alleviate these.

Recommended reading

The following publications may further enhance your understanding of the unit:

- Hartmann HT, Kester DE, Davies FT and Geneve RL. 1997. *Plant propagation: Principles and practices*. 6th ed. New Jersey: Prentice Hall.
- Jaenicke H. 1999. *Good tree nursery practices. Research nurseries*. , Nairobi: ICRAF 83 pp.
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Nursery management and seedling production

Hannah Jaenicke–ICRAF

Introduction

In the following pages nursery management options for research or project nurseries are discussed. Although for all nurseries, be they on-farm or on-station, quality, hygiene and proper planning are of paramount importance; nurseries attached to projects usually have the resources available to invest in more than just the basic inputs. Options are given here which can later be adapted to the local situations without compromising the quality of seedlings produced. A few ideas for experimentation in the nursery can be found in unit 7 'Propagation experiments'.

Although a number of tree species can easily be established through direct sowing in the field, a large number of species requires careful production in a tree nursery, where the young seedlings can be protected and hardened to survive the harsh field environment. Tree nurseries should always be situated close to a reliable water source and/or where protection can be provided. The terrain should be flat and gently sloping, so that drainage water can easily run off. If possible the ground should be covered with a layer of gravel to suppress weeds and to keep the nursery area clean.

The quality of seedlings is determined by the conditions at the site where they will be planted. For example, quality seedlings for dry areas need to have a deep and well-developed root system to be able to grow quickly to the water table. Quality seedlings for a humid site with strong weed competition, however, need to have strong above-ground growth to be able to outgrow the weeds and compete for light and other resources. All seedlings leaving a nursery should be strong enough to start growing quickly after planting out.

Quality seedlings:

- have a well-developed root system and are able to produce new roots quickly,
- anchor in the ground quickly and start assimilating and growing after planting out,
- have a sun-adapted foliage,
- have a balanced shoot/root ratio,
- have good carbohydrate reserves,
- are strengthened by adequate inoculations with mycorrhizae or *Rhizobium* if needed.

¹ This lecture note is adapted from: Simons AJ and Beniast J (eds.) Introduction to Tree Domestication. ICRAF, Nairobi (in prep.)

Good nursery practices

A number of factors influence the production of quality seedlings in a nursery. These are: seedling handling, containers, substrate, fertilizing, nursery hygiene, nursery environment, time management, labelling and record keeping.

Seed germination and seedling handling

Most orthodox seeds are dormant until they come into contact with sufficient moisture to start the germination processes. Some seeds need special treatments to break the dormancy or to speed up and synchronize germination. Soaking the seeds in warm or cool water overnight is usually sufficient to trigger the germination process. Sometimes, special treatment, such as nicking or chilling, is required. This information is usually given on the seed packs. Experiments may be needed for unknown species.

The physical handling of seedlings should be reduced to a minimum during its time in the nursery. The common practice of germinating seeds in germination beds and pricking them out later is discouraged because it can lead to severe root deformities. Better practice would be to sow the seeds directly into the containers. In case of expected low germination, 2-3 seeds can be sown per container. If pricking out is unavoidable, for example when there are only a few very valuable seeds, or when the seed is very small and needs a fine seedbed (e.g. Eucalyptus, Alnus) it is important that it is done as early as possible and very carefully so as not to damage the young seedling and not to bend or overexpose its roots. Similarly, if the transfer of seedlings from small containers to bigger ones is necessary, the utmost care has to be taken to avoid damaging or bending the roots.



The first picture shows 2 plants with deformed roots (left) as a result of poor nursery growth. The plant on the right has a proper rooting system. The second picture shows some root deformities of young seedlings grown in containers in a nursery.

It is also advisable to avoid moving seedlings directly from the shade into the sun; it is better to gradually reduce the shading, so that seedlings remain in the same place. Root pruning is important when seedlings are placed on the ground. The recommended method is to use a wire, which is pulled through the nursery bed to sever the roots. However, the workload in the nursery is often heavy, therefore root pruning is sometimes neglected, leading to plants with a large root system in the ground. These plants suffer severely when removed, and often do not survive in the field. To avoid such problems, use raised beds or frames where possible, onto which containers can be placed. The use of raised beds also improves drainage and air circulation amongst the seedlings, and reduces pest and disease incidence in a humid environment. In a dry environment, you may need to experiment as to whether the use of raised beds is in fact beneficial to the particular species you are working with, or for example, if sunken beds or placing a plastic sheet beneath the plants, can help conserve water and prevent the roots from growing into the ground.

Transport to the field is an important task but is often done carelessly, resulting in loss of seedlings. If you plant bare rooted seedlings, bundle them carefully and wrap them into damp paper, cloth or leaves. If you plant containerized plants, avoid squashing the pots. Bread or soft-drink crates can be used for transport and, if the seedlings are small enough, can be stacked without damaging the plants. Keep the seedlings upright and under a moist cover to prevent them from drying out. If seedlings cannot be planted immediately, keep them under light shade and ensure planting within a few days.

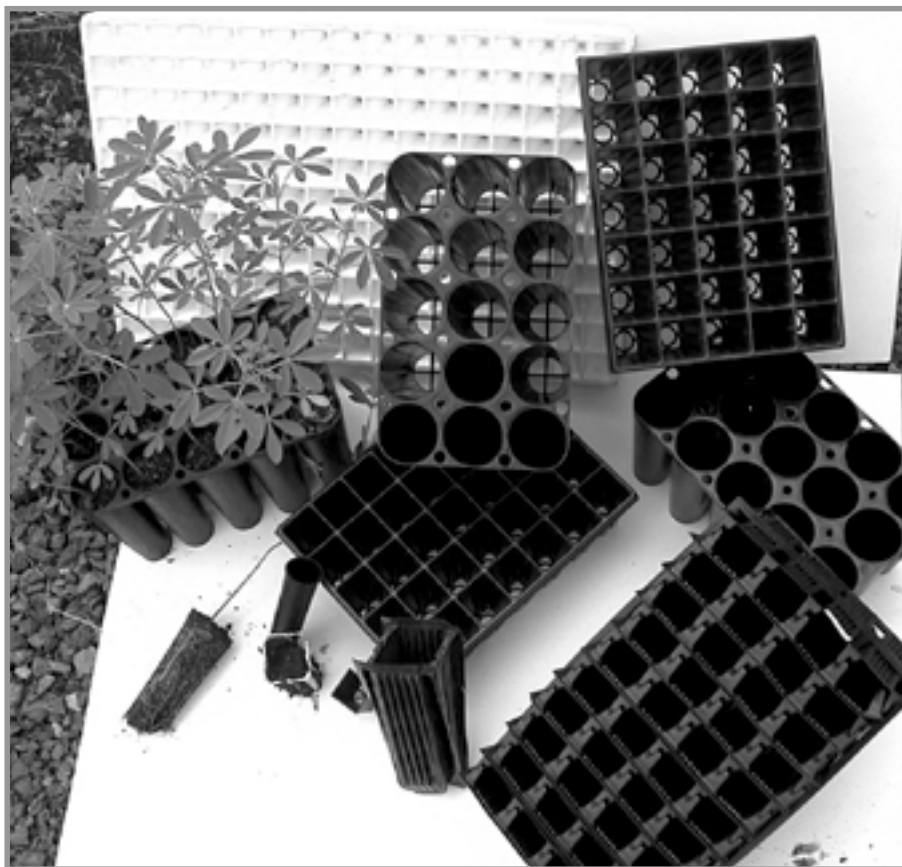
Containers

Trees are often produced as containerized seedlings. Containers for plant propagation come in various forms, sizes, and in different materials - polystyrene, polyethylene, fibre or paper. New forms and materials are constantly being developed and tested. The type of container selected depends on the plants to be raised, their purpose and size. The size of the container also depends on the substrate in use and the fertilization schedule that can be adhered to. Species with a long rotation are usually grown in bigger pots, unless they can be fertilized frequently. Surprisingly, despite decades of research with temperate species, there is still little conclusive evidence concerning the long-term effect with regards to the best type of container to use with species with strong tap roots (Landis and others 1993).

The most commonly used containers in the tropics are polythene bags of different sizes. They are usually locally made, relatively easily available and relatively cheap. However, one of the serious drawbacks is that roots can grow in spirals once they hit the smooth inner surface of the pots. This will lead to plants with restricted growth, poor resistance to stress, and to plants being affected by wind-throw or even early dieback due to ensnarled roots. A modern alternative

for tree domestication programmes are so-called 'root trainers', rigid containers with internal vertical ribs that direct roots downwards, avoiding the spiralling. Root trainers have a large drainage hole at the bottom, allowing for air root pruning if containers are placed on frames above the ground.

Locally made containers, such as used milk cartons, bamboo segments or rolled banana leaves are suitable for on-farm production of trees, though they are often not sufficiently durable for seedlings that need a longer stay in the nursery, such as grafted fruit trees.



Different types of root trainers that can avoid seedling root deformities.

Substrates

The substrate is an essential input into seedling propagation – its importance should not be underestimated. Substrates provide the seedling with nutrients, water and air for good development. They also contain the microorganisms that the seedlings may need. Unsuitable substrates lead to root deformities, pathogen attack and retarded seedling development.

The substrate properties that influence seedling growth are:

- **physical properties**
 - water-holding capacity
 - porosity
 - plasticity
 - bulk density

- **chemical properties**
 - fertility
 - acidity (pH)
 - buffer capacity or cation exchange capacity (CEC)

- **biological properties**
 - availability of appropriate rhizobia and/or mycorrhiza strains

Porosity and water holding capacity are two related characteristics. A substrate needs to hold sufficient – but not too much – water for good seedling development and root growth. It also needs to be sufficiently porous to allow good gas exchange in the root zone. Roots will rot and die without sufficient oxygen and/or with too much water in the root zone. The height of the container also influences the water-holding capacity of a substrate. Waterlogging is more likely to occur in shallow containers, which have a higher capacity of holding water.

Box 1: Classroom demonstration

Container height will affect the water holding capacity and can be demonstrated with an ordinary sponge. Saturate a sponge and hold it flat over a tray. When the sponge stops dripping, turn it on its side – more water will drip out. When it stops dripping, stand it on end and more water will drain into the tray. Each time the height of the water column in the sponge increases, the amount of water it can hold decreases. In other words, deeper containers hold proportionally less water than the same amount of substrate in a shallow container. This explains why native soils, when put into a container, are often waterlogged: their depth has been reduced from metres to a few centimetres.

In some areas, the local soils are inappropriate for seedling production, especially where the soil contains large amounts of clay, which leads to waterlogging and makes the substrate heavy and difficult to transport. Local soils often lack the necessary plant nutrients. To lighten the substrate, additions of either organic matter in the form of decomposed manure, compost, rice husks or other plant residues, or of inorganic materials such as sand or vermiculite are used. Which of these materials are used and in which quantities depends on the local situation, availability of the materials, and on the requirements of the species. Simple experiments can be designed to determine these.

Fertilizing

If a rich organic substrate is used, such as virgin forest soil or compost, fertilizing is usually not necessary during the time a seedling spends in the nursery. However, fertilizing may become necessary when a soil-less or a poor substrate is used, or for species which have higher nutrient requirements or need to remain in the nursery for a long period. It is important to be able to recognize the most common nutrient deficiency symptoms. Apart from the macronutrients N, P, K, Ca, Mg and S, which are needed in relatively larger amounts, there are micronutrients needed in smaller amounts (Fe, Mn, B, Cu, Cl, Zn and Mo) that play important roles in the plant's metabolism. In the following table, a few general symptoms for deficiencies of the macronutrients are given. Micronutrients are generally sufficiently available in the most common substrates.

Table 1: Plant nutrients and their deficiency symptoms

name (symbol)	deficiency symptoms (very general)	Function
nitrogen (N)	Old leaves turn yellow, plant growth retarded, small leaves. Be careful: too much nitrogen leads to overgrown plants, which are highly susceptible to diseases.	Important component of amino acids and proteins.
phosphorus (P)	Small plants with erect growth habit, thin stems, slow growth. Leaves appear dirty grey-green, sometimes reddish-purple.	Provides energy (ATP). Helps in transport of assimilates during photosynthesis. Important in fruit ripening.
potassium (K)	Older leaves show first chlorotic, later necrotic borders. Younger leaves remain small.	Important in maintaining cell turgor, phloem transport, cell growth and cell wall development (K deficiency leads to susceptibility to pests because cell walls are weakened).
calcium (Ca)	Deficiency is often only visible through retarded growth.	Stabilizes cell membranes and cell walls, interacts with plant hormones. Ca is extremely immobile and can only be taken up through young, un lignified roots.
magnesium (Mg)	Old leaves chlorotic from middle or between veins, rarely necrotic. Leaves orange-yellow, drop prematurely.	Component of chlorophyll – photosynthesis is hindered when deficient. Binds ATP to enzymes. Important for protein synthesis.
sulphur (S)	Similar to N-deficiency but symptoms show first on young leaves.	Component of etheric oils, vitamin B, vitamin H, amino acids, and has important functions in protein synthesis.

Organic matter for fertilization is often readily available in rural settings. However, the quality and the nutrients it provides depends to a large extent on the source material: the animal feed in the case of manure, and the plants used in the case of compost. Such organic fertilizers provide not only nutrients, but also condition the soil, and increase both aeration and the water holding capacities of the substrate.

Inorganic fertilizers are often less available and more costly than organic fertilizers. However, they have the advantage of being both fast-acting and having standardized nutrient contents. They are therefore recommended when working in a research setting. The most common inorganic fertilizers used in seedling production are full or NPK fertilizers. The numbers (for example, NPK 17-17-17) indicate the amount of the nutrients in %. For quick action and if micronutrients are needed, foliar feed can be applied to the leaves of the seedlings. Foliar fertilizers are specifically formulated to allow absorption through the leaf cuticle. 'Normal' NPK fertilizers cannot be applied as foliar fertilizer; however, they can be dissolved and added to the irrigation water.

Nursery hygiene

At one time or another, every nursery experiences problems with seedling health. Rather than relying on the use of pesticides, we encourage preventive actions to minimize the damage.

There are two factors influencing plant health:

- **abiotic** factors
 - excessively high or low temperatures
 - drought or waterlogging
 - injury due to chemicals
 - physical damage, for example from strong wind or rain drops
- **biotic** factors
 - all biological organisms that interfere with plant production (bacteria, viruses, viroids, phytoplasms, fungi, insects, mites, nematodes, weeds, parasitic higher plants, birds and mammals)

Abiotic damage can be reduced by correct seedling handling, and by appropriate nursery layout and facilities. Appropriate shading, watering and protection from low humidity or frost are important and part of good nursery management.

The next presentation on phytosanitation describes the most common biotic factors in tree nurseries.

Plant diseases and pests can be checked by proper hygiene conditions in the nursery:

- Keep the nursery area itself free of weeds. Many plant species can be alternate hosts of nursery pests. This precaution includes a sensible selection of ornamentals, shade, hedge and windbreak plants in and around the nursery, as they too can be hosts for pests such as nematodes.

- The substrate can harbour plant pathogens and should therefore be steam pasteurized, if necessary. A simple steam pasteurizer can be constructed from an old and clean oil drum.
- Containers and seeds can be surface sterilized by soaking them in a 10% household bleach solution for 12-24 hours.

Only if these preventive measures are insufficient, the use of pesticides should be considered. Never rely on only one chemical as it may lead to a build up of resistance. Rather, rotate between two or three products. Alternatives to synthetic pesticides are the mechanical removal of infected plant parts or pests, or the use of locally available pesticides, such as tobacco, chilli pepper, neem or pyrethrum extracts.

If you are unsure of the identity of the pest or disease, take samples or photographs and consult local experts or agricultural and horticultural extension services. Burn diseased plants with their substrate and never incorporate such material into the compost.

Nursery environment

Seedling growth is affected by conditions both above-ground such as humidity, carbon dioxide, temperature and light, and below-ground such as water and mineral nutrients. Plant growth can also be influenced by beneficial and harmful organisms.

Young seedlings need a sheltered environment. Sufficient – but not too much – shade is necessary for healthy plant development. If at all possible, a shade net should be installed to provide uniform shade. Local material, such as thatch from grass or banana leaves can also be used, but it can harbour pests and diseases therefore needing frequent replacement. As seedlings grow older, they need more light. Install the shade net in a way that it can easily and gradually be removed, rather than moving the seedlings to a lighter area. In tropical countries it is especially important that the nursery beds and shade nets are placed in a North-South direction, so that seedlings receive both morning and evening sun, but are shaded from the direct midday sun.

Proper watering according to the needs of the seedlings is very important. Water is often a limiting resource and the tendency is for over-watering when it is available. However, too much water can be just as harmful to plants as too little as it leads to water logging and suffocation of the seedling roots. Towards the end of the nursery period, seedlings need to be hardened by reducing watering from time to time. Slight wilting at this stage is not harmful but beneficial to further development.

Cuttings and grafted plants need high air humidity to prevent drying out during the time of root development or graft taking. Simple plastic enclosures or green houses can be built. These structures always need to be well shaded and ventilated to avoid heat damage to the plants.

Time management and planning

Planning the nursery work is essential to avoid unwelcome surprises. Seed and supplies need to be at hand in time for timely seedling preparation. Sufficient time needs to be given to repeat the germination in case of failures. The hardening period should not be too short, to avoid unnecessary loss of seedlings in the field. On the other hand, seedlings should never stay in the nursery into the next season. Such overgrown seedlings lose their vigour and will not grow well in the field. If you can foresee that planting will not be possible due to adverse weather conditions or other factors, consider re-sowing the seeds rather than keeping the overgrown seedlings for the next season. Plant growth can be manipulated in small margins by reducing irrigation to slow it, or adding fertilizer to speed it up. However, forward planning is essential for a successful nursery period. Nursery calendars and inventories are helpful tools in this process.

Box 2: Sample calculations

For 10 000 seedlings in 4x6" containers you need:

Seed: Depends on germination percentage (G), seedling variation (culling, C) and losses (L).

We assume that G = 75%, C = 10% and L = 15%. You need 10 000 seedlings (S)

add for germination failure (GF): $S \times 100 / G \rightarrow 10\,000 \times 100 / 75 = 13\,333$

add for culling at transplanting (CT): $GF \times (100 + C) / 100 \rightarrow 13\,333 \times (100 + 10) / 100 = 14\,666$

add for replacements at out-planting (RO): $CT \times (100 + L) / 100 \rightarrow 14\,666 \times (100 + 15) / 100 = 16\,866$

Total seeds needed for each species **16 866**. You will need 0.85 kg of a species with 20 000 seeds/kg (e.g. *Leucaena leucocephala*), 0.65 kg of a species with 26 000 seeds/kg (e.g. *L. diversifolia*) and 0.5 kg of a species with 34 000 seeds/kg (e.g. *L. trichandra*).

Space: When filled, each container takes about 7 cm x 7 cm or approximately 50 cm². 10 000 cm x 50 cm = 500 000 cm² or 50 m². If the bed is 1.5 m wide, this would translate into 33 m length. Assume you separate the seedlings for easier handling in batches, then you need roughly 35 m bed length.

Substrate: Each container takes approximately 0.4 l substrate. 0.4 x 10 000 = 4000 l (equals two hundred 20 l-buckets or ca. 80 wheelbarrows). A double-cab pickup takes about one ton of substrate, so you need 4 pickup loads.

Water: Of course the amount of water needed depends on the size of the seedlings. A rough estimate is that for 1000 seedlings in containers of 0.4 - 0.5 l volume you need ca 75 - 95 l water per week (Landis et al., 1994). So for 10 000 seedlings you will need 750 - 950 l per week. However, the calculation is based on greenhouse conditions, so assume at least 20% more under open-air tropical conditions. So you would need between 900 - 1050 l, which would be sixty to seventy - 15 l watering cans per week.

Box 3: Sample nursery calendar

In Muguga, Kenya, the best field-planting season is usually between 1 April and 15 May. The researcher wants a *Leucaena* species trial planted with seedlings of about 20 cm size on about 15 April. The nursery manager has calculated the researcher's requirements as follows (Jaenicke, 1999):

	<i>Leucaena leucocephala</i>	<i>Leucaena diversifolia</i>	<i>Leucaena trichandra</i>
days needed from germination to planting out	112	122	100
days needed from sowing to germination	8	8	8
safety margin in case of poor germination or damping-off	15	15	15
total days needed	135	145	123
sowing date	1 December	21 November	13 December

Labelling and record keeping

Proper labelling and record keeping are required in order to keep track of species and seedling batches produced. This is particularly important when several provenances or cultivars of the same species are raised in the nursery. The minimum information required includes:

- Species name and provenance, source of seed (e.g., own collection, name of seed dealer).
- Date of sowing.
- Number or quantity (in g) of seeds sown.
- Location and or condition of germination (e.g., seed bed, heated, sand).
- Germination percentage (or number of seedlings emerged).
- If unavoidable: date of pricking out.
- Type and size of containers.
- Substrate used.
- Any treatment given during nursery period – such as fertilizer (when, which, how much), shade (density), pest and disease control (when, which pest/disease, which method used, product name, concentration).

- Date and number of seedlings removed – and reason (e.g., diseased, damaged, bad development).
- Date and number of seedlings harvested for experimental reasons, sold, planted or given out.

Simple entries in a nursery logbook are sufficient, although a variety of computerized systems have been developed that may be more convenient if a large number of batches are being raised. A batch of seedlings should be given a unique serial number at sowing, which is retained until the last seedling of this batch has left the nursery (Wightman 1999).

Nursery experiments

In a tree domestication programme, part of the activities are concerned with the development of appropriate propagation experimental protocols for new species. It is therefore important to establish a small number of routine experiments to understand key requirements of the species. In particular, you may need to monitor:

- germination requirements (pre-treatments)
- time requirements (time to germination, time to planting)
- possible needs for mycorrhizal or rhizobial inoculation
- substrate and fertilizer requirements
- shade requirements
- the feasibility to use root trainers
- pest and disease incidences.

Simple factorial or split-plot experiments can be designed to test various hypotheses. It is always important to record the nursery conditions of seedlings that are carried on to field experiments.

For further ideas, consult unit 7 on 'Propagation Experiments'.

Troubleshooting

This presentation focused on the optimal set-up and management of a tree nursery for the production of quality seedlings. However, there are bound to be problems and table 2 lists a few causes of bad plant development and suggests ways to address them. Although some of the suggested remedies may be out of bounds for small-scale farmers, every effort should be made to give the young seedlings as good a start in their life as possible.

Table 2: Causes and solutions for poor plant development

cause of poor plant development	suggested remedies
genetic variability of the germplasm	<ul style="list-style-type: none"> - collect from selected trees with clearly pronounced required characteristics - when using clonal material, no genetic variation is expected, unless mutations occur
low quality of germplasm	<ul style="list-style-type: none"> - obtain seed from a reliable supplier - ensure proper storage - when using clonal material, clone-to-clone differences can be >100% in rootability and vigour
<p>root deformities, such as spiralling and bent roots caused by pricking out</p> <p>caused by the container</p>	<ul style="list-style-type: none"> - make a hole big enough for the seedling to be pricked out - direct seeding to avoid the need for pricking out - use root trainers
inadequate light conditions	<ul style="list-style-type: none"> - protect young plants from direct sunlight with light shade - gradually reduce from 40-50% shade to 30% shade before putting plants into the open for hardening off - plant at low enough density to allow for enough light in the propagation beds
inadequate watering	<ul style="list-style-type: none"> - water early in the morning or late in the evening to avoid burning the plants - water the substrate in the pots and not the leaves - use a nozzle or water pressure that is low enough not to spill soil out of the pots - ensure good drainage of the containers
overgrown plants	<ul style="list-style-type: none"> - grade nursery plants into three groups: first quality, second quality and rejects - only plant out or distribute first and second quality and ensure rigorous culling of the rejects

References

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- Landis TD, Tinus RW, McDonald SE and Barnett JP. 1993. *Nursery Planning, Development, and Management, Vol.1, The Container Tree Nursery Manual. Agric. Handbook. 674*. Washington DC: Department of Agriculture, Forest Service.
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Introduction

Throughout their entire life, trees can suffer from a wide range of pests and diseases. In the early growth stage of trees, it is most vital that these are kept at bay. Diseased seedlings will rarely achieve the future growth and potential of their healthy counterparts. Pests and diseases in nurseries may also lead to massive disposal of affected seedlings, which is a waste of time, energy and money. Both from an experimental as well as an economical point of view, it is therefore important to consider pest and disease management as an important and integral part of good nursery management.

Identification of plant disorders is a specialist field and requires a lot of experience. Different pathogens can cause similar symptoms and it may be necessary to take samples of plant and soil material, and if possible the causal agent, and send these to specialists in the field of entomology, nematology and general plant pathology. Quite often, the problem can be readily identified, and no specialist intervention will be needed. The following notes will help nursery managers to identify some common phytosanitary problems at the nursery level and propose methods to alleviate them.

Phytosanitary problems

Fungi

Table 3: Phytosanitary problems caused by fungi

Type	Symptoms	Control
Damping off (<i>Pythium</i> spp., Rhizoctonia <i>solani</i> , <i>Fusarium</i> spp. and others)	<p>General: chlorosis, wilting, constriction of stem and root rot.</p> <p>Pre-emergence: seeds or seedlings are killed before they emerge, difficult to diagnose, low germination may be an indication.</p> <p>Post-emergence: shortly after germination, the young seedlings are infected at the base of the stem, or just below, causing constriction, drooping and ultimately the death of the young plant. Seedlings in a nursery bed will topple when brushed by hand whilst healthy ones will recover.</p>	<p>Cultural: create conditions that are not favourable to the development of the disease (proper drainage, appropriate soil mixture, less organic matter, reduced density, shallow sowing).</p> <p>Chemical: disinfect nursery soil with chemicals approved for this purpose. Cover soil for 24 hours to avoid volatilization of toxic gases, leave soil to aerate for 48 hours before sowing.</p>

Type	Symptoms	Control
	Late damping-off: can take place weeks or months after emergence. Leaf chlorosis and wilting of the terminal bud result from root death. Diagnosis becomes difficult since other pathogens or environmental conditions may induce similar symptoms. Rotting of seedling or cutting roots in the nursery.	Thermal: heat the soil for 2 hours at 60 °C prior to sowing. Biological: not very practical, some soil organisms (e.g. nematodes) may suppress damping-off. Dip cuttings in a fungicide solution.
Powdery mildew (<i>Erysiphe</i> spp., others)	Airborne fungal disease causing leaves to be covered with a white powdery dust. Common on fruit trees such as <i>Prunus africana</i>	Remove and burn affected seedlings and leaves of older plants to avoid spreading of the disease. Apply fungicides.
Leaf blisters (<i>Taphrina</i> spp.)	Airborne fungal disease that causes leaf curling and blistering.	As for powdery mildew.
Sooty mould	Fungi growing on aphid excrement (honey dew) causing a black mould on the leaves, causing them to curl up. Associated with aphids or other sucking insects. Ants milk these insects for the honeydew they produce.	Control aphids and other leaf-sucking insects.

Nematodes

Nematodes are tiny (microscopic) worms and some of the most abundant organisms in the soil environment. Most are harmless, but many species are parasitic to plants, and may cause important losses in yields and quality.



Deformed roots of *Sesbania* sp. as a result of root-knot nematode *Meloidogyne* spp. infestation in the soil.

Nematodes are widely recognized as important pests in crop production, but with the exception of some commercial forestry and fruit species, very little is known about the effect of nematodes on trees. Since soil is an important medium for the spreading of nematodes, vegetative propagation techniques such as cuttings and layering, when done using infected substrates, will spread nematodes to planted fields. Some tree species or provenances can be resistant to certain types of nematodes and can thus be used as rootstocks, for those which are susceptible.

Most important is the root-knot nematode group (*Meloidogyne* spp.). They are widely distributed, especially in tropical regions, and have a very broad host range. In the tropics and subtropics, root-knot nematodes are the major nematode pest and among the leading pathogens that affect crop production.

Symptoms of nematode infestation are often non-specific (chlorosis, wilting, growth reduction, root rot) and often confused with those of other pathogenic or non-pathogenic causes, such as fungi, bacteria, viruses, drought, soil fertility. Only root-knot nematode (*Meloidogyne* sp.) damage can easily be identified since this causes galls or swellings on plant roots that are typical in appearance.

Nematodes can be controlled using chemical, cultural, physical and/or biological means.

Chemical

There are several chemical products called nematicides available, which are formulated as fumigants, liquids or granulates.

Physical/cultural

Sterilizing the nursery soil using heat (steam, solar) is an effective means of controlling most parasitic nematodes and a host of other soil microorganisms. Moist nursery soil can be put in a drum and heated over an open fire for sterilization. Heating at 60 °C for about 2 hours is usually sufficient.

Plant rotation involving species with known nematicidal properties such as marigolds or the neem tree - *Azadirachta indica* - or those that are less susceptible, will reduce the build up of nematode populations. Table 4 lists some agroforestry trees² that are known to be good (susceptible) or poor (tolerant or resistant) hosts for root-knot nematodes:

² Provenances within a same species may show differences in susceptibility

Table 4: Susceptibility of hosts for root-knot nematodes

Susceptible	Tolerant or resistant
<i>Acacia</i> spp.	<i>Anacardium occidentale</i>
<i>Albizia</i> spp.	<i>Azadirachta indica</i>
<i>Carica papaya</i>	<i>Calliandra calothyrsus</i>
<i>Cassia angustifolia</i>	<i>Senna siamea</i>
<i>Desmodium distortum</i>	<i>Crotalaria</i> spp.
<i>Dodonaea viscosa</i>	<i>Eucalyptus camaldulensis</i>
<i>Euphorbia balsamifera</i>	<i>Grevillea robusta</i>
<i>Mimosa scabrella</i>	<i>Leucaena leucocephala</i>
<i>Prosopis juliflora</i>	
<i>Samanea saman</i>	
<i>Sesbania</i> spp.	
<i>Tectona grandis</i>	
<i>Tephrosia</i> spp.	

Several organic soil amendments effectively control nematode populations in the soil: sugar cane molasses, coffee, peanut husks, wood-ash, manure and bone meal. Oil cakes obtained from mustard, neem, peanuts, sesame and castor processing also control nematodes.

Biological

A wide range of soil borne predators such as mites, protozoa, bacteria, fungi and other nematodes can attack plant nematodes. Organic soil amendments may increase the populations of these, but it is not yet practical to consider the use of these predators as an effective means of biological control.

Viruses

Viruses are pathogens that consist of a nucleus of genetic material contained within a protein coat. They need living cells for their multiplication and can be transmitted by sucking or chewing insects (aphids, white flies, leafhoppers etc.) nematodes, weeds and certain propagation methods (cuttings, grafting). Several crop viruses may also affect trees.

Affected plants may show symptoms such as mosaic or other mottling patterns on leaves, chlorosis, stunted or distorted growth of the plants and/or necrotic lesions.

Viruses cannot be controlled with chemicals (pesticides); therefore infected plants must be destroyed to avoid spreading the disease. Care must be taken when vegetatively multiplying plants: use only virus-free certified material as rootstock, cutting or grafting and check motherstock regularly for viral infection using indexing with known indicator plants. Other ways to avoid certain viral infections are to use pesticides against their vectors (aphids, nematodes,) and a quick immersion in hot water ($\pm 50^\circ\text{C}$) of the plant material. Micropropagation is an effective way of propagating virus-free material.

Insects and mites

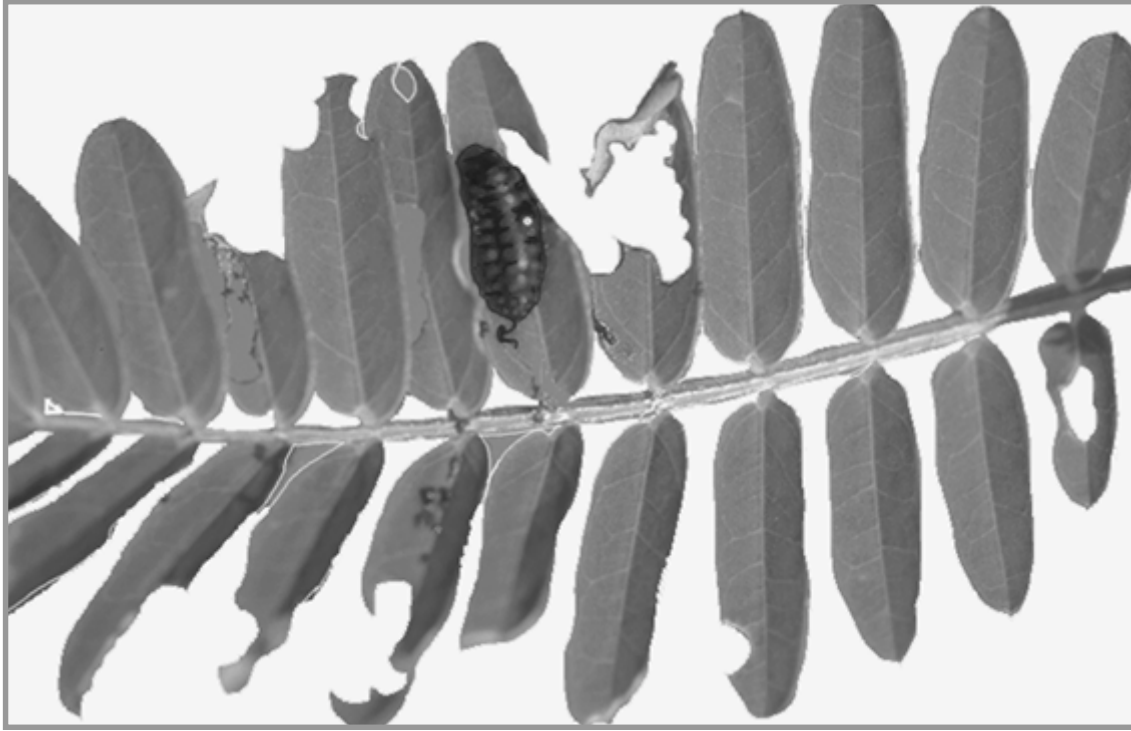
Several insects and mites will attack trees in the nursery and in the field. The damage they cause will depend on their feeding habit which can be biting and chewing (crickets, grasshoppers, locusts, beetles, caterpillars, sawflies etc.) piercing and sucking (aphids, psyllids, leafhoppers, mites, plant bugs etc.) or scraping and sucking (thrips, larvae of fruit flies etc.).

Table 5 describes the main types of damage caused by different types of insects or mites:

Table 5: Damage caused by insects and mites

Type	Damage
Root-feeding insects	Can be very important in nurseries. Predominant are the white grubs (<i>Phyllophaga</i> spp.) which feed on secondary roots and debark the main root. Seedlings turn yellow, lose their leaves and die.
Cutworms and crickets	Mostly <i>Agrotis</i> spp. and <i>Spodoptera</i> spp. They cut the stems of young seedlings and feed on leaves and roots.
Defoliators	Leaves of seedlings can be damaged by a wide range of insects and mites: <ul style="list-style-type: none"> ▪ Large insects such as grasshoppers, crickets and leaf-cutting ants cut large pieces from the leaves. ▪ Caterpillars and other larvae may feed on the leaf blade and leave the veins intact; the black larvae of the sesbania beetle (<i>Mesoplatys ochroptera</i>) are serious defoliators, and may prevent any seedling establishment. ▪ Leaf-rollers and webworms (some caterpillars) roll up parts of the leaf, or web together leaves, to protect themselves while they feed; <i>Sesbania sesban</i> seedlings in western Kenya have been found to be affected by the 'simsim webworm' (Pyrulidae), a pest of simsim (<i>Sesamum indicum</i>). ▪ Thrips and mites scrape the leaves, which become deformed, shrivel and fall.
Leaf miners	Chewing insects which enter and feed on the internal tissue of the leaf; damage is seen as transparent blisters or tunnels; species of the diptera family (<i>Agromyzidae</i>), as well as some species of beetles, lepidoptera and wasps.
Gall formers	Insects that cause the plant to produce tumours, mainly on the leaves, which may twist and fall; they belong to one family of mites (<i>Eriophyidae</i>) and a few insect families (gall midges, gall wasps, sawflies and psyllids).
Sucking pests	Certain bugs, cicadas, leafhoppers, aphids, psyllids and scale insects, affect foliage as well as young stems; apart from their direct damage (leaf fall), they may also transmit viruses, and cause sooty mould infection of the leaves

Next to removing large insects manually and destroying affected plants, chemical control using pesticides is often the main means of insect and mite control. If in doubt, contact a knowledgeable specialist to apply the correct treatment.



Larva and damage of the *Mesoplatys* beetle on young *Sesbania* spp. Leaves.

Other causes for seedling damage

Slugs cause damage similar to that of chewing insects and can destroy whole nursery beds in a short time.

Young seedlings may further be lost to roaming domestic animals, rabbits, lizards or antelopes. Fencing plots may help in certain cases.

Phytosanitary measures

Preventive measures

Ideally, plant health problems should be controlled before they even appear. Far too often however, few resources are allocated for prevention, and only when the first losses occur are expenses incurred and control measures implemented. Prevention requires knowledge on pest and disease biology and routine pest assessments.

Adequate nursery management should take into consideration all the following points:

- Seedbed and potting soil should not only contain necessary nutrients and adequate structure, but also need to be largely free of soil-borne pests and diseases. Soil will almost always harbour pathogenic nematodes, fungi, and bacterial and/or virus pathogens; soil sterilization before sowing is the most effective way to prevent soil-borne disease outbreaks. Heat sterilization of the soil (e.g. steaming in drums) is usually superior to chemical sterilization, although the latter, especially when solids (granules) are used, does not require expensive equipment. More intensive sterilization is generally required for fungal control, as compared to nematode control; simple heating of the soil in a drum for a few hours often gives adequate control of parasitic nematodes. Outbreaks of fungal diseases are largely dependent on environmental conditions, and therefore very difficult to predict. Nematodes are more likely to be influenced by host susceptibility. Planting known hosts in suspect nursery soil will allow the assessment of a potential nematode problem and influence a decision to apply preventive soil treatment.
- In order to avoid a high level of soil parasites, especially nematodes, seedbed rotation, similar to the practice of crop rotation in the field, may prove to be a cheap and useful alternative or addition to soil sterilization. A rotation scheme should be developed for all seedbeds, based on the nematode host status: susceptible → poor host → poor host → non-host or resistant → susceptible. This requires that the nematode susceptibility or resistance of the trees are known which is not always the case.
- Seedbeds may be protected by mixing seedlings and plants with pesticide properties. For instance, marigold (*Tagetes* spp.) and simsim (*Sesamum indicum*) intercropped with root-knot nematode susceptible plants can reduce infestation of the latter, probably through nematicidal root exudates. Marigold will also deter many insects, such as ants and termites.
- Seed soaking and dipping of cuttings and bare root seedlings in fungicide or nematicide solution, e.g. 10% household bleach, is another technique to protect plant material from soil-borne diseases. Most seed treatments require special machinery, although in some cases simple soaking of the material can be done.
- Baits and traps can be used to divert insects and mammals from planting material (and can be mixed with poison); cutworms are attracted by molasses, white flies by yellow surfaces; army worms and other marching caterpillars can be trapped by digging a trench about 60 cm wide and 45 cm deep along the side of the seedbed; incoming worms can be killed by rolling a log backwards and forwards over them, or by filling the trench with straw and setting it alight.

- Removal and burning of infested material, such as nematode infested roots or diseased plants and leaves, will destroy at least part of the inoculum potential and lessen chances of re-emergence of the problem. Even plants, which were affected but have since recovered, should be removed. Pathogen populations, though decimated, will still be present and waiting for the appropriate conditions to re-establish themselves. A general recommendation could be to remove all plant material and leaf litter on the soil surface, along with the older, lower leaves on the stem of the plant. This requires full time vigilance in the nursery.
- Management of the seedling environment is one of the central issues in the control of diseases by cultural practices. Since seedlings, during and after emergence from the soil, are particularly prone to pathogen attack, any measure that shortens this danger period and/or manipulates the environment to the disadvantage of the pathogen is beneficial. Depending on soil type and moisture status, depth of sowing can be adjusted to shorten the period of emergence and thus reduce the risk of damping-off diseases. Frequency and dosage of watering, drainage and aeration can all be adjusted to reduce risks of pest and disease outbreaks. Proper site location, planting density and weed control can also contribute to create optimal conditions for plant growth and thus control certain pest and disease outbreaks.

Curative measures

In reality, pest and disease control is still largely dependent on the use of pesticides. They are widely available and their effect is immediate.

Biological control, despite ever increasing research, has only limited practical use, and is still to be widely commercialized. Nevertheless one should be aware that many insects are not pests, but are in fact beneficial, and may aid in the control of real pests (e.g. ladybirds are predators of aphids). When selecting a chemical for controlling a known pest, preference should be given to the most specific product available. Some insecticides are highly specific against aphids, but will not affect beneficial insects, such as ladybirds.

A lot of the negative side effects of pesticides related to environmental pollution are less of a problem in nurseries because of the small scale of such operation. However, effective and efficient application requires knowledge of the pesticide used, its dosage, application method and safety precautions. This information should be indicated on the label of the pesticide container. In any case, protective clothing such as rubber boots, gloves and a dust mask should be used.

Pesticides are applied either into the soil or onto the aerial parts of the plant, and their activity is either through direct contact with the parasite, or it is taken up by the plant and provides protection from the inside (systemic).

Soil pesticides are available as granulates, liquids and fumigants. The latter require special equipment and are probably only justified for large-scale commercial nurseries. Liquids can be applied together with the irrigation system. The most easy to apply are granulates which are incorporated in the soil. All pesticides disinfect and sterilize the soil medium. In the closed soil environment of nurseries (seedbeds and pots) they will almost always provide very good control. There are a number of recommended soil pesticides available which are active against both nematodes and soil insects. Some recommended multipurpose fumigants are general biocides, which will control soil-borne diseases and weeds, as well as nematodes and soil insects. Always consult with specialists in chemical pest control since the use of pesticides is subjected to strict regulations and recommendations.

Whereas sterilizing the soil medium can largely prevent soil-borne diseases, air-borne pests and diseases are more difficult to prevent and may occur unexpectedly, often as a result of changes in the weather. Mostly sprays are used, although dusts may be more appropriate when water for spraying is scarce. Leaf application of pesticides should not be carried out when rain is expected since this may wash the chemical off. A large number of insecticides are available, from very specific ones to broad-spectrum insecticides. Fungicides are fewer and usually broad spectrum. They are more versatile and the same product may be used on soil, leaves and seeds. Most have no injurious effect on other organisms.

A lot of plants contain chemicals that have pesticidal (insecticidal and/or nematocidal) properties, and are referred to as natural pesticides. Watery suspensions of leaves, seeds or fruits can be used as alternatives to chemical sprays. Best known are pyrethrum (flowers), neem (seeds), marigold, chilli pepper (against aphids), derris roots (rotenone), garlic and tobacco.

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Nursery management - practical

Objectives

The objective of this practical exercise on nursery management is to allow participants to observe and discuss possible problem areas in tree nursery management while visiting a tree nursery.

An additional objective can be to practice some of the basic nursery activities such as pricking out, composting, watering, etc.

Prerequisites

The following prerequisites, tools and materials are needed for this practical:

- A well-equipped and maintained tree nursery.
- Plants grown in various mixtures of different potting mixtures, e.g.: forest soil, sand, compost, sawdust, manure, vermiculite, cocopeat.
- Plants with root deformities raised in nursery containers which students can open to inspect root system development.
- Plants grown in root trainers to demonstrate their benefit.
- Sufficient seedlings of an agroforestry tree species ready for pricking out.
- Polybags and root trainers filled with an appropriate potting mixture.
- A composting area to demonstrate compost making.

Assignments

- 1) Participants and resource persons visit an established tree nursery and discuss the following aspects:
 - a) Compare the observed nursery management practices and procedures to your own. What do you do that is different? Why?
 - b) Do you observe any management practices that you do not agree with? Why? Suggest changes or improvements.
 - c) Identify practices and procedures that are new to you? Explain their advantages over other ones participants may know of.
 - d) Discuss cost/benefit of certain nursery management practices and investments.
- 2) Assessing different potting mixtures. Compare and describe the development of seedlings grown in various potting mixtures. Then open the containers and describe the root system development of the plants. Identify reasons for the different development.

- 3) Root deformities. Open five containers and inspect the root system. Make drawings of the roots and discuss possible reasons for their growth form. Discuss how root deformities can be avoided.

- 4) Pricking out:
 - a) carefully wet the soil of the seedling box or seedling bed to allow easy removal of the plants;
 - b) lift the seedlings with a little shovel or a flat piece of wood. Select only healthy strong seedlings;
 - c) always hold the seedlings by their leaves - never on their stem which may not recover if pressed;
 - d) if roots are too long, prune them with a sharp knife;
 - e) place the seedlings into a flat recipient with water and cover with a moist cloth or straw;
 - f) using a sharp stick or a dibbler , prepare a hole in the container that is big enough to accommodate the roots without bending them;
 - g) carefully insert the seedling into the hole and lift it slightly to allow the roots to straighten;
 - h) close the hole by pressing the soil gently against the roots so that the seedling sits firmly in the container;
 - i) water and put the containers in a shaded area.

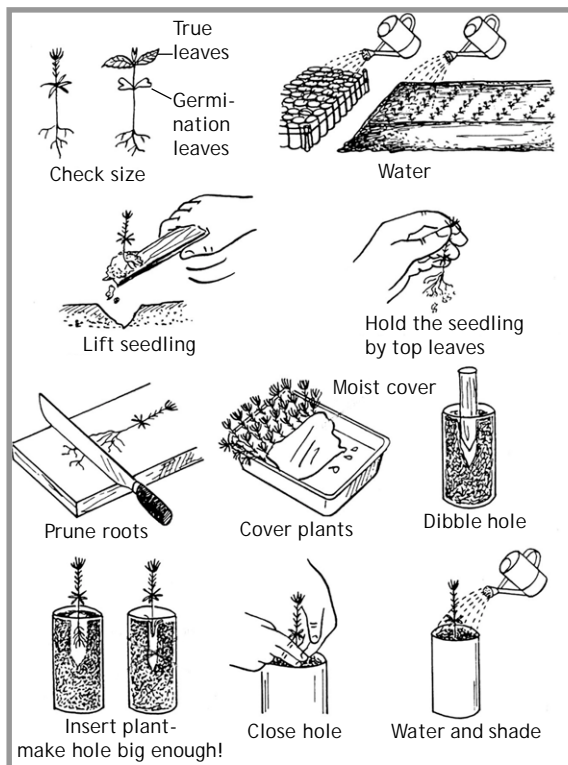


Figure 2-1. Pricking out (ILO, 1992).

- 5) Composting: Participants observe a composting operation and discuss the benefits of organic matter in potting mixtures.
- put your hand inside the compost heap and describe the temperature;
 - put a thermometer into the compost and write down the temperature reading after 5 minutes;
 - remove some of the composted material, smell it and describe.

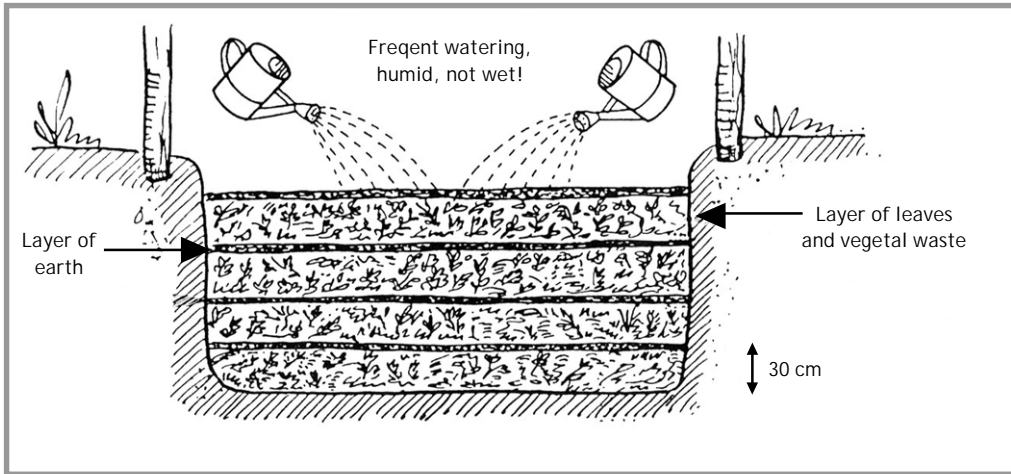


Figure 2-2. Making compost in a compost pit or ditch (ILO, 1992).

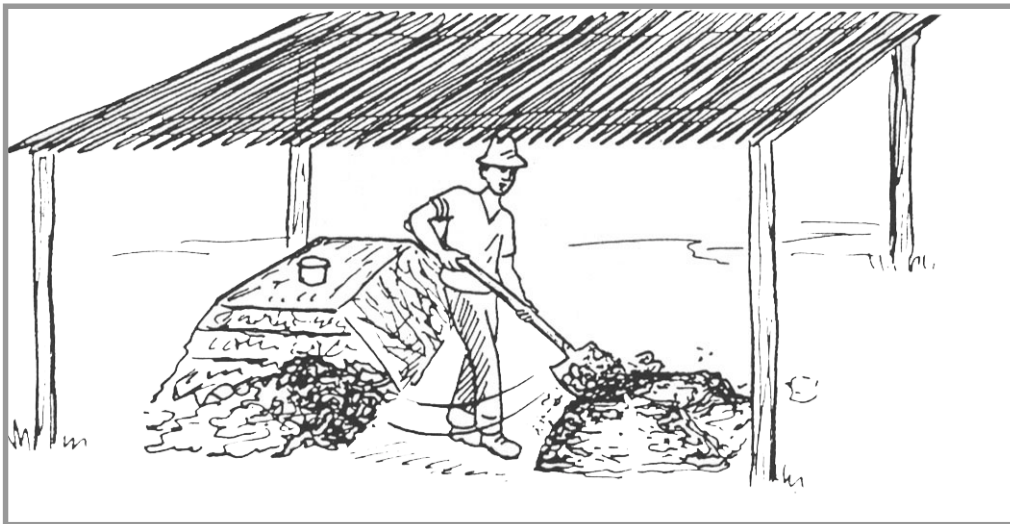
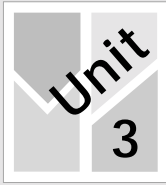


Figure 2-3. Making compost in a compost mound. When the compost shrinks after heating up, turn it over and arrange a new heap.



Cuttings

Training Guidelines

Instructional objectives

At the end of the unit on cuttings, participants will be able to:

- List and describe the different phases of the rooting process of cuttings.
- Explain the physiological background of the rooting process.
- Describe and build a non-mist propagator used for the rooting of cuttings.
- Propagate selected agroforestry trees using stem or root cuttings.

Instructional methods

The unit consists of a 60 to 90 minute theoretical presentation supported by the usual audio-visual aids (video, slides, transparencies). This is followed by a practical on preparing for and taking of cuttings, non-mist propagation of these and post-rooting care of the young plants.

Instructional materials

A lecture note supports the theoretical presentation, and the materials needed for the cuttings practical and demonstration are listed in the detailed description of the practical.

Unit summary

The lecture describes how and why agroforestry trees can be propagated using root or stem cuttings. The rooting process can be seen as the succession of the following stages; propagation, induction, rearrangement of tissues, initiation of roots, elongation and development of roots and the development of a new plant as a whole.

Several factors affect this process: hormonal balance and induction, water, mineral and energy status, the number of leaves and the phytopathological status of the plants. Some of these factors can be modified exogenously to promote rooting and new plant development.

One of the main factors affecting the success of the rooting of cuttings in the tropics is the water status of the plants and the environment; if the cuttings and plants are too dry they will wilt, too moist and fungal or bacterial diseases may affect them. It is therefore important to control the ambient air humidity of the cutting environment, and this can be achieved using mist or non-mist propagation structures. The lecture describes a non-mist propagator and students may construct one during a practical session or have a demonstration of what this looks like.

It is important to take good care of the mother or stock plants from which cuttings will be taken and the lecture describes the different steps leading to the taking of cuttings. Care of the cuttings will involve the reduction of the leaf surface, the use of root promoting hormones and where needed, fertilizer and pesticide use to ensure good development of the cuttings.

Once the cuttings have rooted, they can be potted and hardened-off in preparation for their planting in the field.

Recommended reading

The following publication may further enhance your understanding of the unit:

- Longman KA. 1993. *Rooting Cuttings of Tropical Trees*. Tropical Trees: Propagation and Planting Materials Vol. 1. London: Commonwealth Science Council.



Cuttings principles and techniques

Ze'ev Wiesman-Ben Gurion University, Israel and Zac Tchoundjeu-ICRAF

Introduction

Taking stem cuttings is perhaps the most common way to vegetatively propagate shrubs or trees. The process is relatively simple requiring only a limited area for reproduction, whilst a single mother- or stock plant can yield many cuttings. A large number of ornamental plants are propagated this way, but little is known about the use of this method for most agroforestry trees. The following paragraphs briefly describe some of the underlying principles of the cutting and rooting process, highlight the different factors influencing this and look at the different steps leading to the successful propagation of trees and shrubs through this technique.

Rooting process

The following diagram illustrates the different stages in the rooting process of cuttings and indicates which exogenous and/or endogenous factors influence these stages.

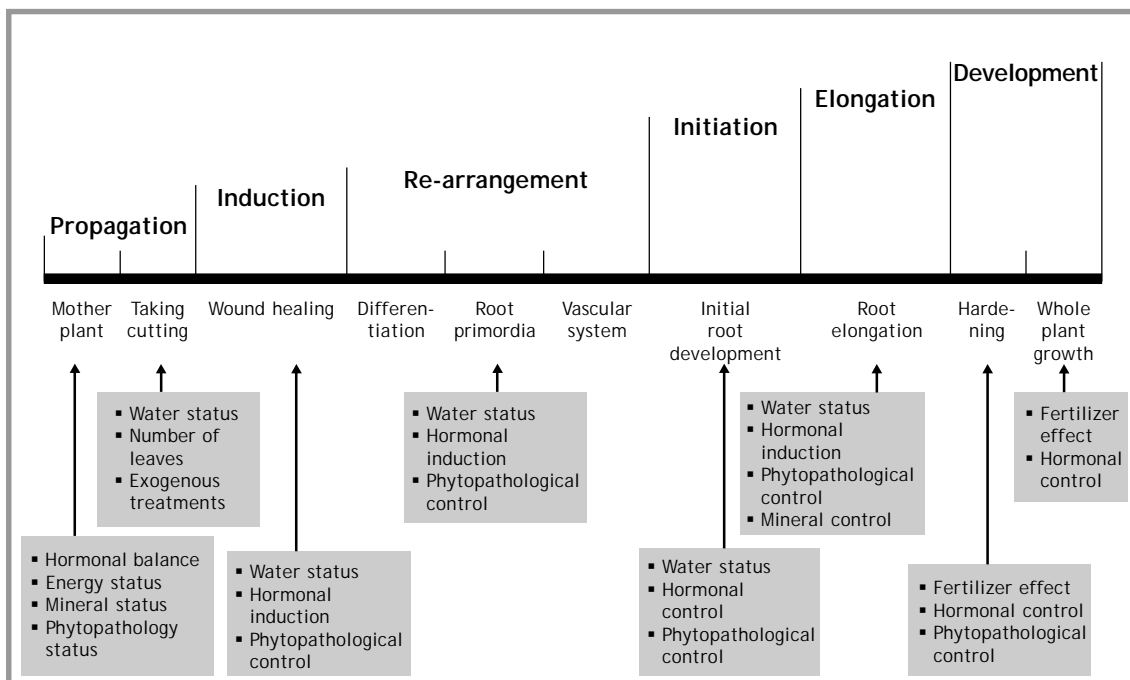


Figure 3-1. Different stages in the rooting process, and the factors influencing them.

The rooting of stem cuttings is a complex process resulting from a combination of many factors. The success of taking cuttings starts with the status of the stock- or motherplants and this is affected by several endogenous and exogenous factors. Once the cuttings are harvested from the mother plant, several measures need to be taken to ensure proper conditions for the

rooting process. This starts with a healing process, the formation of new cells, the induction of root formation, the linking up or bridging of these roots with the existing vascular tissue of the cutting stem, elongation of these newly formed roots and finally the development of a new functional plant from the cut stem pieces. Again, several exogenous and endogenous factors influence the success of this process.

Factors affecting the rooting process

In the following paragraphs, the most important factors, which influence the success of rooting cuttings, will be briefly described. They are: the rooting substrate, humidity, plant hormones, leaf area, light and temperature, and plant hygiene.

Rooting substrate

Determination of appropriate substrates is essential for the rooting of stem cuttings. Most tropical tree species require a light medium with good drainage to prevent waterlogging and subsequent rotting of the cuttings. The following substrates were found to satisfy these requirements:

- rotted sawdust
- fine river sand
- river sand and saw dust mixture (50:50 v/v)
- coarse gravel
- coarse gravel and sawdust mixture (50:50 v/v)
- vermiculite

In order to avoid pest and disease attacks, the substrates should be washed properly before use and sterilized if possible. They should be renewed at least once per year.

Humidity

As soon as a cutting is removed from a stock plant, it will not be able to take up the water needed for its survival and development. It thus becomes critical to maintain an optimal level of ambient humidity to make sure that the cuttings will not wilt and dry out due to low humidity, or become diseased because of a too high humidity. Water is an important external factor affecting the success of rooting of the cuttings.

Hormones

As mentioned in the introduction, plant hormones are of paramount importance in the multiplication process. Certain hormones such as auxins (IBA, IAA, NAA) will influence root development, and others such as gibberellins will influence stem elongation and bud development. Depending on the balance of these hormones in the motherplant and in the cuttings,

the rooting process will be affected either positively or adversely. Therefore, it is sometimes necessary to increase the amount of root promoting hormones. Synthetic plant hormones can be applied to promote the root development process either through their direct action on the root development process or through an antagonistic action on root inhibiting hormones. The appropriate balance of plant hormones in the cutting will affect wound healing, the development of root primordia, initial root development, root elongation, hardening and further development of the rooted cutting. The hormonal balance in the stock plant will influence that of the cuttings, and thus timing of taking cuttings is an important consideration in the cutting process. In general, it will be important to go through a set of experiments to determine appropriate auxin concentration for rooting unknown species. The starting point may be the use of 50µg of IBA, NAA, IAA or a mixture of IBA and NAA. However it must be noted that not all species require auxin for rooting.

Leaf area

Plants need nutrients (nitrogen, phosphorous, potassium, etc.) and metabolites (proteins, lipids, carbohydrates) for their growth and development and thus it is important that motherplants and cuttings are in optimal condition as far as their nutrient and energy status is concerned. In cuttings, this metabolic activity takes place in the leaves remaining on the cutting.

The initiation of roots in a cutting relies on the photosynthetic activity of the leaf area of the cutting. It is therefore important to maintain a sufficiently large leaf area on a cutting so that the leaves can continue to produce the metabolites necessary for root initiation through photosynthesis. At the same time, the cuttings will lose water through transpiration of this leaf area. The recommended leaf area of a cutting will need to ensure that there is an optimal balance between these two processes and this will vary from species to species. For example, the optimum rooting percentages of *Khaya ivorensis* under intermittent mist were obtained when leaves were trimmed to 50-100 cm² on a cutting. In contrast to *K. ivorensis*, *Lovoa trichilioides* cuttings needed larger leaf areas (200 cm²) for optimum rooting in a non-mist propagator. In the absence of any information on optimal leaf area of the species, a leaf area of 50 cm² is recommended which can then be compared to other sizes to determine the optimum leaf area for cuttings of the species under investigation.

Light and temperature

Ambient light and temperature conditions will also influence the rooting process. Control of these factors often requires equipment and infrastructure that may not be readily available in all nurseries (electricity, additional light or complete darkness, heating cables in the rooting substrate). Research is needed to determine the influence of these factors on the rooting of cuttings of different agroforestry species, and in finding ways to circumvent technical difficulties.

While irradiance probably affects rooting directly via its effect on photosynthesis, it is not

clear how light quality influences rooting. In *Triplochiton scleroxylon*, the measurement of rates of net photosynthesis in stock plants, grown under different levels of irradiance, indicated that rooting ability is strongly correlated with the photosynthetic activities (Leahey and Storeton-West 1992).

Phytopsanitary aspects

The health status of stock plants and cuttings is also important. Care must be taken not to collect cuttings from diseased stock plants, especially where fungi, bacteria or viruses are concerned. This may not only be detrimental to the rooting process itself but will also result in further spreading the disease if infected cuttings are transplanted to the field. In some cases, cuttings can be treated with a pesticide or soaked in a surface sterilant, such as diluted household bleach (see also unit 2).

Preparing cuttings

Management of stock plants

Some important rules for the management of stock plants are:

- Establish stock plants as close as possible to the propagation area.
- Prune the stock plants regularly (thrice a year) to encourage production of good shoots and maintain juvenility of the vegetative material. Always conserve one pair of feeding leaves on each plant.
- Use fertilizer to accelerate growth on nutrient deficient soils.
- Recommended plant spacing for most species; 1-2 m between rows, 0.5-1 m within each row.
- Separate different clones from each other and label them clearly. Allow some clones to grow as to express the clonal characteristics of the mature trees.
- Grow stock plants under light shade, for example intercropped with *Calliandra* or *Leucaena*.

Taking cuttings

- Cuttings should be taken early in the morning before the sun is hot, as this will keep transpiration and thus drying out to a minimum.
- Trim leaves before the shoots are detached from the stock plants as this reduces water-loss. Leaf areas for optimum rooting vary with species, however, 50 cm² seems to be the recommended leaf area prior to full investigation on this factor for different agroforestry species. The leaf area should allow for a balance between photosynthesis and transpiration when cuttings are under the non-mist propagator.
- Use a polyethylene bag that is moistened inside to carry the shoots.
- Keep the collected shoots under shade, without throwing or squeezing the bags.
- If you are carrying the shoots over a longer distance, keep them in a cool box – but ensure that the shoots do not directly touch the cooling elements.

- In the nursery, have all equipment and tools ready and well arranged in advance in order to keep cuttings moist and transfer to propagators without delay. Delay can cause the cuttings to dry out and is often responsible for rooting failure of cuttings in arid and semi-arid zones.

Propagation facilities

Mist propagation

A critical factor in the successful rooting of cuttings is the maintenance of a humid environment to reduce water-loss through transpiration. Mist propagation is a technically advanced system to achieve this. It uses a high-pressure irrigation system that produces a fine mist through special mist jets placed above the cuttings. The frequency and duration of a mist application can be controlled using a timer, a moisture sensitive switch or a so-called 'electronic leaf'. Since this system is expensive and requires reliable electricity and water supplies, it is not recommended for places where these utilities may not be available or can be unreliable.

Non-mist propagation

A suitable alternative for maintaining a moist environment is the non-mist propagator. This is a simple wooden frame enclosed by clear or white polyethylene sheeting. The propagator is filled with a moist rooting medium and contains a reserve of water (Photo below). To minimize the effect of light (quality and quantity) on rooting ability, propagators should be placed under uniform shade. If possible, a 60% shade cloth should be used to protect the propagators.

As described above, the temperature of the propagation environment is also an important factor in rooting success. In non-mist propagators, it usually varies between 28–30°C. In hot and dry zones, frequent watering of the propagator itself can reduce excessively high temperatures.

Humidity also varies within the propagator. Humidity levels are about 90–100% after watering, however the level drops rapidly to as low as 40% when the propagator is opened. To maintain high humidity, the cuttings and the air space within the propagator should be sprayed once a day with a hand-sprayer. Temperature and humidity are the main factors that should be constantly monitored within the propagators.



A non-mist propagator.

Post propagation care

Potting

Potting-up is a delicate process in vegetative tree propagation, where one can easily lose all the rooted material. The same care as described earlier for pricking out seedlings (Unit 2) should be applied. Remove the rooted cutting gently from the rooting substrate using a small flat piece of wood, shake off loose rooting substrate and place the cutting into a container which is already partly filled with a suitable, light but nutrient-rich substrate. Cover the exposed roots with substrate, press substrate firmly around the cutting, and water. Newly potted cuttings need to remain in a humid and well-shaded environment until shoot growth commences. Watering at this level should be done with care, preferably with a sprayer or a watering hose with a fine nozzle.

Hardening

Hardening-off is to gradually accustom potted cuttings to grow under ordinary nursery or field conditions. This is done through a stepwise decrease in the humidity previously needed for the rooting of the cuttings. Under the harsh environment of the Sahel, potted cuttings of *Prosopis africana* were kept in closed propagators for three weeks, whereas *Bauhinia rufescens* needed only two weeks (Tchoundjeu 1996). Afterwards, the propagators were opened during the night (1 week), then night and day, except on very hot days. In the last phase, plants were moved to the nursery still under dense shade. Species differ in their requirements: *Prunus africana* roots well but the hardening is more difficult, while *Pterocarpus erinaceus*, *Bauhinia rufescens* and *Tamarindus indica* root well and are relatively easy to harden.

References

- Leakey RRB and Storeton-West R. 1992. The rooting ability of *Triplochiton scleroxylon* cuttings: the interaction between stock plant irradiance, light quality and nutrients. *Forest Ecology and Management* 49: 133-150.
- Longman KA. 1993. *Rooting Cuttings of Tropical Trees. Tropical Trees: Propagation and Planting Materials* Vol. 1. London: Commonwealth Science Council.
- Tchoundjeu Z. 1996. Vegetative propagation of Sahelian agroforestry tree species: *Prosopis africana* and *Bauhinia rufescens*. In: Dieters MJ, Nikles DG, Harwood CE and Walker SM (eds.) *Tree Improvement for Sustainable Tropical Forestry*. Proceedings QFRI-IUFRO Conference, Caloundra, Queensland, Australia: . 27 October - 1 November 1996.



Cuttings practical

Objectives

The objective of this practical session is to allow participants to see the effects of auxin in rooting cuttings and to use a non-mist propagator for setting cuttings.

The participants should work in groups of 3-4. Each group should have the chance to assist in the building of a poly-propagator and set cuttings applying different root promoting hormones.

Prerequisites

The following tools and materials are needed for this practical:

- **Polypropagators** with different rooting substrates: sand, fine gravel, sawdust etc.
- Small containers with **rooting powders** in different strength for each group: for example IBA 0.1 %, 0.3 %, 0.8 %. Other products if available (e.g. IAA)
- Large **plastic bags** for collection of the cuttings.
- **Cool box** and **ice blocks** to store the cuttings if not immediately used.
- **Secateurs**, **scissors** and sharp **knives** for preparation of the cuttings.
- A small bottle of 80 % **ethanol** or **methyated spirit** to disinfect the tools.
- A **handsprayer** for each group.
- **Calliper** and **ruler** to measure the cuttings before setting.
- **Labels** and **assessment sheets**.
- A series of selected agroforestry **stock plants**. Seedlings or coppiced plants with shoots of about 30 cm are suitable.

Assignments

Building a polypropagator

Although the task of this practical is not to actually build a complete propagator, it is advisable to allow the students to finish filling a propagator with its substrates to understand the process and be aware of potential pitfalls. The following steps give an overview of the process.

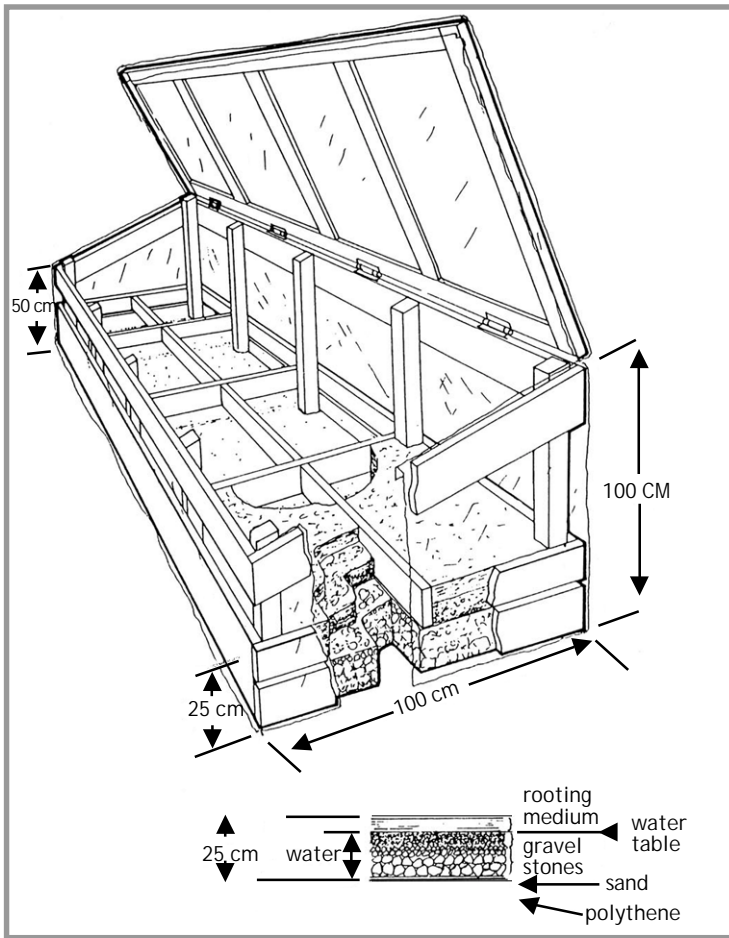


Figure 3-2. Schematic representation of a non-mist propagator (Longman 1993).

The following materials and steps are required to build a polypropagator of 1 x 3 x 1 m:

- 1 The frame should preferably be made of durable, termite resistant wood, especially the parts that are resting on the ground. Alternatively, the wood should be treated with a preservative; however, you will need to ensure that the preservative does not damage the cuttings. You need approximately 8 m of 250 x 25 mm, 10 m of 50 x 50 mm and 32 m of 50 x 25 mm timber.
- 2 Strong quality polyethylene sheeting: 10 m length of 2 m wide material.
- 3 0.5 m³ of broken cement blocks or stones (30–120 mm), 0.25 m³ of gravel (5–10 mm), 0.25 m³ of coarse sand.
- 4 Fixing materials: nails, office stapler and drawing pins to join and fix polyethylene sheeting, hinges and screws, and clips, to secure covers.
- 5 A double piece of polyethylene without holes should be used for the base of the propagator. This should be left loose enough so that when it rests on the ground, it will hold the filling/drainage without excessive strain.
- 6 Align the long axis of the propagator in an east-west direction. It is vital to level the ground, and spread sand to prevent the polyethylene sheet from getting pierced or stretched by stones. Use a level gauge to make sure that the propagator stands level, as one compartment should not have more water than the others.

7. Put a short piece of plastic pipe or bamboo (25-30 cm long and about 5 cm in diameter) in the corner vertically. This will help to check the water level easily, and fill water if needed, without soaking the rooting medium.
8. All the filling/drainage material should be thoroughly washed before use. It is also important when attaching polyethylene sheeting, to make double overlapping joints between one sheet and another, as this will help to conserve high humidity within the propagator.
9. Add the different substrates carefully so as not to damage the polyethylene sheet: a thin layer of river sand, a thick layer of stones, a thick layer of gravel, a thin layer of sand (adding up to a total of about 15-25 cm), then add water until the filling/drainage layer is fully saturated.
10. Add about 10 cm depth of rooting medium on top. The rooting medium should be moist but not waterlogged, or the cuttings will not thrive.

Maintenance will involve regularly checking the water level (each week); water will be added when needed using a plastic pipe. The outside of the propagator should be cleaned regularly in order to allow enough light to enter the propagator. It is important to patch up any holes in the polyethylene sheet with a small piece of sticky tape in order to conserve high humidity within the propagator.

Collecting and setting cuttings

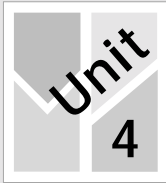
1. Trim the leaves of the selected shoots before cutting them off the stock plant, discard the terminal buds and leaves if they are too soft.
2. Place the shoots quickly into polyethylene bags containing a label, marked with the species name and clone number; and moist paper or other damp material. In absence of damp material, humidify the inside of the bag using a sprayer. Keep the bag closed at all times.
3. To avoid heating up of the cuttings during transport, store them in a cool box but avoid direct contact with the cooling element.
4. In the nursery, put the shoots into a bucket of water or spray them frequently until they are used.
5. Using a sharp knife or secateurs, cut single or double node cuttings. Cut the basal end of the cutting squarely – avoid cutting the base slanted as this may result in a one-sided root system.
6. Dip the basal 0.5-1 cm of the cutting into the required rooting powder. Each participant should have the chance to set 3-4 cuttings of each treatment: for example, control (no treatment), 0.1% IBA, 0.3% IBA, 0.8% IBA.
7. Insert the cutting into a prepared hole in the rooting substrate to a depth of about 2-3 cm, making sure that the leaf is well above the substrate, and firm the cutting in with two fingers.
8. Label the cuttings with: species name, clone number, date of setting and treatment(s) applied. Enter the information on your assessment sheet.
9. Spray the cuttings before closing the lid of the propagator tightly.

Example assessment sheet

Species				
Date of setting				
Substrate				
			Rooting yes/no	
Clone number	Cutting number	Treatment	Date 1	Date 2

cuttings

.....



Grafting

Training guidelines

Instructional objectives

At the end of the unit on grafting, participants will be able to:

- List and explain the main reasons for grafting agroforestry trees.
- Explain some of the underlying physiological principles of grafting and describe the conditions for its successful application.
- List and describe the most common grafting techniques.
- Practise grafting with some selected agroforestry tree species.

Instructional methods

The unit consists of a 60 to 90 minute theoretical presentation, with the usual audio-visual support tools, followed by a 4-hour practical exercise including demonstrations.

Instructional materials

Lecture notes support the theoretical presentation and the materials needed for the practical exercise and demonstrations are listed in the detailed description of the practical.

Unit summary

Grafting is one of the more complex and labour intensive vegetative propagation techniques. Grafting entails the union of the stem part of one plant with the root part of another one to form a new plant. When the stem part of one plant consists of a single bud, the technique is referred to as budding.

As in other vegetative propagation techniques, there are several reasons for grafting or budding agroforestry trees. The most important ones are: to multiply trees that cannot easily be multiplied through sexual or other asexual methods, to replace

the existing root system of a tree with a better one, to decrease the time needed by a tree to reach maturity (flowering, fruiting), to repair damage to older trees or to rejuvenate them with young and improved material.

In grafting, cut material from stems and roots are put together in such a way that new cells, developed as a result of the healing process of the wound, will eventually join together and form new tissues that will allow the grafted plant to grow and develop as a normal one.

When grafting or budding agroforestry trees, it is important to consider the compatibility between the plant materials, as well as their physiological age. Other factors that will affect the success of these techniques relate to the conditions under which they take place such as humidity, temperature, contact surface between the materials and hygiene.

The most common grafting techniques for agroforestry trees are top-wedge grafting, splice grafting, whip and tongue grafting and approach grafting. The most common budding techniques are T- and patch budding.

Recommended reading

The following publications may further enhance your understanding of the unit:

- Edmond JB, Senn TL, Andrews FS and Halfacre RG. 1975. *Fundamentals of horticulture*. USA: McGraw-Hill, Inc.
- Hartmann HT, Kester DE, Davies FT and Geneve RL. 1997. *Plant propagation: Principles and practices*. 6th ed. New Jersey, USA: Prentice Hall.
- Macdonald B. 1986. *Practical woody plant propagation for nursery growers*. Oregon: Timber Press.
- Mudge KW, Mwaka A, Isutsa D, Musoke R, Foster D and Ngoda BJM. 1992. *Plant propagation - a teaching resource packet*. USA: Cornell University.



Grafting principles and techniques

Hannah Jaenicke–ICRAF

Introduction

Grafting, the technique of combining two or more different plants, has been practised for many centuries. Ancient Chinese, Greek and Roman literature refers to grafting, as does the Bible. In the early 19th century, well over 100 different methods of grafting were known (Thouin 1821).

Initially, grafting was practiced on trees that were culturally and economically important, such as olives and citrus in the Mediterranean by the Greeks and Romans. In later centuries, the grafting of ornamentals, such as roses, and of the many other plants imported from foreign countries into European gardens, became important. In the tropics, grafting is practised on a relatively small number of commercially important trees, such as mango, citrus, rubber, and avocado. However, it is also a viable option to domesticate several under-utilized agroforestry tree species.

Grafting is a technique of vegetative propagation that is relatively labour intensive and requires skilled and experienced people for successful and satisfying results.

Definitions

The following definitions are needed to understand grafting and budding techniques and their underlying principles:

- **Grafting:** the technique of connecting two pieces of living plant tissue together so that they will unite and form a functional plant.
- **Scion:** the aerial part of a tree that will form the crown of the new plant. This part contains the dormant buds of the tree whose desired characteristics need to be multiplied.
- **Budding:** a special form of grafting in which the scion consists of either a single or several buds. It is a more economical form of grafting, as more scions can be produced from a single mother tree.
- **Rootstock:** the below-ground or lower part of a tree, sometimes including part of the stem and some branches, that will form the root system of the new plant. This part may also contain dormant buds, which should not be allowed to develop in the new plant since they (suckers) do not have the desired characteristics that need to be multiplied.
- **Vascular cambium:** a thin layer of meristematic cells between a tree's bark (*phloem*) and wood (*xylem*). Meristematic cells are capable of dividing into new cells that may differentiate into new tissues and organs.

- **Callus (tissue):** a mass of undifferentiated cells formed around a plant wound. In grafting or budding, this callus will form around the wounds at the union of the scion and the rootstock. From the callus cells, new vascular tissue develops that will allow scion and rootstock to function as one plant.

Reasons for grafting and budding

The following are the main reasons why you may want to consider grafting or budding agroforestry trees:

- To multiply a tree that cannot be multiplied through sexual or other asexual propagation methods.
- To obtain a tree that combines both the good characteristics of one tree and the rootstock of another one.
- To decrease the amount of time that a tree needs to attain maturity (flowering, fruiting and seeding).
- To rejuvenate older trees through the use of young, improved material from another tree.
- To repair damage caused to certain parts of a tree.
- To detect viral diseases

Multiplication

Many species of tropical trees cannot easily be propagated from stem or root cuttings. Tree domestication programmes, aiming at the capturing of genetic superiority as expressed in mature trees, revert to grafting as an intermediate step to rejuvenate, or reinvigorate, the desired material. A scion taken from the desired tree is grafted onto a vigorous rootstock seedling. Shoots produced by this plant can be rooted, or, if rooting inhibition still occurs, grafted onto a new rootstock. As shown in Figure 4-1, on the following page, several grafting cycles may be required before satisfactory rooting occurs (Siniscalco and Pavollettoni 1988).

Rootstock

Often, a desirable cultivar does not possess a necessary below-ground characteristic that another cultivar or provenance may have. For example, some clones are tolerant or resistant to drought, saline conditions or soil-borne pathogens. These clones can be considered to provide rootstock for other individuals that have desirable above-ground characteristics, such as fruit quality.

In some species, especially citrus, the rootstock can also influence the above-ground fruit quality. Another commercially important influence of the rootstock is the vigour of the combined plant. For many fruit species, dwarfing rootstocks have been developed.

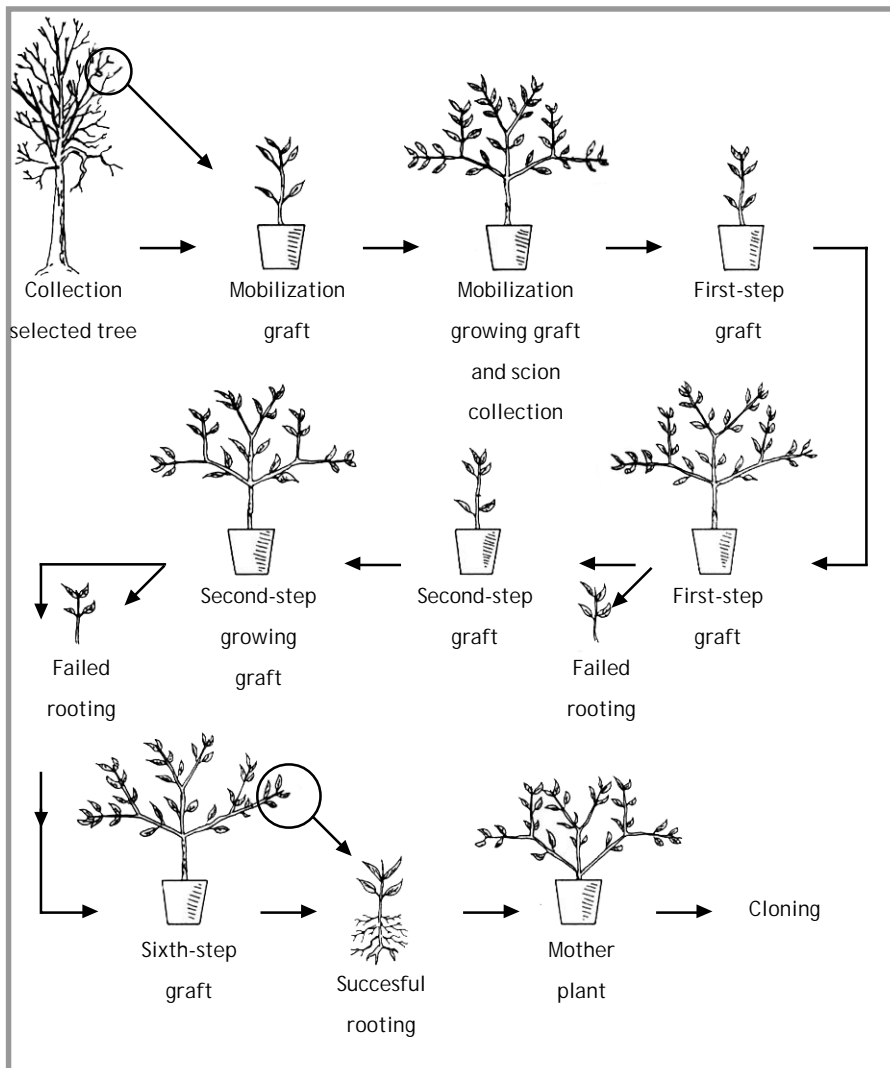


Figure 4-1. Scheme for rejuvenation techniques used in serial graftage of ten-year-old *Eucalyptus x trabuttii* onto juvenile seedling understock. Six serial grafts were needed before mature grafted scions could be used as cuttings and rooted (Siniscalco and Pavolettoni 1988).

Maturity

Vegetative propagation in general is a tool to decrease the time to maturity, of the plants produced. This is the case with cuttings from mature trees or with air layering. Grafting a mature scion onto a young vigorous rootstock has the same effect and it is often more successful than rooting mature cuttings. However, an additional advantage of grafting techniques can be that the time to maturity of young seedlings, for example from a breeding programme, can be greatly reduced, when they are grafted onto a well-established, mature rootstock. The basic principle behind this phenomenon is that a strong root system is already developed and the plant's energy can be utilized in flower and fruit production.

Rejuvenation

A mature tree of an unimproved provenance can be grafted with scions from an improved variety. Examples are mango and citrus, that are frequently sown from seed and do not have the desired fruit quality. Top-grafting of improved scion material onto these established trees can be economically beneficial.

In dioecious plants, which have male and female flowers on separate trees, a problem known to producers is an unfavourable ratio of male to female trees. An unproductive male tree can be changed to a female tree by grafting scions from a female mother plant onto the male stem. If there are not enough pollinators available, (e.g. after unproductive male trees were felled), a scion of a male tree can be inserted into the crown of a female tree.

Another possibility, especially interesting for farmers with very small plots, is the fact that several different varieties of the same species can be grafted onto the same rootstock. In this manner, early and late varieties could be harvested from the same tree.

Damage repair

Occasionally the roots or trunk of a tree are severely damaged by browsing animals or drought. If it is considered worthwhile to save the tree, such damage can be repaired using the bridge grafting technique.

Virus detection

In some commercially important species (e.g. citrus), viral diseases are a serious problem. If there is doubt about whether a mother tree is diseased, a test-bud of that plant can be inserted into a clean, highly susceptible 'indicator' plant. This plant will show the symptoms and thus large-scale spreading of the virus can be prevented.

Physiology

Grafting can be seen as the healing of a wound into which a piece of another plant has been inserted. Physiologically, the same mechanisms as in wound healing, the rapid division of meristematic cells and their following differentiation into the damaged organs, takes place. A successful graft not only has the physical stability of an undamaged plant, but it also functions as one unit after phloem and xylem cells unite.

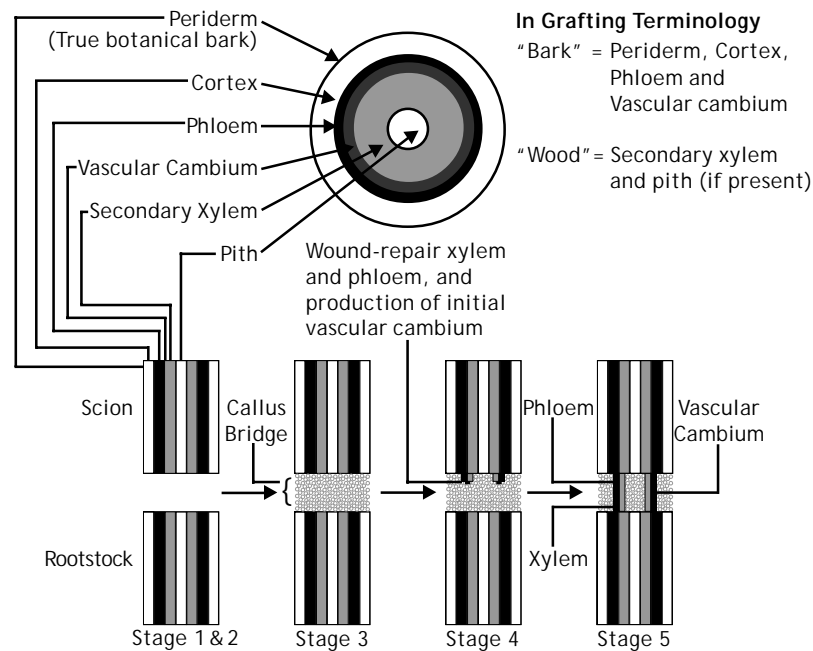
Healing process

The usual sequence in the healing of a graft union is as follows:

- **Lining up of vascular cambiums.** The person carrying out the grafting places the freshly cut scion into direct contact with the freshly cut rootstock. It is of utmost importance that the cambial layers of both plants are in direct contact.

- **Wound healing response.** Necrotic (black) material is formed from the cells damaged by making the cuts.
- **Callus bridge formation.** The next, undamaged layer of cambium cells, produces a large number of parenchyma (tissue) cells that form a callus, and provide a mechanical link between the scion and the rootstock.
- **Cambium formation.** Certain callus cells line up with the cambial layers of both scion and rootstock, and differentiate into new cambium cells.
- **Vascular tissue formation.** Secondary phloem and xylem cells are formed from these new cambium cells, finally establishing a firm vascular connection between the two plants.

Figure 4-2. Top: Grafting terminology of the bark and wood and associated tissues with schematic drawing of a stem cross section of a young woody plant stem. Bottom: Schematic longitudinal section of the stages of graft union formation. (Hartmann et al. 1997).



It is important to note that the graft union is entirely made by the formation and differentiation of new cells. Existing cells of both scion and rootstock do not move or grow together.

Healing conditions

For a successful graft union to be established several factors are important.

The cambial layers of both scion and rootstock must be in intimate contact to allow the newly formed cells to grow into a joined secondary vascular system.

The newly formed cells have a relatively thin cell wall and are unprotected against desiccation, thus the graft union needs to be kept sufficiently moist. This is usually done by wrapping and/or waxing the graft union. However, sufficient oxygen is also necessary as the rapid development of cells is a metabolically highly intensive process. Cases are known (e.g. grapes) in which the waxing of the graft union is detrimental to success, possibly because of suffocation of the tissue.

The ambient temperature will affect the wound healing process. For most plants, the optimum lies somewhere between 15 and 30 °C but for some tropical plants it might be higher. Below that, metabolic activity is too low to guarantee sufficient cell growth; above that, cell death leads to failure.

The high humidity and temperatures that are required for successful grafting are also conducive to bacterial and fungal growth. It is therefore imperative that utmost care and cleanliness be practised when grafting.

The physiological activity of scion and stock plant can have an influence on the wound healing process. Most deciduous plants in temperate regions are best grafted when they have a high metabolic activity and assimilate translocation is high. This is usually the case just before or after budbreak. For many evergreen tropical species, many of which flower and fruit simultaneously, such a distinct pattern cannot be established. In cases where a prolonged dry period induces dormancy, the period just before the onset of the rains may be the best time for grafting.

In addition to these factors affecting the success of grafting and budding, there are species and clone differences. Some species are easily propagated by grafting, though others tend to have a low success rate. It is hypothesized that the reason may be the different speed and vigour of callus formation after wounding, or the ability to exactly line up matching cell formations, in particular the endoplasmatic reticulum of both graft partners (Kollmann 1992). In species that form wound callus readily, the newly formed cells are protected from desiccation and thus survive better than in plants in which callus formation is slow and poor. Tissue incompatibilities (see below) can also be a cause for poor success.

Rootstock-scion relationships

An important reason for grafting is to make use of the influence of a rootstock to a scion in terms of pest and disease resistance, growth or development. Certain rootstocks can be tolerant or resistant to nematodes, fungal, bacterial and viral pathogens, drought or salinity. Rootstocks can also affect tree growth and development, maturity, fruit quality and productivity.

Cases of resistance to factors affecting the root system directly are relatively easy to understand. The genetic constituency of some plant cultivars, varieties or provenances allows survival and growth even under unfavourable conditions. This may be due to mechanical resistance, chemical inhibitors, greater vigour or better nutrient uptake capabilities of the root

tissue. These characteristics do not disappear when a scion from a different plant is grafted on top of the rootstock since the genetic information is not changed.

There are, however, influences that are more complex and that need explanation beyond the simple mechanical characteristics. These are the rootstock influences on growth, productivity and quality. Several explanations for these phenomena have been offered, but they are not consistent and seem to vary between plant species. A common explanation is that the vascular connection transports assimilates and storage products, as well as endogenous growth regulators and other substances (influencing growth, flowering or fruiting behaviour) both upwards and downwards through the plant. Cell division-promoting cytokinins are usually produced in the root tips and transported upwards, therefore an influence from the root system could be expected. It has also been hypothesized that a graft union might result in slightly impaired vascular flow, which could influence the amount of water and growth regulators translocated, thus bringing the plant into a slight stress situation, inducing prolific flowering. Early experiments supporting this theory have already shown that by autografting (grafting a scion onto its own rootstock), increased fruit production could be achieved (Hodgson and Cameron 1935).

It is important to note that the scion can also have an influence on the rootstock. These influences are often undesirable, such as the infection of a susceptible rootstock with a virus transmitted from the scion.

Incompatibility

Problems of graft incompatibility are often cited as the most severe hindrance to full acceptance of grafting, however they do occur and need to be taken into account (Feucht 1987). A general rule states that: the closer the partners are botanically related, the more successful the graft unions should be. Grafting within a clone is the process of grafting a scion back onto the plant from which it came, or grafting it onto another plant from the same clone and is usually successful.

Grafting between clones of the same species (i.e. *Mangifera indica* cv 'Kent' onto *Mangifera indica* landrace) is also considered a normal and generally successful practice. However, incompatibility reactions have been observed within some species.

Grafting between species of the same genus is occasionally successful, but often fails. In temperate horticultural practice, almond, apricot and plum (*Prunus amygdalina*, *P. armeniaca* and *P. domestica*, respectively) are grafted onto peach (*P. persica*). Almond and apricot, however, cannot be inter-grafted. Any of the species within the genus *Citrus* can usually be inter-grafted without problems.

Grafting between genera within a family is rarely successful. An example is the trifoliolate orange (*Poncirus trifoliata*) that is used commercially as dwarfing rootstock for orange (*Citrus sinensis*).

Grafting between families has been shown to work in isolated experimental cases, and mostly with annual plants in which delayed graft incompatibilities may not show. A noteworthy

example, which helped a great deal in our understanding of the processes during graft union formation, is the grafting of *Vicia faba* on *Helianthus annuus* rootstock (Kollmann 1992).

Incompatibility symptoms often do not show immediately; they can appear as late as many decades after the union was formed, for example when storm damage can cause the trunk to break at the point of the graft union. The most common incompatibility symptoms are:

- Failure to form a successful graft union.
- Early defoliation of deciduous plants, decline in vegetative growth due to shoot die-back and general ill-health of the plant.
- Premature death of trees after a few years or while still in the nursery.
- Marked differences in growth rate or vigour between scion and rootstock; overgrowth at, above, or below the graft union.
- Differences between scion and rootstock in onset of vegetative growth after dormancy due to drought or low temperatures.
- Graft components break apart cleanly at the graft union.

Apart from breakage, isolated cases of the above symptoms are not indicative of incompatibilities. Sometimes, graft incompatibilities can be avoided by the use of a mutually compatible interstock, which is an insertion between the intended rootstock and scion of a third cultivar. This interstock then provides a bridge but still allows the characteristics of both scion and rootstock to be expressed.

Grafting and budding techniques

Top-wedge grafting

This is the method most commonly used, as it is simple and usually successful with both seedlings and older trees. It is often used in topworking older trees as it can be used with scions considerably thinner than the rootstock. In topworking older trees, two small scions are usually inserted at either side of the cleft. In these cases it is important that the scions are cut so that the outside of the wedge is slightly thicker than the inside to allow for the larger circumference.



Figure 4-3. Top or wedge graft. (Mudge et al. 1992).

Splice and whip and tongue grafting

A long, slanting cut is made in both scion and rootstock and these are tied together. This method is simple but needs some practice to allow for evenly slanting cuts and for matching scions and rootstocks. When tying-in, care is needed to prevent inadvertently slipping when joining the pieces. It is the technique of choice for material with a very pithy stem.

A more secure version of the splice graft is the whip and tongue graft in which a second short vertical cut is made 2/3 from the tip of the cuts in both scion and rootstock. The 'tongues' of both scion and rootstock are then slit into each other and the graft securely tied in. The advantage of this form of grafting is a larger portion of cambial cells to match and an initial good hold of the scion into the rootstock. The method requires soft material and is often used with young plants that have only limited lignification.



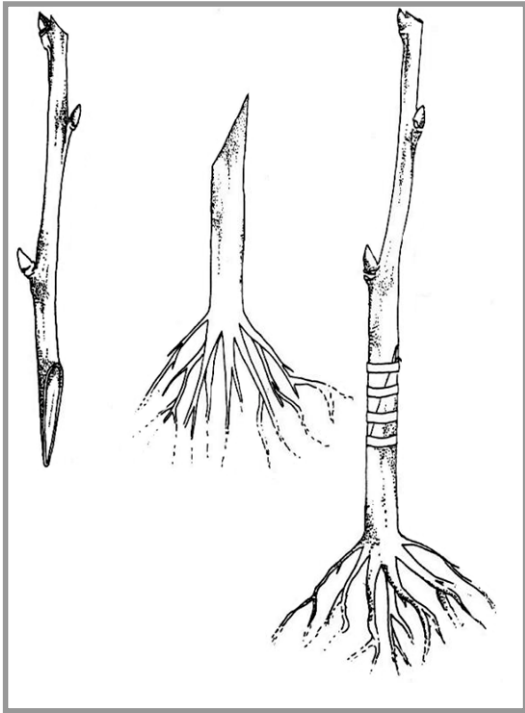


Figure 4-4. Splice grafting. (Macdonald 1996).

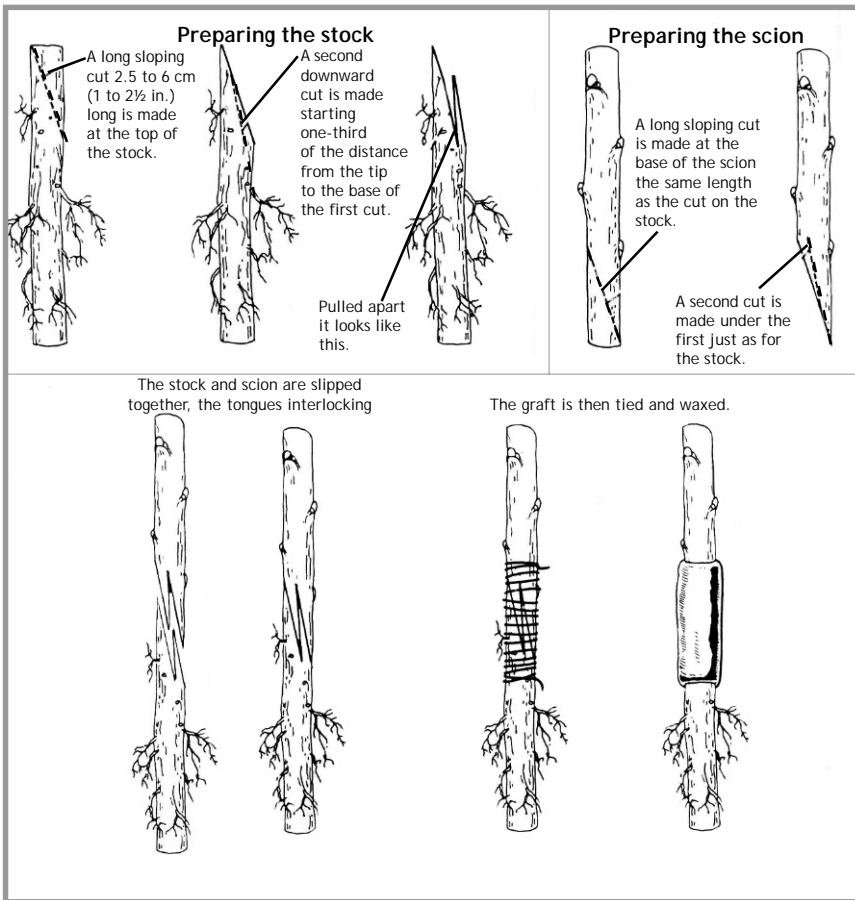


Figure 4-5. Whip and tongue grafting. (Hartmann et al. 1997).

Approach grafting

This is a form of grafting particularly suitable for difficult combinations. Both scion and rootstock remain intact plants until a secure graft union has been formed, thus allowing both to use their own vascular system for assimilation and water uptake.

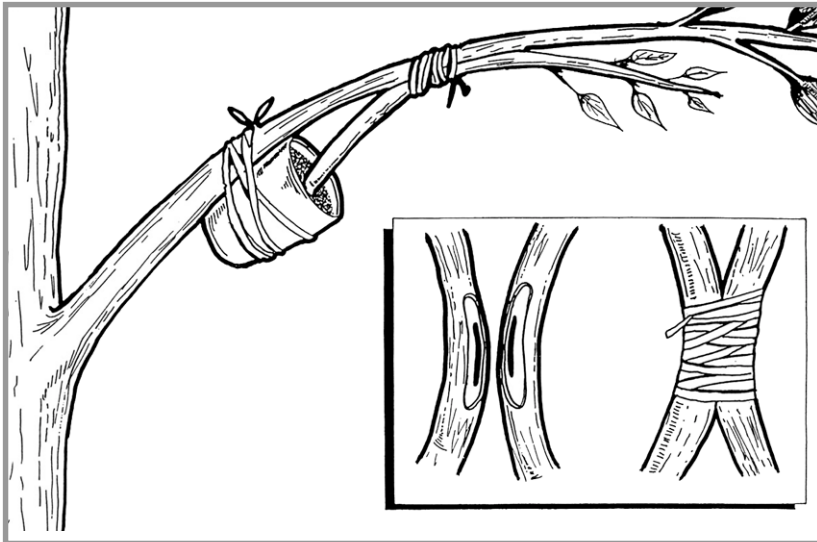


Figure 4-6. Approach grafting. (Mudge et al. 1992).

T-budding

Most forms of budding should be done when the bark slips off easily from both scion and rootstock, which is at a time of high metabolic activity. T-budding is most commonly used in the propagation of citrus. It is generally limited to small nursery stock of between 6-25 mm diameter, which are actively growing, so that the bark slips easily from the wood.

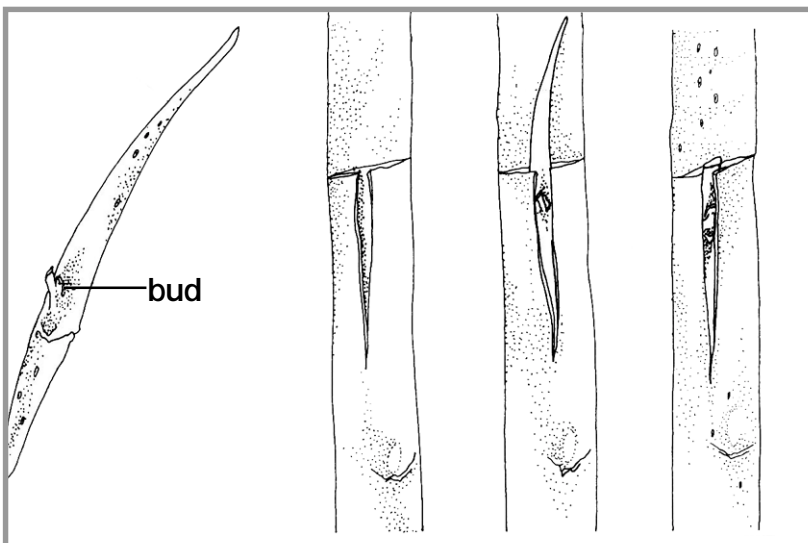


Figure 4-7. T-budding. (Macdonald 1986).

Patch budding

This is a method widely used for tropical trees with thick bark, such as the rubber tree. It can be used on stock plants as big as 10 cm in diameter.

A rectangular piece of bark is cut out of the rootstock, usually with a special double-bladed knife. A matching piece of bark, including a bud, is cut from the budwood and matched into the prepared rootstock.

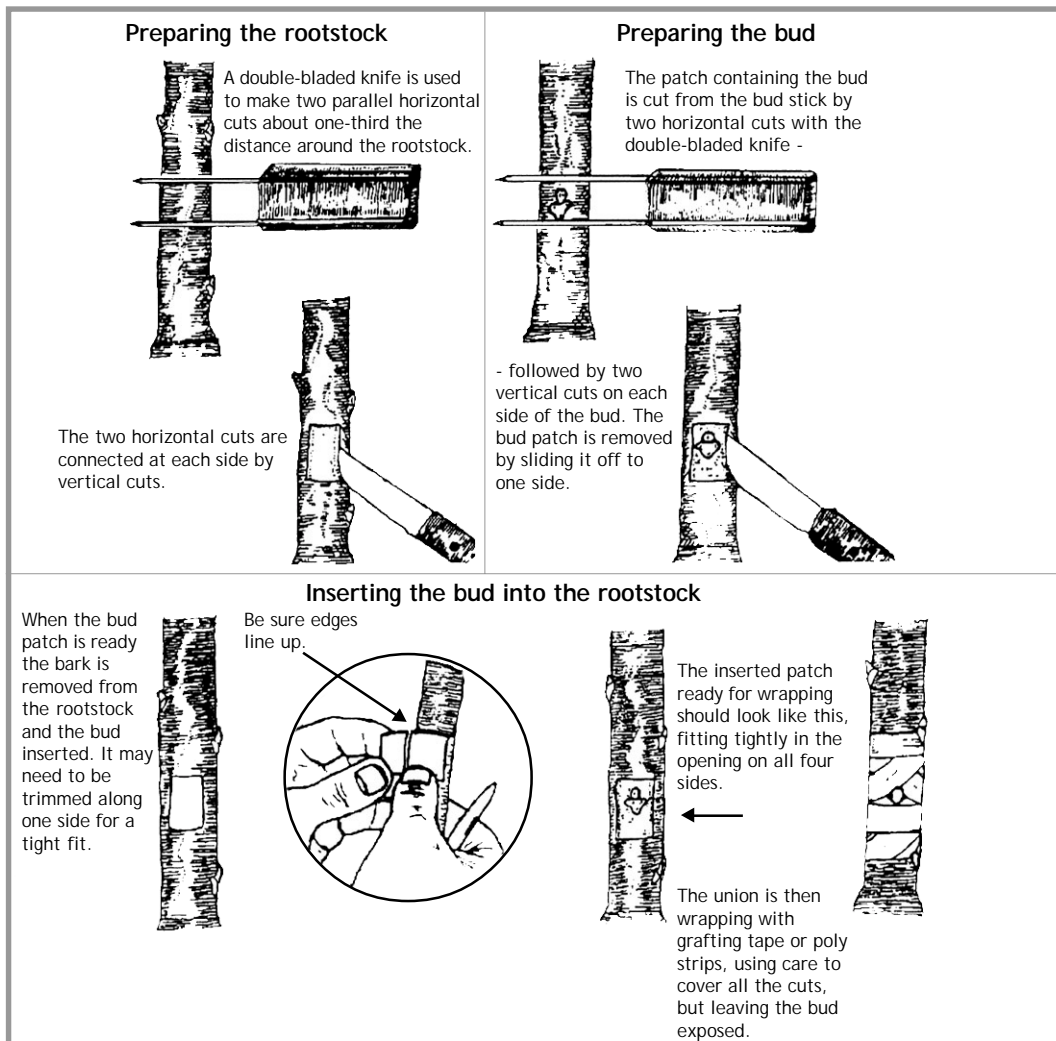


Figure 4-8. Patch budding. (Hartmann et al. 1997).

References

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- Thouin A. 1821. *Monographie des greffes, ou description technique*. Royal Horticultural Society Library (cited in Hartmann et al. 1997).



Grafting practical

Objective

The objective of the practical exercise on grafting and budding is to allow participants to practice several grafting techniques on selected agroforestry tree species or ornamentals.

The students should work in pairs and each pair should aim at practicing about four grafts of each technique described in this practical. It is advisable to have successful examples of the different techniques available for demonstrations, especially if the training is to be completed in one afternoon and the students will not be able to observe the results of their practical work by the end of the course.

Prerequisites

The following tools and materials are needed for this practical:

- 1 Good quality, sharp **grafting or budding knives**. Any sharp knife may do but the higher expense of a special grafting knife may pay in the long run in terms of durability and consistent quality of the cuts. Grafting knives are available for right-handed and left-handed people. Budding knives with a specially curved blade and a tool to lift the bark flap for T-budding are available as are special double-bladed knives for patch budding. In order to ease the fitting of scion and rootstock cuts, special tools have been manufactured that cut a notch into the scion and a corresponding groove into the rootstock. These tools only operate well when scion and rootstock are of approximately the same diameter. For softer tissue woody materials, surgical scalpels or razor blades can also be used, but will require more caution on behalf of the users.
- 2 A fine-grained **sharpening stone** is needed to keep the blades of the grafting and budding knives sharp after repeated cutting of woody material.
- 3 **Surgical spirit** to disinfect the knives.
- 4 **Secateurs, hand sprayers** and **plastic bags** to collect scions.
- 5 **Cool box** with **ice** packs for short-term storage of the scions.
- 6 Different types of **grafting/budding wraps** (polyethylene strips, raffia, latex bands, self-adhesive or degradable bands), ± 1 cm wide.

7. Special **wax or white latex paint** to cover the grafting/budding union as to avoid desiccation of the tissues.
8. Small (10 x 20 cm) transparent **polyethylene bags** to cover the top part of small seedling grafts, and **fine string**.
9. A series of **potted seedlings** of selected agroforestry trees or ornamentals to be used as **rootstocks, scions** and **buds**.

Assignments

Sharpening grafting/budding knives

A fine-grained sharpening stone with a flat surface should be used. The stone is wetted with water, or, for better results, with oil thinned with paraffin. The blade of the knife is pulled at an angle of about 30 degrees over the stone until a sharp edge is obtained. If the knife is very blunt, a medium-grain stone can be used initially, using a fine-grained one for finishing off. The whole width of the stone should be used so that its surface remains flat.

Collecting scions and budwood

Collect young, vigorous shoot tips of about 20 cm from a suitable mature tree in the surrounding removing all leaves and the tip. Collect the shoot tips in a small plastic bag in which you have sprinkled some water, label carefully with species name and cultivar or clone number, and immediately store in the cool box. For the purpose of these exercises, scionwood can also be collected from seedlings.

Top wedge grafting (see Figure 4-3 page 65)

- a) Using a sharp grafting knife, top the seedling stock where it is about pencil thickness and about 20-30 cm above the soil line.
- b) Cut a vertical slit, 2.5 cm down through the remaining stem, using a very thin flat blade knife, taking care to avoid splitting the stock below the cut.
- c) Take a scion of the same thickness and cut the basal end to a tapered wedge shape slightly longer than the slit in the stock. Insert the wedge firmly into the slit, matching the vascular cambia of both stock and scion. To allow for good callus formation, it is important that a small semi-circle of cut scion is visible above the rootstock. This semi-circle is called a 'church window'.

- d) Bind the graft firmly with grafting tape or polyethylene strip, making sure that the scion does not slip during tying in.
- e) Cover the scion and several centimetres of rootstock below the union with a transparent polybag in which you have sprinkled a few drops of water. Tie tightly around the stem. Cut a small corner off the bag and blow up like a balloon. Then twist the corner closed and tie with a small piece of string. Doing this increases the humidity and CO₂ levels inside the bag, and also prevents the bag from clinging to the scion, thus avoiding possible infections.
- f) Place the grafted plant in shade and keep well watered. Regularly remove all side shoots that develop below the graft.
- g) When the scion shoots begin to grow, gradually ventilate (cut slits) and then remove the bag.

Whip and tongue grafting (see Figure 4-5 page 66)

- a) Using a sharp grafting knife, top the seedling stock where it is about pencil thickness and about 20-30 cm above the soil line.
- b) Make a slanting cut of about 2.5-6 cm into the rootstock
- c) Make a similar cut on the scion
- d) On both cuts, a reverse cut is made about one third of the distance from the tip. It should be about half of the length of the first cut and should be parallel to it.
- e) Rootstock and scion are then inserted with the tongues interlocking and matching the cambium layers well.
- f) Bind the graft firmly with grafting tape or polyethylene strip, making sure that the scion does not slip during tying in.
- g) Cover the scion and several centimetres of rootstock below the union with a transparent polybag in which you have sprinkled a few drops of water. Tie tightly around the stem. Cut a small corner off the bag and blow up like a balloon. Then twist the corner closed and tie with a small piece of string. Doing this increases the humidity and CO₂ levels inside the bag, and also prevents the bag from clinging to the scion, thus avoiding possible infections.

- h) Place the grafted plant in shade and keep well watered. Regularly remove all side shoots that develop below the graft.
- i) When the scion shoots begin to grow, gradually ventilate (cut slits) and then remove the bag.

Approach grafting (see Figure 4-6 page 67)

- a) Select two seedlings of similar size.
- b) Cut a slice of bark and wood 2.5-5 cm long from both stems at the point where the union is to form. This cut should be the same size and form in both partners to allow for a good match of the cambial layers. It should be smooth and as flat as possible.
- c) Bind the union together and wax, or wrap tightly with parafilm.
- d) Keep the two plants well watered and protected until the graft has succeeded.
- e) After a solid union has formed, which can take several months, the rootstock is cut above the union, and the scion below it to complete the graft.

T-budding (see Figure 4-7 page 67)

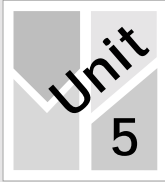
- a) Select a healthy seedling with a smooth bark. T-budding works best if the bark slips easily off the wood.
- b) Make a vertical cut into a smooth part of the rootstock and then a horizontal cut above it to form a T.
- c) Slip the bark open at the corners.
- d) Prepare a budwood by cutting all leaves off a small branch but leave 1 cm of the leafstalks for easier handling of the buds.
- e) From the budwood , slice off a bud starting about 1 cm below the bud and end 2.5 cm above it. Make a horizontal cut at the end and slide the shield off the wood. This shield should be as thin as possible but should still be firm.
- f) Insert the shield into the T by pushing it downwards under the bark flaps.



- g) Tie the bud in with grafting tape or parafilm, making sure that you leave the bud exposed.
- h) After the bud has healed in, cut the rootstock off above the bud.
- i) Remove any growth from below the bud.

Patch budding (see Figure 4-8 page 68)

- a) Select a stock plant with a diameter of up to 10 cm on which the bark slips easily off the wood.
- b) Using a double-bladed knife, make two parallel horizontal cuts of about 2.5 cm length.
- c) Connect these with two vertical cuts using a single bladed knife and remove the piece of bark.
- d) Prepare a matching piece of bark from your budstick.
- e) Slide the bud carefully off the budstick sideways to ensure that the woody core of the bud does not break off.
- f) Insert the patch into the prepared rootstock. It is very important that the pieces match snugly at the top and bottom end, so that vascular tissue can form.
- g) Tie in firmly to make sure that there are no air pockets under the patch which would dry out the patch. Leave the bud exposed.
- h) After the bud has healed in, cut the rootstock off above the bud.
- i) Remove any growth from below the bud.



Layering

Training guidelines

Instructional objectives

At the end of the unit on layering, participants will be able to:

- List and explain different layering techniques.
- Explain the underlying principles of layering and describe the conditions for its successful application.
- Successfully use air layering to propagate selected agroforestry trees.

Instructional methods

The unit consists of a 30 to 45 minute theoretical introduction and some discussion. If possible, a 4-hour practical or some demonstrations on layering methods should complement the presentation.

Instructional materials

Lecture notes support the theoretical presentation and the materials needed for the practical exercise and demonstrations are listed in the detailed description of the practical.

Unit summary

Under the heading of layering, all types of propagation are summarized in which roots are formed while the stem is still attached to the mother plant. Only after the root formation, the layer is detached and planted as a separated plant.

In horticulture, the most common layering techniques include air layering, simple layering, and stooling. In tropical fruit propagation, air layering plays an important role.

Layering is often used in species that are particularly difficult to root, as the intact stems allow a continuous supply of water, nutrients and plant hormones to the place of root development. Dehydration, a common problem in cuttings, is prevented,

as is nutrient leaching, which often occurs under mist propagation. As layering beds are often used for many years, utmost hygiene has to be practiced to prevent the spreading of pests and diseases, especially nematodes and viruses (see also unit 2).

Simple layering is usually done with many-stemmed shrubs that produce long and soft stems. Young stems are bent down and pegged into the ground about 15 to 20 cm below the tip, thus forming a 'U'. During the season, the stems grow and will produce roots where it is pegged down. To improve on the rooting success, the stems can be wounded, and/or auxins applied. The stems are usually allowed to grow for one to two seasons before cutting the rooted stem off and planting it under shade.

Air layering can be done with almost any woody plant and it is an excellent method to propagate small numbers of individual trees. Usually, the bark of last season growth is girdled and two handfuls of a light, moist substrate, such as moss, sawdust or coconut peat is applied around the wound. This is then tied into plastic to avoid drying out and wrapped in aluminium foil to prevent heating up. Auxins can be applied to enhance rooting success. Species that are commonly propagated by air layering include *Mangifera indica*, *Ficus* spp., *Citrus auriculiformis* and *Persea americana*.

Stooling, or mound layering is done with plants that have been severely cut back. New shoots developing are continuously covered with moist soil, sawdust or other light substrate to about half their height. At the end of the season, roots will have formed at the base of the shoots, which can then be cut off and planted as separate plants.

Recommended reading

The following publications may further enhance your understanding of the unit :

- Hartmann HT, Kester DE, Davies FT and Geneve RL. 1997. *Plant propagation: Principles and practices*. 6th ed. New Jersey USA: Prentice Hall.
- Macdonald B. 1986. *Practical woody plant propagation for nursery growers*. Oregon, USA: Timber Press.
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Layering principles and techniques

Zac Tchoundjeu and Hannah Jaenicke—ICRAF

Introduction

The term layering is used for all types of propagation in which roots are formed while the stem is still attached to the mother plant. Only after the root formation, the layer is detached and planted as a new plant.

Layering is often used in species that are particularly difficult to root from cuttings, as the layered branches allow a continuous supply of water, nutrients and plant hormones to the place of root development. Dehydration, a common problem in cuttings, is prevented, as well as nutrient leaching, which often occurs under mist propagation. As layering beds are often used for many years, utmost hygiene has to be practiced to prevent the spreading of pests and diseases, especially nematodes and viruses (see Unit 2). As layering methods are often used with species that are otherwise difficult to root, it can take several months until roots have formed on the layered branch.

The most common layering techniques for agroforestry trees include, air layering, simple layering and stooling. In tropical fruit propagation, air layering is the most important technique.

Even though these methods have been developed in the temperate regions with a distinct dormant season due to cold temperature, they can easily be adapted to tropical conditions where the rains largely determine the growing seasons.

Air layering or marcotting

Air layering or marcotting can be done with almost any woody plant and is an excellent method to propagate small numbers of individual trees. It involves the girdling of a relatively young shoot, thus leading to an accumulation of rooting promoting plant hormones at the cut, without hindering water and nutrient supply to the tip. The shoots should be young and vigorous yet woody enough to withstand the treatment; best is the previous season's growth. It seems that the individual development of the shoot is more important than the season in which the marcot is set (Garner et al. 1976).

A cut is made at a convenient place on the shoot. The ideal length of the shoot above the marcot is between 20 and 60 cm so as to avoid large rooted shoots that may have establishment difficulties. A complete ring of bark of about 1–5 cm is removed by making two encircling cuts and removal of the intermediate ring. It is important that a large enough ring is removed to prevent callus from closing the wound, yet excessive damage to the shoot should be avoided. Cutting into the wood should also be avoided as it may interrupt the water supply and also

increases the risk of breakage of the shoot. Root promoting substances, such as slurry of auxin powder can be applied and mixed with a fungicide if necessary.

Two handfuls of a suitable rooting substrate are then applied around the wound. This is then tied into plastic and wrapped in aluminium foil to preserve moisture and prevent over heating. A suitable rooting substrate should be light in weight, porous to allow sufficient oxygen around the wound but yet with a high water holding capacity. Moss, coconut (coir) fibre, sawdust, vermiculite or mixtures of soil with any of these substrates have proved to be suitable. A little soil from under established trees can be added to the substrate to help in the rooting process, especially for species that require microsymbionts.

In order to improve the survival rate of the rooted marcot, leaves are trimmed or completely removed and the shoot partially severed a few days before harvesting. At harvest, the marcot should be immediately placed into a container with water and then potted up, using an appropriate light, but nutritious potting medium, and placed under shade, preferably under humid conditions, such as in a polypropagator.

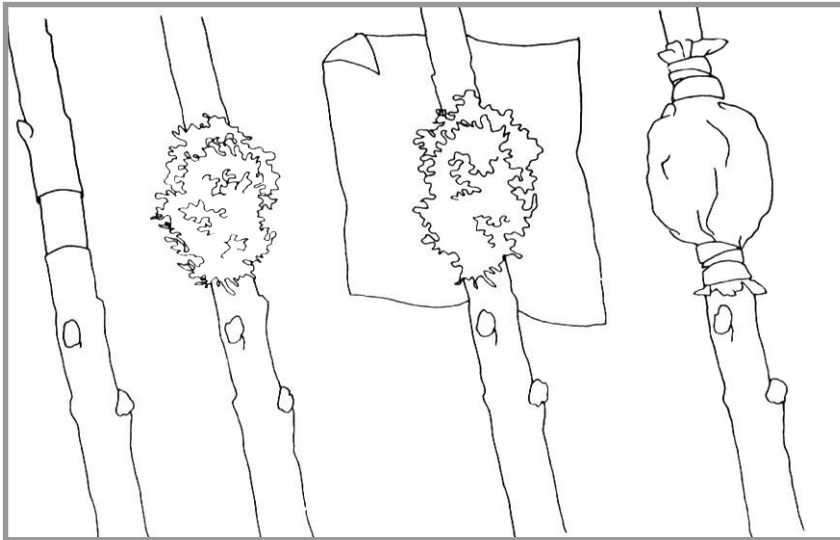


Figure 5-1. Air layering or marcotting. (Macdonald 1986).

Species that are commonly propagated by air layering include mango, *Ficus* spp., *Citrus auriculiformis* and *Persea americana*. It is a method that is most appropriate for humid environments but if care is taken, it can also be successful in drier climates. As for other vegetative propagation methods, sufficient moisture is the key to success and the set layers need to be inspected regularly and moistened as necessary.

Simple layering

Simple layering is usually done with many-stemmed shrubs that produce long and soft shoots after coppicing. Plants are coppiced at the end of the dormant season and the developing young shoots are bent down and pegged into the ground about 15 to 20 cm below the tip, thus forming a 'U'. During the season, the shoots grow and will produce roots where they are pegged down. To improve on the rooting success, the shoots can be wounded, or auxins applied. The stems are usually allowed to grow for one to two seasons before cutting the rooted stem off and planting it under shade. For this method to be successful it is important that the substrate used for layering is kept moist, but not waterlogged at all times and that soil-borne diseases are avoided.

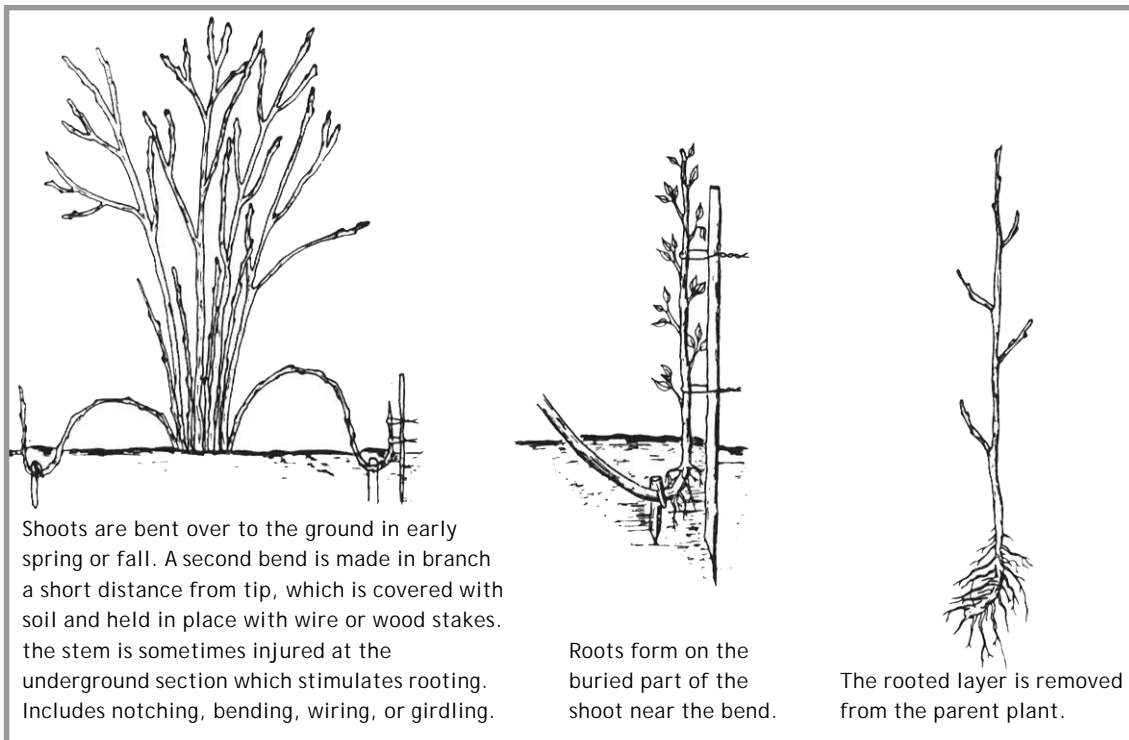


Figure 5-2. Simple layering (Hartmann et al. 1997).

Stooling or mound layering

Stooling, or mound layering is done with plants that have been severely cut back (to between 2.5-5 cm above soil level) and that have the natural vigour to produce many strong coppice shoots. New shoots developing are continuously covered with moist soil, sawdust or other light substrate to about half their height. If they are covered too high, leaves may be covered leading to weakening of the shoot. At the end of the season, roots will have formed at the base of the shoots, which can then be cut off and planted as separate plants. Also with this method, the substrate has to be kept moist and free of pathogens.

To establish a stool bed, seedlings should be planted in rows wide enough to allow sufficient space for the mound or stool. 1-1.5 m apart has proved sufficient (Garner et al. 1976). They are allowed to establish for one growing season and then cut back to between 2-5 cm above ground to initiate the development of vigorous coppice shoots for rooting. It has been shown that girdling the newly developing shoots by forcing them to grow through a wire mesh can enhance the rooting success. Depending on the species, a 0.5 cm square mesh can be placed over the stump before the shoots develop. The shoots are then forced to grow through the mesh and are girdled as they thicken.

This form of layering can also be performed in containers with single plants (Munson 1982 in Hartmann et al. 1997).

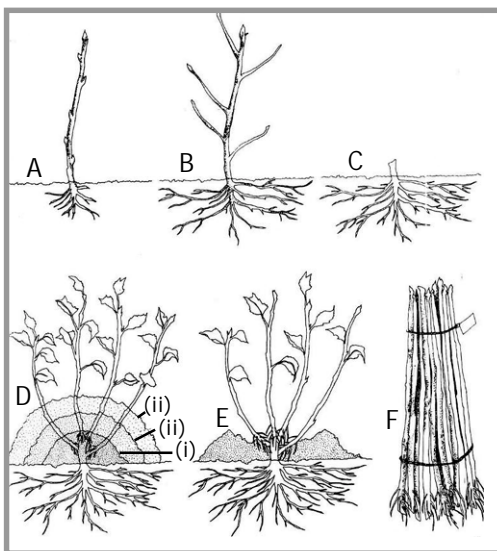


Figure 5-3. Stooling. (Macdonald 1982).

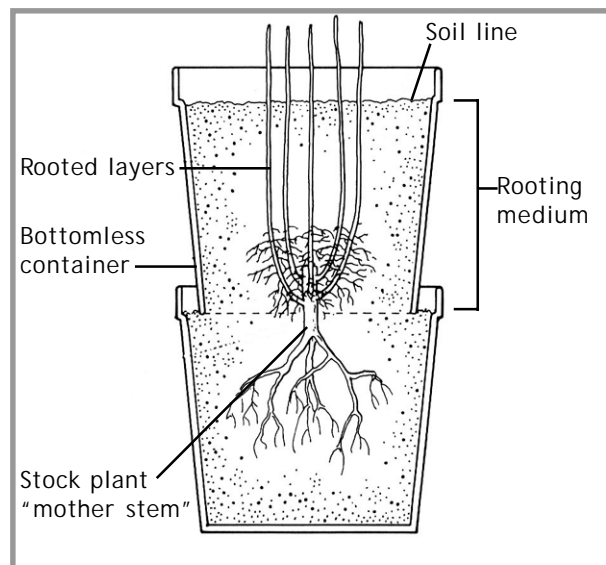


Figure 5-4. Containerized layering. (Hartmann et al. 1997).

References

- Garner RJ, Chaudri SA and staff of the Commonwealth Bureau of Horticulture and Plantation Crops. 1976. The propagation of tropical fruit trees. *Horticultural Review* No. 4. FAO and CAB.
- Hartmann HT, Kester DE, Davies FT and Geneve RL. 1997. *Plant propagation: Principles and practices*. 6th ed. New Jersey, USA: Prentice Hall.
- Macdonald B. 1986. *Practical woody plant propagation for nursery growers*. Oregon USA: Timber Press.
- Mudge KW, Mwaka A, Isutsa D, Musoke R, Foster D and Ngoda BJM 1992. *Plant propagation— a teaching resource packet*. USA: Cornell University.
- Munson RH. 1982. Containerised layering of Malus rootstocks. *Plant Propagator* 28(2): 12-14.



Layering practical

Objective

The objective of the practical exercise on layering is to allow participants to practice air layering on selected agroforestry tree species or ornamentals.

The students should work in pairs and each pair should aim to practice at least two air layers as described in this practical. It is advisable to have successful examples of the different techniques available for demonstrations, especially if the training is to be completed in one afternoon and the students will not be able to observe the results of their practical work by the end of the course.

Prerequisites

The following tools and materials are needed for this practical:

- Good quality, sharp **knives** or surgical scalpels to perform a clean cut.
- A fine-grained **sharpening stone** is needed to keep the blades of the knives sharp after repeated cutting of woody material.
- **Surgical spirit** to disinfect the knives.
- Moist **peat moss, coconut fibres, sawdust, or soil mixed with these substrates** to form a light rooting medium that can hold sufficient moisture.
- **Plastic sheets** of about 6x9" that can be cut from small plastic bags to wrap the air layer.
- **Fine string** and **electrician's tape** to close the air layer tightly.
- Pieces of **aluminium foil** to cover the finished air layers.
- A **hormone preparation** (IBA in talc mixed with a little water to make a slurry) with or without **fungicide** is optional depending on the species used and the environmental conditions at the site.
- Various agroforestry trees or ornamentals can be used for this exercise

Assignment

Air layering or marcotting

- 1 Using a sharp knife, make a cut into the bark of a shoot on the portion of last season's growth about 20 cm below the tip.
- 2 Make another cut about 3 cm below the first one and connect both with a horizontal cut. Then slide the bark off the branch. Make sure there is no vascular connection left through the ringbarked portion.
- 3 If desired, apply a small amount of hormone preparation to the upper end of the cut.
- 4 If the shoot is rather vertical, it is advisable to prepare a small pocket with the plastic sheet before putting the rooting substrate. Place the plastic sheet **below the cut** so that one small end faces the cut. Use a piece of string to secure it tightly and then fold the plastic over the cut so that a pocket is formed. Ensure that the opening is on top for easy filling.
- 5 Take 1-2 handful of the moist rooting substrate and cover the cut so that 1/3 of the substrate covers the bark above the cut. The substrate must be moist like a squeezed-out sponge, but not wet.
- 6 Pull the plastic tightly over the air layer and close the top end with string. Secure the ends and the middle opening with electricians tape.
- 7 Cover the air layer with a piece of aluminium foil.
- 8 Label the air layer with the date when set. Periodically open and check whether it is still moist enough.

(See Figure 5-1 page 78)



Micropropagation

Training guidelines

Instructional objectives

At the end of the unit on micropropagation, participants will be able to:

- Discuss the potential of micropropagation for agroforestry trees.
- Explain the underlying physiological bases for micropropagation and describe the conditions for its successful application.
- List and describe some of the techniques used in micropropagation.

Instructional methods

The unit consists of a 60-minute interactive theoretical presentation, with the usual audio-visual support.

Where possible, students can visit a micropropagation laboratory to view demonstrations of some of the techniques highlighted during the presentation.

Instructional materials

Lecture notes and other relevant handouts (laboratory layout, composition of selected propagation substrates) support the presentation.

Unit summary

Micropropagation, also referred to as tissue or *in vitro* culture, is a relatively new vegetative propagation technique, which uses a plant's potential to regenerate a complete new plant from single cells or small amounts of living tissue through the cultivation of these in controlled environments. Since this technique requires a substantial investment in infrastructure, equipment and materials, its application is mostly justified in the case of high value plants where traditional vegetative propagation methods are considered unsuccessful. The method also allows for the production of virus-free plant material and large amounts of new plants issued from a limited amount of initial material.

Plant material propagated *in vitro* using laboratory techniques and controlled conditions is very susceptible to attacks by certain microorganisms (such as bacteria and fungi) and thus it is very important to work in a clean, aseptic and even sterile environment.

Not all plants can be successfully propagated through micropropagation and even for those which can, it is important to consider criteria such as the age of the plant, the season for multiplication, the part of the plant to be considered.

The most common techniques used for micropropagation of plants are: embryo culture, axillary shoot formation, adventitious shoot formation, micrografting and somatic embryogenesis. The lecture note focuses on a description of the general procedures of micropropagation.

Recommended reading

The following publications may further enhance your understanding of the unit:

- Hartmann HT, Kester DE, Davies FT and Geneve RL. 1997. *Plant Propagation. Principles and Practices*. 6th ed. New Jersey: Prentice Hall.
- Skoog F and Miller CO. 1957. *Chemical regulation of growth and organ formation in plant tissues cultured in vitro*. Symposium of the Society for Experimental Biology 11:118-131.
- Vasil IK and Thorpe TA (eds.) 1994. *Plant cell and tissue culture*. Dordrecht: Kluwer Academic Publishers, 593 pp.



Introduction

Micropropagation, or *in-vitro* propagation are terms used for procedures to propagate plants from plant cells, tissues or organs under aseptic conditions in a controlled artificial environment. The term 'tissue culture' is used for a wider aspect of culturing plant (and animal) cells, including for research that not necessarily aims at the production of a functional organism. The umbrella term for these procedures is 'biotechnology'.

The development of micropropagation started at the beginning of the 20th century when some researchers observed that plant cells have the biological capacity to reproduce an entire plant *in-vitro*, given the right balance of plant hormones, minerals, vitamins and sugars. Initially, tissue culture was purely an academic research exercise to elucidate the mystery surrounding the growth and development of plants. In recent years, tissue culture techniques have been used on a commercial level to produce large quantities of clean propagules of horticultural crops such as bananas, pineapples, citrus and several ornamental plants, as well as commercial trees such as eucalyptus, rubber, oil palm and others. Many of the commercial nurseries, which use tissue culture techniques to produce propagules of various crops and trees, are found in developed countries and very few in developing countries. This is mainly due to the limited number of well-trained personnel in tissue culture technology, lack of awareness of the potential for micropropagation and inadequate capital to set up the facilities for micropropagation of important crop plants and forestry trees. However, micropropagation is an alternative and viable option for rapid multiplication of propagules to satisfy the high demand for crop and tree planting material by farmers.

Terminology

The following are the definitions of some of the terms used in the presentation needed to understand micropropagation techniques and their underlying principles.

- **Axillary buds:** dormant buds in the axils of leaves. These buds are stimulated to grow through hormonal changes, for example, when the main stem is coppiced.
- **BA/IBA:** plant hormones (benzyl adenine, a cytokinin, and indole-3-butyric acid, an auxin).

- **Callus:** undifferentiated cells growing after wounding. Under the influence of hormones and enzymes, callus can differentiate into various plant organs.
- **Explant:** any plant section/segment collected from existing plant for use in tissue culture work.
- **Haploid:** having one of the usual two sets of chromosomes.
- **Incubate:** to grow under special conditions, including temperature, light and nutrients.
- **Micropropagation:** propagation of plants under controlled, artificial conditions using plant growth media containing plant growth regulators and a balanced mixture of plant nutrients.
- **Organogenesis:** the initiation of any organ from explants *in vitro*.
- **Plantlets:** the very young plants developing from incubated cultures.
- **Polyembryony:** the development of more than one embryo after sexual reproduction.
- **Primary culture:** the initial culture of an explant from original parental plant material.
- **Pro-embryo:** the product of the first (transverse) cell division after fertilization. The apical cell later develops into the embryo, the basal cell into the so-called suspensor which functions to absorb and transmit nutrients to the proembryo.
- **Propagules:** the fully-grown plants arising from vegetative parts of plant segments that are ready for transplanting in the field.
- **Regeneration:** the growing of shoots or roots from explants.
- **Seedlings:** young plants grown from seed.
- **Subculture:** the culturing of plant material originating from primary or subsequent cultures.

Physiology

Tissue culture techniques are based on the principle of omni- or totipotency, meaning that indefinite culture of plant cells is possible under ideal conditions. In recent years, the tissue culture of animal cells has also become possible. In order for cells to survive outside the organism, the surrounding environment must mimic the optimal conditions in terms of light, temperature, humidity and supply of nutrients, vitamins and hormones. The balance of auxins and cytokinins enables the manipulation of shoot or root formation *in vitro*. Auxins are needed to promote the development of roots, an auxin-free substrate is necessary for maturation of tissues, and a substrate containing cytokinins and gibberellins is needed to start the differentiation of shoot tissue. By careful subculture on or in substrates containing different ratios of these hormones and increasing levels of nutrients, it is therefore possible to control the stages of development. Hardening of the young plantlets is similar to 'normal' seedlings in a greenhouse under shade and high humidity conditions until strong enough to withstand field conditions.

Reasons for micropropagation

The main reasons why micropropagation of agroforestry trees can be considered are:

- To multiply a tree which cannot readily be multiplied by seed or conventional vegetative methods.
- To rapidly propagate large quantities of propagules of superior tree provenances.
- To clean pathogen infected clonal plant material.
- To rejuvenate older trees through repeated *in-vitro* micrografting.
- To multiply pathogen-free propagules for farmers.

Requirements

Micropropagation work requires a small number of well-trained and skilled workers, some laboratory space and time to produce large quantities of propagules.

Unlike the conventional vegetative propagation of agroforestry trees in a nursery, micropropagation requires an organized laboratory space:

- **Preparation room:** A clean lab space or room where culture media are prepared and sterilized, and explants are cleaned and sterilized before they are transferred into vessels such as test tubes, jars or bottles, containing medium.
- **Transfer or inoculation room:** A clean lab space with a laminar-air-flow hood, which provides a sterile or pathogen-free working bench/space.

- **Incubation or culture room:** A clean lab space with shelves, controlled light and temperature where the plant cultures are left to grow and develop.
- **Greenhouse:** A clean room with benches, humidity chamber and fan or air flow control mechanism where regenerated plants (propagules) are acclimatized before taken to the field.

The most critical requirement for successful *in vitro* propagation is maintenance of aseptic conditions as well as the control of temperature and humidity during the entire process of micropropagating plants.

The regeneration of any explant (cell, tissue, organ) into an entire new plant needs the balanced supply of hormones, nutrients and sugar suspended in either an inert solution or a solid supporting material e.g. agar-agar. The critical hormones inducing development of shoots are cytokinins and auxins are needed for root development. These hormones have to be supplemented with basal salts of the essential mineral nutrients and carbohydrates from sugar e.g. sucrose. For some plant species the enhancement of growth and development of *in vitro* plants, requires additional supplementation of other hormones such as gibberellic acid and some vitamins. The basal salts medium most commonly used in tissue culture work is that of Murashige and Skoog (1962). Other modified media have been formulated e.g. the woody plant medium by McCown and Lloyd (1981).

Techniques

The following are the general steps in the micropropagation procedure that apply to all techniques described further on:

- Defining the purpose for carrying out micropropagation.
- Selecting appropriate techniques.
- Preparation of appropriate culture media, sterilized in an autoclave or pressure cooker.
- Collection of plant materials (leaves, stems, roots, flowers or fruits) from selected field or greenhouse plants/trees.
- Preparation of explants collected from the field for inoculation into the culture media: washing, cutting into small sections, sterilizing with sterilant e.g. sodium hypochlorite.
- Preparation of the laminar-air-flow hood for inoculation work: cleaning with disinfectant (e.g. alcohol) of the dissection stage, hood sides and air filters, disinfecting surgical tools by flaming.

The following table adapted from Hartmann et al. (1997) gives some examples of tissue culture techniques, which are described in more detail below.

Table 6: Some of the techniques used for tissue culture of trees

Structures formed	Regeneration method	Explant source	Uses
seedlings	embryo culture (e.g. embryo rescue)	embryos isolated from the fruit and seed coverings	Used when other methods of vegetative propagation and micropropagation of plant organs or tissues fail.
plantlets	axillary shoot formation (e.g. meristem culture, shoot apex culture)	shoot tips less than 1 mm in size	Used for virus elimination.
	adventitious shoot formation (e.g. organogenesis, androgenesis)	leaf pieces, petioles, pollen mother cells	Used for the propagation of monocots (e.g. palms). It is one of the key steps in the production of genetically transformed plants. Androgenesis is used to obtain haploid plants - mainly for research purposes.
	micrografting	small scion shoot tip usually grafted to a seedling rootstock	Used for virus elimination or as alternative to conventional grafting in exceptional cases.
somatic embryos	somatic embryogenesis	developing embryos or parts, e.g. the nucellus or ovule	Used to reproduce clonal copies of the mother plant and in breeding and genetic transformation

Embryo rescue

This is a technique used in cases where the zygotic embryo fails to survive after initial development in the maternal tissue. The technique involves surgical excising of the embryo in the early stage of development before it dies, and to culture it in appropriate media formulation containing a balance of auxins and cytokinins with gibberellins to enable the embryo to develop outside the maternal tissue or embryo sac.

This technique is useful where vegetative propagation and micropropagation of other plant organs or tissues fail. The method is also useful in generating off-springs from inter-species crossing, where the embryos fail to develop due to incompatibility problems or to shorten breeding cycles in breeding programmes.

Axillary shoot formation

Shoot apical meristem culture

This is a technique used to clean diseased plant material. The dome of the apical meristem is surgically removed under a microscope and cultured in appropriate media. The meristem is

free from systemic pathogens because the vascular bundles are not yet differentiated and contaminated plant sap flowing in phloem tissues does not reach the meristem cells. Therefore, the ensuing plants from cultured meristem culture are clean and disease free. It is necessary, however, to test the regenerated plants to ensure that they are really free from the specific pathogens that affected the original parental trees. If found to be free from infection, then the plants can be used as stockplants for further multiplication using other propagation techniques that are appropriate for the species.

Axillary shoot culture

Axillary shoot culture is the regeneration of plants from segments of roots, stems or leaves under *in vitro* conditions. Explants are collected from the suitable parental material. The sterilized explants are inoculated in primary culture containing Murashige and Skoog (MS) basal salt medium supplemented with cytokinin. The cultures are left to develop multiple young shoots. The shoots are then subcultured in fresh medium of MS supplemented with cytokinin for greater shoot proliferation. Subculturing is done 3 to 5 times in order to increase the number of shoots from the original primary culture.

The normal plants are then separated and cultured in root development media. For many plants, plain MS basal salt medium is sufficient to root the shoots, but some plant species would require supplementation with auxins for successful rooting.

The rooted plants are then hardened under 'high light' conditions before being taken to the greenhouse for acclimatization and subsequent transferring to the field for planting.

The axillary shoot proliferation and rooting of shoots is easier for juvenile (seedling) shoots than for shoots from adult trees. The latter need to be rejuvenated by repeated micrografting before they can be successfully used in axillary shoots proliferation. The other problem of culturing field materials is tissue contamination. This can be overcome by careful handling during washing and sterilization and using the correct sterilization procedure and right concentration of the sterilant. Hormone and mineral requirements for different agroforestry trees may also vary, and it is necessary to modify the media formulation depending on need, if failure to regenerate shoots and rooting of shoots is experienced with a particular species.

Adventitious shoot formation

This is a technique in which the explants are cultured in callus media, usually MS basal salt medium supplemented with high levels of auxin. The cultures are incubated under dark conditions so that they can form callus, which is massive undifferentiated cell proliferation.

The callus primary cultures are then subcultured in fresh media containing cytokinin to induce them to form embryos which are further cultured in media supplemented with small amounts of auxins, gibberellin and cytokinin to promote shoot elongation and rooting of elongated shoots. The normal plants are hardened and acclimatized like normal seedlings.

This technique leads to production of many variants and may not exhibit traits of parental material. It is slow and difficult to be successful for many woody perennial plants.

Androgenesis

A special form of adventitious shoot formation, this is a technique where the explants are anthers or immature pollen grains. The explants are cultured with anther culture media and incubated under light for the anthers or pollen grain to regenerate multiple plantlets. The plantlets are subcultured in rooting media (plain MS) to develop roots. When roots have developed, the plants are hardened and acclimatized as outlined above. The plants regenerated from this technique are haploid and useful in tree breeding work. However, the media formulation requirements of many species vary and the success rate is low for many of them.

Micrografting

This is a technique used to rejuvenate old trees and confer competency to regenerate easily when propagated. Initially, seedling rootstocks are raised aseptically and then small scions (terminal shoots) are collected from the superior old trees and micrografted onto the rootstocks under aseptic conditions. The micrografted cultures are incubated and left to grow. The ensuing scion growth is excised and re-micrografted on freshly raised rootstock and incubated. The micrografting is repeated for several cycles and at about 5 to 8 cycles the ensuing scion growth are excised and tested for rooting competency, by rooting them in plain MS media. Rejuvenated scion shoots will root readily. Then the rejuvenated shoots can be propagated using appropriate techniques e.g. axillary shoot proliferation, since the competency to regenerate would have been restored after repeated micrografting *in-vitro*.

Somatic embryogenesis

This technique is used for mass propagation and for genetic improvement programmes. It is based on the potential of callus cells to differentiate into embryos and complete plants when subjected to the right external conditions. Somatic embryos develop in a similar way to zygotic embryos, however without the development of endosperm or seed coat. A variety of types of somatic embryogenesis have been defined, depending on the explant. Individual protocols for the induction of embryogenetically determined cells have to be developed for each genotype.

A few generalized stages are as follows:

- 1 Selection and culture of appropriate explant material is the most critical step. The production of a callus culture follows.
- 2 The embryogenic potential in the cell explants is stimulated by transferring the cells to a basal medium with a high concentration of auxin (e.g. coconut milk). After one to two weeks, small pro-embryos may appear.
- 3 Differentiation and maturation of somatic embryos happens when the pro-embryos are shifted to an auxin-free basal medium with a high concentration of ammonium nitrogen. In this stage, some manipulation may be required for the maturation of embryos, such as the addition of abscisic acid, or dehydration to mimic *in vivo* conditions.
- 4 Plantlet formation can be induced, by transferring the embryos onto a medium containing a low level of cytokinin.
- 5 After leaves and roots have formed, the plantlet can be transplanted to a nursery substrate and handled like any other seedling.

Conclusion

As with any other propagation method, there are dangers and problems associated with micropropagation techniques. Several technical problems may arise from rapid proliferation of pathogens, shoot tip die-back or lack of differentiation potential after prolonged culture. As in other vegetative propagation conditions, diseases can be spread rapidly together with the explants, if not thoroughly screened.

Micropropagation techniques are very useful for agroforestry in a few cases, for example if very expensive or rare specimens need to be propagated for which traditional propagation methods have failed. Another reason to justify the development of expensive facilities and protocols is the economic return of the products – this has been the case for oil palm, banana, coffee, and rubber amongst others.

References

- Hartmann HT, Kester DE, Davies FT and Geneve RL. 1997. *Plant Propagation. Principles and Practices*. 6th ed. New Jersey: Prentice Hall.
- McCown BH and Lloyd G. 1981. Woody plant medium (WPM). A mineral nutrient formulation for microculture of woody plant species. *HortScience* 16: 453.
- Murashige T and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473-497.



Propagation experiments

Training Guidelines

Instructional objectives

At the end of the unit on propagation experiments, participants will:

- Recall the main concepts and principles of experimental design
- Apply these concepts and principles to typical vegetative propagation experiments.
- List and explain some of the common problems and constraints in propagation experiments.
- Describe how management can improve precision of a trial.
- Illustrate the concepts and principles of experimental design in vegetative propagation with a case study of an experiment.

Instructional methods

The unit consists of a 90-minute presentation, interspersed with problems discussed in the group. It can be followed by a presentation of a case study on vegetative propagation presented by a scientist or technician who has been actively involved in this type of work.

Laboratory and nursery practicals covered in other units during the course will also serve to illustrate experimental design for propagation research.

Instructional materials

The theoretical presentation is supported by a lecture note, which includes the discussion problems, and a case study report.

Unit summary

The principles of experimental design (randomization, replication, control of variation) are reviewed in the context of 'typical' tree improvement trials. The application of the same principles to vegetative propagation trials, are then considered.

In vegetative propagation trials, the 'experimental objects' will often not be field plots but propagules of some type, such as cuttings. The trial may be established in a nursery, propagator or laboratory. Each of these will have their own patterns of variation that must be allowed for, in the experimental design. When doing experiments with rooted cuttings, factors which can influence outcome include: clone, position of cutting on shoot, light conditions experienced by the stock plant, time of year, rooting medium, temperature and moisture. These all need to be considered when designing trials. Standard methods can be used to determine the required size of experiments. When the outcome is a 'yes/no' measure (e.g. did the cutting root?) the number required is often larger than expected. Lack of sufficient facilities for replication when working with controlled environments is common. The fact that an experiment is in the lab or nursery does not remove the need for randomization. Staff selecting material for trials may inadvertently introduce biases, which can be avoided by simple techniques. Since the experimental objects are often small, numerous and hard to distinguish, it is essential to maintain careful records of the experimental design to link with the recorded data.

Recommended reading

We are not aware of any books specifically on design of propagation experiments, but many of the points important for other trials are equally important for vegetation propagation research. Hence general books are useful.

- Coe R, Stern R and Nelson L. 1997. *Design of agroforestry experiments - guidelines for training workshop resource persons*. Nairobi: ICRAF.
- Mead, R., Curnow, R.N. and Hasted, A. 1983. *Statistical Methods in Agriculture and Experimental Biology*. Chapman and Hall.
- Williams ER and Matheson AC. 1994. *Experimental design and analysis for use in tree improvement*. Australia.:CSIRO.
- Dyke GV. 1988. *Comparative experiments with field crops*. London: Griffin.



Design and management of experiments

Richard Coe—ICRAF

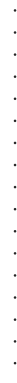
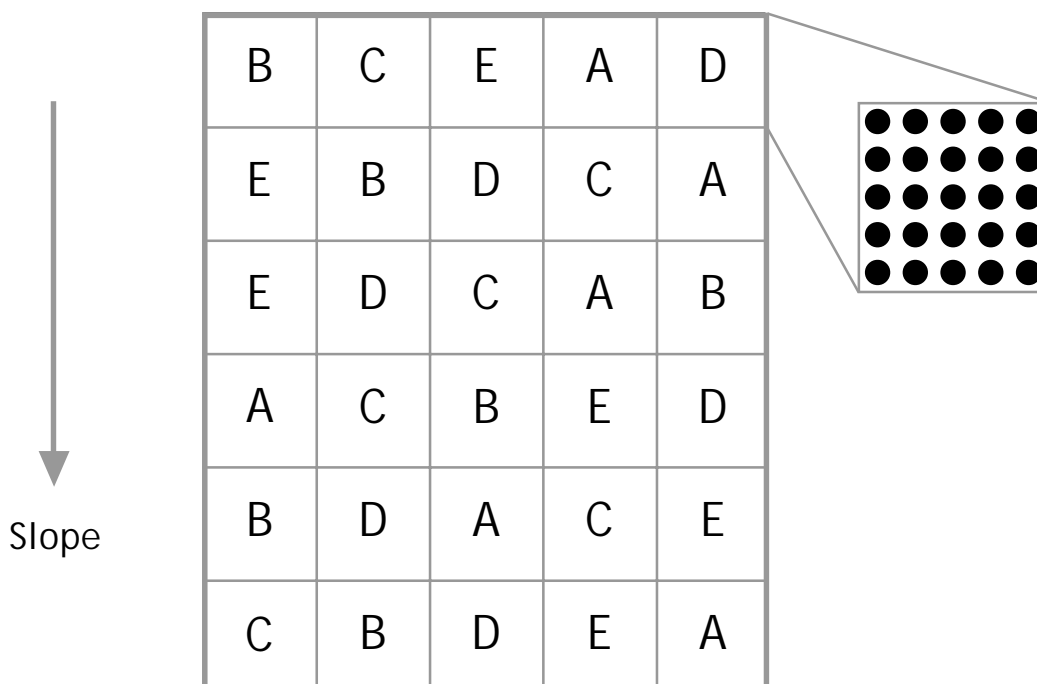
Introduction

The design of an experiment incorporates the complete set of instructions for carrying out an experiment. In many textbooks 'Experimental design' means a description of the statistical aspects, such as the number of plots, only. However these cannot usefully be separated from other aspects of the trial.

Designing an experiment is the responsibility of the principal investigator, and should be done in consultation with anyone with relevant expertise. It is useful if everyone involved with the trial understands not only the design, but also some of the reasons for the trial being designed in that way. This will help ensure that the protocol is followed accurately, and that appropriate action is taken when something goes wrong and adjustments have to be made.

Example: a simple tree improvement trial is set up to compare the performance of 5 different provenances (A,...E) of *Sesbania sesban*.

Trees are planted in plots, spaced at 1m x 1m with 25 trees in one plot all being the same provenance. There are six such plots of each provenance, arranged in the field as shown below. The nine central trees in each plot are used for assessment.



Design basics

Objectives

The objectives of a trial are chosen in accordance with the overall research strategy. They determine the rest of the design, and must be stated in a sufficiently concise way to do that. In the example, the objectives are: (1) to determine whether there are useful differences between provenances and (2) which provenances have the highest growth rate combined with pest resistance.

Treatments

The treatments are the conditions compared in the experiment. The only consistent differences between plots should be the treatments. In the example, the treatments are the provenances. There should be no differences between plots in factors such as spacing, fertilizer or planting methods that would confuse (or be confounded with) provenance differences.

Plots or experimental units

These are the 'objects' to which treatments are applied. In the example, they are field plots containing 25 trees.

Replication

Despite all efforts to control it, there will be variation between different plots of the same treatment. In order to determine the precision of the treatment comparisons it is necessary to measure this variation. Hence it is necessary to have repeated plots of the same treatment, or to 'replicate' treatments. A complete set of 1 plot of each treatment is referred to as a replicate. In the example there are 6 replicates.

Randomization

The treatment applied to a particular plot should be determined at random. Each plot should have an equal chance of receiving any particular treatment. Randomization ensures there is no bias in the allocation of treatments, for example giving the same treatment to all the 'better' units. In the example, it would be wrong to put provenance A only along one side of the field (it might be unfairly subjected to wind or pest exposure) or any other predetermined position. Randomization is also the basis of valid statistical analysis. 'Random' is not the same as 'mixed up' or 'haphazard'. Randomization should be carried out using random numbers from a table or computer program.

Blocking and control of variation

Reducing or controlling the amount of random (non-treatment) variation is important. One very important way is by blocking. At the start of the experiment the plots are grouped into sets thought to be similar, each set called a block. Careful allocation of treatments to the blocks (while still randomizing allocation within blocks) can result in large gains in precision. In the example the blocks are formed across the slope since there are likely to be trends in fertility and moisture up and down the slope. Treatments have been allocated to blocks so that one complete replicate falls in each block.

Measurements

The measurements taken should also be determined by the objectives. There is no point collecting data not required by the objectives, and the trial will not reach its objectives if needed data are not recorded. In the example, we need to measure growth rate and pest incidence, but not flowering patterns and branch angles.

Application to propagation experiments

Treatments

The treatments in propagation experiments may be very varied, but are likely to relate to genetics (concerned with differences between clones, varieties or species) or management (concerned with the effects of changing propagation methods).

Problem: It is known that the hormone IBA increases success of rooting of cuttings in many species. Typically 20 μg per cutting is a suitable dose, with 300 μg being too much, depressing rooting ability. Now work is starting on *Melia volkensii*, which has not been carried out before. Decide the treatments to include in a trial to determine suitable IBA rates for melia.

Very often there will be interactions between genetic and management components. For example, we find that one clone responds more to rooting hormone than another. Management trials, aimed at developing improved methods of propagation, will need results that apply to any clone. This implies including several clones in the trial.

The information given is that 20 μg is probably better than no hormone, but 300 μg is too much. This suggests there is a response to hormone of the type shown:



The problem then is to confirm that this is the form of the response and get a good estimate of the position of the peak. We have to choose hormone levels that will give data that allows the curve to be well estimated.

At least 3 levels of hormone are needed to draw the curve. If only 3 are used, it is not possible to see the asymmetry of the curve. Therefore 4 are recommended, or possibly 5. The lowest level has to be 0 μg , a 'control' that will confirm that the hormone is doing something. The highest should be well past the expected optimum, so that we are sure the response is really decreasing - say 300 μg . One level should be at the expected optimum - 20 μg . Two more levels should be put either side of the expected optimum, so that we have good data to find the shape of the curve even if the optimum is rather different from the expected. Hence we arrive at 5 treatments:

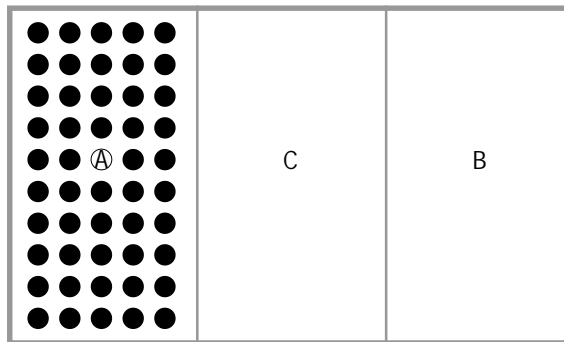
0, 10, 20, 150 and 300 μg .

This design avoids the two most common problems, which occur in experiments of this type: Using too many different levels (e.g. 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μg). It is always better to have several replicates of a few levels rather than a few replicates of many levels. Only using levels up to the expected optimum. It is essential to experiment well past the optimum in order to see where the top of the response is.

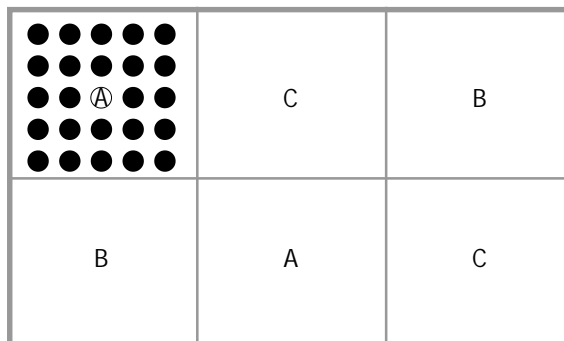
Plots or experimental units

Consider a trial to compare the rooting ability of cuttings of three different clones (A, B, C). Fifty cuttings of each clone are used. Possible arrangements are :

1. Groups of 50



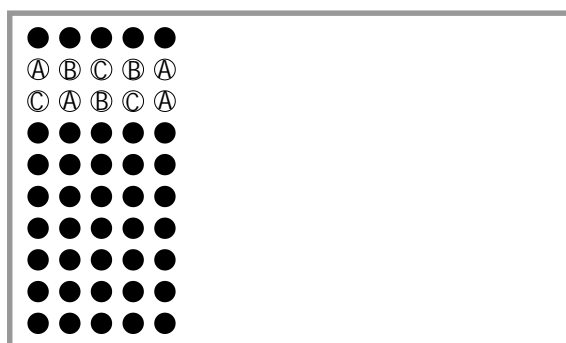
2. Groups of 25



3. Groups of 10

● ● (A) ● ● ● ● ● ● ●	C	B
B	A	C
B	C	A
	B	A

4. Single cuttings



All these take the same resources (number of cuttings, area of propagator) but are not the same. Arrangement 1 is not replicated, so cannot give clear conclusions. Statistical theory shows that Arrangement 4 will give the best (most precise) answers, but is hardest to manage. In this arrangement, there is greater potential of losing track of particular cuttings. Arrangement 3 is probably the best compromise.

Problem. We want to do an experiment to compare the response to rooting hormone of 5 different clones of *Prunus africana*. Fifty cuttings of each clone are available. What would be a good way of arranging the cuttings and treatments in non-mist propagators?

Assume that two levels of hormone are to be used, zero and something known to produce a response, labelled - and +. Therefore there are 2 hormone x 5 clones = 10 treatments.

One option would be to use 'units' of a single cutting, randomising the clone and hormone treatments completely and giving a replication of 50. The problems with this are:

- The design is complex to lay out and record.
- It is possible that some of the hormone could be diffused in the rooting substrate from a + cutting to an adjacent - cutting.

The second problem requires the isolation of cuttings of + and - treatment, either by physical separation or by barriers. Plastic boxes are often used to create barriers. Leaving a lot of space between cuttings means the whole experiment takes up more space than might be available. Using boxes is not practical if each of the 250 cuttings has to have its' own box.

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Grouping cuttings and using boxes with a separate box for each group, can address both these problems. For example, we could put one cutting of each clone in each box, then give half the boxes hormone and leave the other half without. That uses a total of 50 boxes. The design is of the 'split-plot' type, with clones being compared within boxes and hormone between boxes. The analysis of variance (ANOVA) looks like:

Source	df
Hormone	1
Main plot error	48
Clone	4
Clone x hormone	4
Split plot error	192
total	249

A design with fewer boxes might involve 5 cuttings of each clone in each box. Assuming the 5 cuttings of each clone are grouped together in each box, the ANOVA would look like:

Source	df
Hormone	1
Main plot error	8
Clone	4
Clone x hormone	4
Split plot error	32
total	49

This looks reasonable, but an intermediate design with, say 3 cuttings per clone per box, would give more main plot error degrees of freedom, which would be necessary if the design was to be blocked at all.

Replication

In some trials the main data collected is of the 'yes/no' type (Did the cutting root? Did the graft take?). Each experimental unit then provides much less information than if a quantitative measurement is taken (How much root biomass is there at 4 weeks? What is the starch content?). The result is that surprisingly large numbers of units are needed to get clear answers.

An example is shown in the table. Suppose treatment A gives 20% rooting success. Treatment B is hypothesized as an improvement. How many cuttings of each do we need to test the hypothesis? The answer depends on the size of the improvement:

Rooting success for A	20%			
Rooting success for B	30%	50%	70%	90%
Number of cuttings of each required	390	52	19	10

Randomization

Because of the large numbers of units (such as pots or cuttings) often involved in propagation trials, there is a tendency to avoid randomization. The reason given is that the organization of the trial is simpler if all the pots of one treatment are kept together. The problem is simply that, without randomization, we cannot conclude that it is treatments that cause the effects noted. It may simply be due to position in the lab or nursery.

Blocking

Blocking a lab, nursery or propagator experiment is as important as blocking a traditional field experiment.

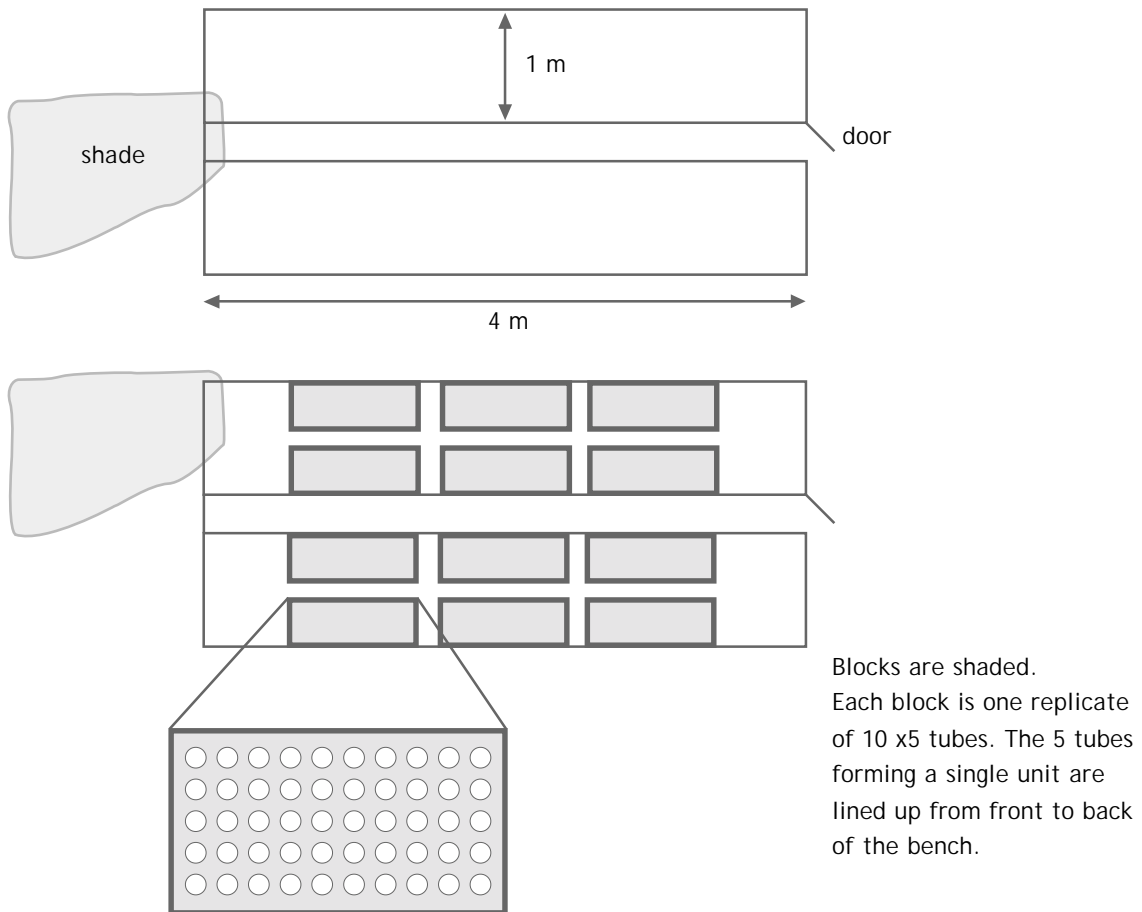
Any experimental facility has systematic variation. You only have to look at a production nursery, in which all seedlings are subject to the same 'treatment', to see the variation that exists. The variation will be due to many factors – exposure to direct sun and wind, closer to sources of water etc. The same is true of a lab bench, a closed propagator, a green house or even a controlled-environment growth chamber.

The patterns of variation are learnt by experience, but can often be predicted. For example, the edges of non-mist propagation tend to be different from the middle. These patterns must be used to define blocks.

When the trial is analysed the contribution made by the blocks can be assessed and used to improve the design of the next trial.

Problem: An experiment is to be carried out in a greenhouse, shown below. Plants will be in standard polytubes, spaced 10 cm x 10 cm. The trial will use 'units' of 5 tubes. There are 10 treatments and 12 reps, requiring 5x10x12=600 tubes in total. It is known that plants near the door do poorly. Those near the front of the benches tend to outperform those near the back. A tree outside the greenhouse shades the NE corner. How would you block the experiment?





The ends of the green house, subject to shade and disturbance from the door, do not need to be used: there is sufficient bench space in the middle 3m. Then each bench is divided into blocks of 5 x 10 tubes. The blocks are arranged as shown, so that differences between the front and back of the bench are accounted for, as are any difference between the left and right benches. Within a block, the 5 tubes making up one unit are in a line from the front to back of the bench. This way any remaining differences between front and back are evenly distributed amongst all units, minimizing the between unit variation.

Common problems

Limitation of research facilities.

Experiments done in greenhouses, propagators or growth chambers commonly use the 'environment' (light, water or temperature regime) as one of the treatments. The problem is that in most places it is not possible to replicate these treatments. For example, we only have one propagator with a heating cable. There is no way around this problem. The result is that statements about the effects of these variables will always be provisional. 'Replication in time' is sometimes used, but does not really remove the problem - a particular propagator can continue to be good or poor at repeated time points, irrespective of the treatments.

Lack of material

Consider a trial with rooted cuttings that requires 4 treatments and 50 cuttings per treatment. We know cuttings from different clones can respond rather differently. The idea is to 'balance' clones across treatments. Ideally we would have 4 (or a multiple of 4) cuttings of each clone, allocated equally to the treatments. If less than four are available for some clones, we have to try to 'balance' the allocation as well as possible. What must certainly be avoided is the use of 1 clone mainly for one treatment and another clone for a different treatment. In that case, clone and treatment effects will be mixed up or confounded. The problem is made more complex when it is noticed that there is not only a clone effect, but also an effect depending on where the cutting is taken from (near the growing point or nearer the base).

Managing trials to give good results

The way a trial is managed and looked after can have a large effect on its quality.

Uniform management

The most important principle is that of uniform management. The only difference between the ways in which units are managed, should be in the treatments. Take care to avoid such things as always opening 1 propagator for inspection and leaving others closed, or giving more water to one end of the bench than the other.

Selection of material

It has been shown that when material is being chosen for an experiment (e.g. seedlings from a nursery, cuttings from a bucket), there is a tendency to select the 'best' material first. Furthermore, many people will set up an experiment by treatment (establish treatment 1, then move to treatment 2). The result can be an important bias - the best material all ends up on one treatment. The way to avoid this is first segregate the material into classes, then make sure each treatment is represented in each class. The simplest option is to allocate each class of the planting material to one block of the field or greenhouse. Differences in material then just contribute to block differences, and are not confused with treatments.

The same applies if, for example, several batches of planting medium have to be used.

Organizing the work

The way work on an experiment is organized, can also affect the results. For example two technicians are doing grafting, and one does treatments A and B, the other C and D. The result will be that 'technician effects' (which may well be important) are confused with differences between (A and B) and (C and D).

A better solution could be for one technician to do half of A, B, C and D and the other

technician to do the other half. Even better would be for one technician to work on one set of blocks and the other to work on the remaining blocks.

Similar considerations apply when planning the order in which an experiment is set up. If you leave treatment D until last in the day you may bias results – the cuttings have been left longer than for other treatments. Again the solution is to set up 1 block, then move onto the next.

Labelling and recording

In many field experiments it is quite clear what the treatment is, that has been applied to any one plot. This may well not be the case with propagation experiments – cuttings can look identical even when coming from different clones, for example. This means careful labelling and recording is essential.

Make sure there is an accurate plan of the experiment, showing the exact location of each plot and indicating the treatment applied. Make sure the plan can be orientated correctly either by adding the north direction, or by adding 'landmarks' (door of the greenhouse, nursery tap,...). The plan should be accurate enough that each plot can be identified even if the labels get moved or lost.

Mistakes

When setting up a trial with hundreds of cuttings it is possible that mistakes will be made. It is most important to record exactly what was done rather than what should have been done. It is possible to allow for mistakes at analysis, but only if they are not hidden.

References

- Coe R, Stern R and Nelson L. 1997. *Design of agroforestry experiments – guidelines for training workshop resource persons*. Nairobi: ICRAF.
- Mead R, Curnow RN and Hasted A. 1983. *Statistical Methods in Agriculture and Experimental Biology*. Chapman and Hall.
- Williams ER and Matheson AC. 1994. *Experimental design and analysis for use in tree improvement*. Australia: CSIRO.
- Dyke GV 1988. *Comparative experiments with field crops*. London: Griffin.



Annex 1 *Course brochure*

Short Training Course on

Vegetative Tree Propagation for Arid and Semi-Arid Lands

16 to 20 February 1998 in Nairobi, Kenya

Background

The International Centre for Research in Agroforestry (ICRAF) and the International Programme for Arid Land Crops (IPALAC), via a grant from UNESCO, are jointly offering a course on tree propagation for the arid and semi-arid lands.

Multiplication of agroforestry trees and shrubs is one of the major constraints to the large scale dissemination of agroforestry technologies.

Propagation by seed is the common way by which plants regenerate naturally. For research and rapid improvement of undomesticated species, however, vegetative propagation methods offer several advantages. For example, in wild populations, a large variation in important product characteristics (e.g., fruit quality, bole straightness, biomass) may be expressed. Furthermore, individuals may be recognized within a population that produce a higher quality of the desired product(s). It would therefore be advantageous to propagate these individuals vegetatively to 'capture' the genetic variation expressed which may otherwise get lost or diluted during sexual propagation.

Vegetative propagation methods have been developed and used since centuries. Especially in temperate regions, vegetative propagation has been a most important approach in the domestication of fruit species and particular methods have been developed for different species. Tropical fruit species have been subjected to vegetative propagation in a few cases that have found a lucrative export market, like citrus, mango, avocado, macadamia nut. Tropical timber species have also been cloned, mainly for plantations where uniform trees are needed.

Many indigenous trees with a potential high monetary or nutritional value are so far only used from natural stands. By integrating these high value trees into agroforestry systems smallholder farmers in the tropics could greatly benefit. Vegetative propagation is seen as a possibility to select superior germplasm and bring this important resource into the farmers' fields.

Objectives

The overall objective of the course is to enhance the knowledge and practical skills of technicians responsible for vegetative tree propagation in agroforestry research and development projects.

The Course

The course will take place from 16 to 20 February 1997 at ICRAF's headquarters in Nairobi, Kenya.

The course will cover the following subjects:

- 1 Concepts and principles
- 2 Tree nurseries
- 3 Cuttings
- 4 Grafting
- 5 Layering
- 6 Micro propagation
- 7 Propagation experiments

Instruction will mainly consist of practical work and demonstrations introduced through short theoretical presentations.

Resource persons to teach the course will be representatives from the International Program for Arid Lands Crops (IPALAC) in Israel, ICRAF's regional office in West Africa and from ICRAF headquarters.

Participants

Participants will be selected from national institutions and non-governmental organizations in East and Southern Africa. The following criteria will apply:

- Participants need to have a minimum degree of Diploma in forestry, agriculture or a related field.
- They need to be active in nursery operations involving vegetative propagation techniques.
- Their application needs to be endorsed by their employing institution which will have to ensure that the knowledge and skills acquired during the course will be used.
- Special consideration will be given to the participation of qualified women candidates as to maintain an equal gender balance.

Conditions

Sponsorship for nominated candidates will be on a competitive basis and the organizers will also consider a limited number of qualified candidates with individual sponsorship.

Application Deadline

Application forms should reach ICRAF not later than 15 December 1997.



Annex 2 Application form



INTERNATIONAL CENTRE FOR RESEARCH IN AGROFORESTRY
UNITED NATIONS EDUCATION AND SCIENCE ORGANISATION
INTERNATIONAL PROGRAMME FOR ARID LANDS CROPS

Short Training Course on:
'Vegetative Tree Propagation for Arid and Semi-Arid Lands'
16 to 20 February 1998, Nairobi, Kenya

APPLICATION FORM

I. TO BE COMPLETED BY THE APPLICANT

A. PERSONAL INFORMATION

1. Name: Mrs./Ms./Mr.	2. First name(s):
3. Title:	
4. Employing institution: <i>(name)</i> <i>(street/P.O.Box):</i>	
<i>(city):</i>	<i>(country):</i>
<i>(telephone):</i>	<i>(fax):</i>
<i>(telex):</i>	<i>(cable):</i>
<i>(email):</i>	
5. Home address:	
<i>(city):</i>	<i>(country):</i>
<i>(telephone):</i>	
6. Birth date:	7. Nationality:
8. Passport number:	9. Place of issue:
10. Date of issue:	11. Date of expiry:
12. Mother tongue:	13. Working language:

B. EDUCATION

14. Highest degree obtained (Ph.D., M.Sc., B.Sc., Certificate, Diploma, other):

15. Year obtained:

16. Institution:

17. Discipline(s):

18. List relevant in-service training activities you participated in since you graduated:

C. PROFESSIONAL EXPERIENCE

19. Number of years of professional experience:

20. Number of years with present employer:

21. Name and title of your present supervisor:

22. Brief description of your present duties:

23. Briefly describe your professional experience prior to your present employment:

24. Have you ever published any of your work for research, training or extension? If so, list these publications:

25. Briefly explain what you expect to gain from attending this training course and what contribution you can make. If needed, use the reverse side of this page for any other information that may support your application. Do note that this information will be taken into account when considering your application.

The undersigned certifies that the above information is correct and complete, and acknowledges that ICRAF will not be held responsible in case of accident, illness, theft or death while travelling to and from, or staying in Kenya to attend the course.

Date:

Signature:

111

II. TO BE COMPLETED BY EMPLOYING INSTITUTION

The undersigned, Dr/Mrs/Ms/Mr:

Title:

Name and address of employing institution:

Approves the application of the above candidate and his/her sponsor.

Date:

Signature:

Official stamp:

III. ADDITIONAL INFORMATION - JUSTIFICATION TO ATTEND

Use this page, and eventually additional pages, to briefly describe how you will benefit from this course and what you will contribute to it. This justification to attend will allow course organizers to consider your application and to select the best applicants.



Annex 3 Course programme

“Vegetative Tree Propagation for Arid and Semi-Arid Lands Training Course”



16-20 February 1998 ICRAF, Kenya

COURSE PROGRAMME

Monday 16 February	
09.00 - 09.30	Course Opening - <i>P. Sanchez; T. Lehmberg</i>
09.30 - 10.00	Introduction to vegetative propagation - <i>Z. Wiesmann;</i>
10.00 - 10.30	Benefits and risks of vegetative propagation in agroforestry - <i>H. Jaenicke</i>
10.30 - 11.00	<i>Coffee/Tea break</i>
11.00 - 12.00	Propagation facilities and non-mist propagators - <i>Z. Tchoundjeu</i>
12.00 - 13.00	Physiology and growth regulators - <i>Z. Wiesmann</i>
13.00 - 14.00	<i>Lunch break</i>
14.00 - 18.00	Nursery Practical I (stem and root cuttings)
Tuesday 17 February	
09.00 - 10.30	Experimental design for tree propagation research - <i>R. Coe</i>
10.30 - 11.00	<i>Coffee/Tea Break</i>
11.00 - 12.00	Case study from the Sahel - <i>Z. Tchoundjeu</i>
12.00 - 13.00	Nursery management - <i>H. Jaenicke</i>
13.00 - 14.00	<i>Lunch break</i>
14.00 - 18.00	Nursery practical II (nursery management)
Wednesday 18 February	
09.00 - 10.30	Grafting techniques - <i>H. Jaenicke</i>
10.30 - 11.00	<i>Coffee/Tea break</i>
11.00 - 12.30	Other vegetative propagation techniques - <i>Z. Tchoundjeu; H. Jaenicke</i>
12.30 - 14.00	<i>Lunch break</i>
14.00 - 18.00	Nursery practical III (grafting & layering)
Thursday 19 February	
09.00 - 10.30	The Kibwezi Irrigation Project - <i>Z. Carmi</i>
10.00 - 10.30	<i>Coffee break</i>
10.30 - 12.30	Use of artificial media in plant propagation - <i>Z. Carmi</i>
12.30 - 13.30	<i>Lunch break</i>
13.30 - 18.00	Field visit (Roslyn River Garden Centre and Kenya Forestry Research Institute)
Friday 20 February	
09.30 - 10.30	Friday Seminar - <i>Z. Wiesmann</i>
10.30 - 11.00	<i>Coffee Break</i>
11.00 - 12.00	Micropropagation - <i>M. Kwapata</i>
12.00 - 13.00	Pest and disease control in tree nurseries - <i>J. Desaegeer</i>
13.00 - 14.00	<i>Lunch break</i>
14.00 - 15.00	Molecular analysis methods to support vegetative propagation - <i>I. Dawson</i>
15.00 - 16.30	Nursery Practical IV (assessments)
16.30 - 17.00	Course evaluation and closing - <i>J. Beniast; H. Jaenicke; Z. Wiesmann</i>

ANNEX 4 a - THEORETICAL SESSIONS EVALUATION



INSTRUCTIONS:

- This form will allow you to evaluate each theoretical presentation made during the course. For each presentation, indicate the date, subject and name of the person teaching it. Evaluate the presentation using the criteria listed on the next page.
- Do NOT evaluate presentations that you have not attended.
- Use the appropriate evaluation forms to evaluate the field visit(s) and the training exercise.
- Please return the filled-out evaluation form to the course organizers at the end of the course.
- Thank you for your collaboration.

NOTE :

This course evaluation is anonymous and only serves to help resource persons to better prepare and deliver their presentations in the future. The other evaluations (final, field visit(s) and training exercise, will be summarized in the course report.

KEY TO THE EVALUATION OF THE THEORETICAL PRESENTATIONS:

New knowledge acquired :

- 1 = very little
- 2 = strengthens existing knowledge
- 3 = a lot

Presentation : quality of the presentation

- 1 = poor
- 2 = average
- 3 = excellent

Time : duration of a session as compared to the overall length of the course:

- 1 = too long
- 2 = adequate
- 3 = too short

Importance : importance of the subject for your daily work

- 1 = not important
- 2 = important
- 3 = very important

Supporting training notes : quality of the supporting documents

- 1 = poor
- 2 = average
- 3 = excellent
- = none given out

Audiovisual support: quality of slides and transparencies

- 1 = poor
- 2 = average
- 3 = excellent
- = none used

COMMENT(S) : Formulate your comments or suggestions for a specific presentation. If necessary, use additional pages but indicate clearly what presentation they refer to.

Date	Subject	Name resource person	New knowledge acquired	Time	Importance	Quality of the presentation	Training materials support	Audio-visuals used	Comments

KINDLY RETURN YOUR FILLED-OUT FORMS TO THE COURSE ORGANIZERS. THANK YOU FOR YOUR COLLABORATION

ANNEX 4 b - EVALUATION FIELD VISIT

Date:

Site visited:

Resource person for the visit:

Tick the appropriate box and formulate your comments/suggestions

<p>a) Did you acquire any new knowledge?</p> <p><input type="radio"/> Yes</p> <p><input type="radio"/> A little</p> <p><input type="radio"/> No</p>	<p>b) The time allocated to the visit :</p> <p><input type="radio"/> Too long</p> <p><input type="radio"/> Adequat</p> <p><input type="radio"/> Too short</p> <p><input type="radio"/> The visit can be cancelled</p>
<p>c) What was the relevance of the visit in the context of the overall course programme?</p> <p><input type="radio"/> Very relevant</p> <p><input type="radio"/> Somewhat relevant</p> <p><input type="radio"/> Not relevant</p>	<p>d) The timing of the visit in the context of the overall programme was:</p> <p><input type="radio"/> Appropriate</p> <p><input type="radio"/> More or less appropriate</p> <p><input type="radio"/> Not appropriate</p>
<p>e) The documentation in support of the visit was:</p> <p><input type="radio"/> Excellent</p> <p><input type="radio"/> Average</p> <p><input type="radio"/> Poor</p> <p><input type="radio"/> Not available</p>	<p>f) The organization of the visit was:</p> <p><input type="radio"/> Excellent</p> <p><input type="radio"/> Average</p> <p><input type="radio"/> Poor</p>
<p>g) Your comments and/or suggestions:</p> 	

ANNEX 4 c - EVALUATION FIELD EXERCISE

Your group number:

Group resource person:

Tick the appropriate box and formulate your comments/suggestions

<p>a) Did the training exercise enhance your knowledge of the corresponding unit ?</p> <p><input type="radio"/> Yes <input type="radio"/> More or less <input type="radio"/> No</p>	<p>b) The total time dedicated to the training exercise was:</p> <p><input type="radio"/> Too long <input type="radio"/> Adequat <input type="radio"/> Too short <input type="radio"/> The exercise can be cancelled</p>
<p>c) Did the theoretical presentations on this unit prepare you for this exercise?</p> <p><input type="radio"/> Yes <input type="radio"/> More or less <input type="radio"/> No</p>	<p>d) The timing of the exercise in the context of the overall programme was:</p> <p><input type="radio"/> Appropriate <input type="radio"/> More or less appropriate <input type="radio"/> Not appropriate</p>
<p>e) The information needed to implement this exercise was :</p> <p><input type="radio"/> Excellent <input type="radio"/> Average <input type="radio"/> Poor <input type="radio"/> Non-existent</p>	<p>f) The organization of the exercise was :</p> <p><input type="radio"/> Excellent <input type="radio"/> Average <input type="radio"/> Poor</p>
<p>g) Did you receive proper guidance and instructions from your group resource person?</p> <p><input type="radio"/> Yes <input type="radio"/> More or less <input type="radio"/> No</p>	<p>h) The choice of the site(s) for the field exercise was :</p> <p><input type="radio"/> Appropriate <input type="radio"/> More or less appropriate <input type="radio"/> Not appropriate</p>
<p>i) Your comments and/or suggestions:</p> 	

ANNEX 4 d
COURSE EVALUATION
PERSONAL ACTION PLAN FORM

NAME :	
INSTITUTION :	

ACTIVITY 1 (use the same type of table for a maximum of 3 action items or activities)

Title of the activity:	
Person(s) responsible(s):	
Resources needed:	
Expected outcome(s):	
Means of verification :	
Timeframe:	

ANNEX 4 e - FINAL EVALUATION

Please tick the appropriate box(es) () and or answer the questions. This evaluation is anonymous but important since it will allow us to further improve future training courses on this subject. Use the back of these pages and/or additional sheets if necessary.

Thank you for your collaboration.

1. PRE-COURSE ARRANGEMENTS

1.1. When were you informed about this training opportunity (date):

1.2. How were you selected to participate?

- I applied as an individual candidate
- I was nominated by my employer
- I was nominated by the national contact person
- I was nominated by an ICRAF representative
- Other (indicate how) :

1.3. Did you receive the following information about this course :

- Information brochure
- Application form
- Nomination letter specifying conditions of participation
- Information on your travel to Nairobi/Kenya
- Other(s) (specify) :

1.4. Did this information allow you to prepare yourself to attend this course?

- Yes
- No

1.5. Other comments and/or suggestions that will allow us to improve pre-course arrangements:

2. COURSE STRUCTURE

2.1. Was the timing of this course appropriate (February)?

- Yes
- No. If no, why and what would be a better time of the year to organize such course:

2.2. Evaluate the following general aspects of this course:

Aspect to evaluate	TOO LONG	ACCEPTABLE	TOO SHORT
a) Total course duration (1 week)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b) Theoretical presentations	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
c) Group work	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
d) Field visit(s)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
e) Exercise	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

2.3. Look at the course programme and rank the units according to your personal preference regarding the **overall quality** of the topics and their **usefulness** in the context of your day-to-day work. Use a figure from 1 (excellent/very useful) to 5 (poor/not useful). **You cannot use the same rank more than once!**

122

Unit	Quality	Usefulness
1 Concepts and principles		
2 Tree nurseries		
3 Cuttings		
4 Grafting		
5 Layering		
6 Micro propagation		
7 Propagation experiments		

2.4. Other comments/suggestions on the structure of the training course:

3. COURSE OBJECTIVE

3.1. Consider the course objective and tick the appropriate figure to evaluate the **appropriateness** and the **effectiveness** of this objective. Note the meaning of these terms:

Appropriateness: opportunity and usefulness of the objectives in the context of your daily work

Effectiveness: the way the objectives have been achieved whether appropriate or not

(1=NOT appropriate/effective, 5= VERY appropriate/effective)

COURSE OBJECTIVES	APPROPRIATENESS					EFFECTIVENESS				
	1	2	3	4	5	1	2	3	4	5
<i>The overall objective of the course is to enhance the knowledge and practical skills of technicians responsible for vegetative tree propagation in agroforestry research and development projects.</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

3.2. Did the programme developed for this course address these objectives?

- Yes
- More or less
- No

123

3.3. Will the topics taught during this course allow you to implement the 'Personal Action Plan' developed during this course?

- Yes
- More or less
- No

3.4. Your comments and/or suggestions related to the course objectives and programme:

4. TRAINING MATERIALS

4.1. Did you receive sufficient training materials (quantity) in support of the various topics covered during this course?

- Yes
- More or less
- No

4.2. What is the overall quality of these materials?

- Excellent
- Good
- Average
- Poor (explain) :

4.3. Will the training materials received during this course allow you to improve your agroforestry training or education activities?

- Yes
- More or less
- No

124

4.4. Other comments/suggestions regarding the training materials for this type of course:

5. LOGISTIC ASPECTS

5.1. Consider the following logistic arrangements of this course and evaluate them ticking a figure between 1 and 5 (1=very poor, 2=poor, 3=average, 4=good, 5=excellent)

Aspect to evaluate	1	2	3	4	5
International travel arrangements (if applicable)					
Reception in Kenya (if applicable)					
Registration formalities at ICRAF					
Hotel in Nairobi (if applicable)					
Meal arrangements (lunches and coffee breaks)					
Daily subsistence allowance and claim settlements					
Training infrastructure (rooms, audio-visual equipment) at ICRAF					
Secretariat					
Daily transport arrangements (to ICRAF/hotel, visit, exercise)					
Other(s)Please specify: - - - -					

125

5.2. Your suggestions/comments regarding logistic arrangements for this type of training course:

6. INTERACTION PARTICIPANTS/RESOURCE PERSONS

6.1. Evaluate the following aspects by ticking the appropriate box (1= very poor, 2=poor, 3=mediocre, 4=good, 5=excellent):

Aspect to evaluate	1	2	3	4	5
Total number of participants (± 20)					
Educational background and experiences represented					
Interaction between participants					
Interaction between participants and resource persons					

6.2. Your comments/suggestions on these interactions:

126

7. OVERALL EVALUATION

7.1. Considering all aspects (technical and logistic), what is your overall evaluation of this training course:

- Excellent
- Very good
- Good
- Mediocre
- Poor (explain):

7.2. Will your participation in this training course have helped you to better teach agroforestry in your future training/education activities?

- Yes
- More or less
- No (explain why):

7.3. From your personal point of view, list the three main **strong/positive** points of this training-of-trainers course in order of importance:

1.

2.

3.

7.4. From your personal point of view, list the three main **weaker** points of this training-of-trainers course in order of priority and suggest ways of improvement:

1.

2.

3.

127

7.5. Suggest some improvements that can be made for this type of training course:

A

abiotic 9
 abiotic factors 25
 abscisic acid 7
 abscission layers 7
Acacia spp. 34
 adventitious roots 8
 adventitious shoot formation 84, 90
Agromyzidae 35
Agrotis spp. 35
 air layering 14, 75, 77
Albizia spp. 34
 Alnus 20
Anacardium occidentale 34
 analysis of variance (ANOVA) 100
 androgenesis 91
 antigibberellins 7
 aphids 35
 apical dominance 4, 6, 7, 8
 apical shoots 8
 apomixis 4
 approach grafting 56
 auxins 7
 axillary buds 85
 axillary shoot culture 90
 axillary shoot formation 84, 89
Azadirachta indica 33, 34

B

BA/IBA 85
 bananas 85, 92
 bare rooted seedlings 21
Bauhinia rufescens 50
 beetles 35
 biological control 38
 biological properties 23
 biotechnology 85
 biotic 9
 biotic factors 25
 blocking of variation 97
 bridge grafting 60
 bud development 46
 budding 56, 57

buffer capacity 23

bulk density 23

C

calcium 24
Calliandra 48
Calliandra calothyrsus 34
 callus 58, 86, 90
 callus bridge formation 61
 cambial cell growth 7
 cambium formation 61
Carica papaya 34
Cassia angustifolia 34
 caterpillars 35
 cation exchange capacity 23
 chemical properties 23
 chemical sterilization 37
 chilli pepper 39
 chilling 20
 citrus 85
Citrus auriculiformis 76
Citrus sinensis 63
 coffee 92
 compatibility 56
 containers 20, 21
 control of variation 97
 coppice shoots 8
 crickets, 35
Crotalaria spp. 34
 culture room 88
 curative measures 18, 38
 cuttings 3, 43
 cytokinins 7

D

damage repair 60
 damping-off 31, 32
 derris roots 39
Desmodium distortum 34
 dioecious plants 60
 diseases 10
 documentation 14
Dodonaea viscosa 34
 dormant 20

E

embryo culture 84, 89
 embryo rescue 89
 endoplasmatic reticulum 62
 endosperm 7
 epicormic shoots 8
 Eriophyidae 35
Erisyphe spp. 32
 Ethylene 7
Eucalyptus 20, 85
Eucalyptus camaldulensis 34
Eucalyptus x trabutii 59
Euphorbia balsamifera 34
 experimental design 95
 experimental protocols 29
 experimentation 19
 explant 86, 88

F

factorial or split-plot experiments 29
 fertility 23
Ficus spp. 76
 fruit flies 35

G

garlic 39
 genetic diversity 9
 genotype 6
 germination 20
 gemplasm collection 11
 gibberellins 7
 girdling 80
 grafting 3, 8, 55, 57
 grasshoppers 35
Grevillea robusta 34
 growth inhibitor 7
 growth regulators 2, 6

H

haploid 86
 hardened 26
 hardening 47
 hardening-off 50
 healing conditions 61
 healing process 60
 heat sterilization 37
Helianthus annuus 64

I

I. wombolu 14
in vitro culture 83
 inbreeding depression 13
 incompatibility 63
 incubate 86
 incubation room 88
 inoculation room 87
 insects 35
 interstock 64
Irvingia gabonensis 14

K

Khaya ivorensis 47
 kinetin 7

L

L. diversifolia 27
L. trichandra 27
 labelling 28
 lateral shoots 8
 layering 4, 8, 75
 leaf area 47
 leaf blisters 32
 leafhoppers 35
Leucaena 28, 48
Leucaena diversifolia 28
Leucaena leucocephala 27, 28, 34
Leucaena trichandra 28
 locusts 35
Lovoa trichilioide 47

M

macronutrients 24
 magnesium 24
Mangifera indica 63, 76
 marcotted 12
 marcotting 14, 15, 77
 marigold 33, 39
 McCown and Lloyd 88
 measurements 97
Melia volkensii 97
Meloidogyne spp. 32, 33
 meristem 8
 meristematic cells 57
Mesoplatys ochroptera 35, 36
 micrografting 84, 89, 91

micronutrients 24
microorganisms 22
micropropagation 4, 83, 86
Mimosa scabrella 34
mist propagation 49
mites 35
mono- or oligoclonal populations 9
monocots 89
mother plants 44
mound layering 76, 79
Murashige and Skoog 88
mycorrhizae 19, 23

N

natural pesticides 39
neem 39
neem tree 33
nematodes 32
nicking 20
nitrogen 24
non-mist propagation 49
non-mist propagator 43
nursery 17
nursery environment 26
nursery hygiene 25
nursery logbook 29
nutrient deficiency 24

O

objectives 96
offsets 4
oil palm 92
ontogenetic 8
organogenesis 86, 89
orthodox seeds 20
outbreeding 11
outbreeding species 15

P

P. armeniaca 63
P. domestica 63
P. persica 63
palm 85
pathogens 31
Persea americana 76

pest and disease management 31
pesticides 39
phenotypic selection 12
phloem 57
phosphorus 24
Phyllophaga spp. 35
physical properties 23
physiological age 56
physiology 60
phytohormones 1
phytosanitary measures 36
phytosanitation 17, 31
pineapple 85
plant bugs 35
plant hormones 2, 6
plantlets 86, 89
plasticity 23
plots or experimental units 96
polyembryony 4, 86
polypropagator 51
Poncirus trifoliata 63
porosity 23
potassium 24
potting-up 50
powdery mildew 32
preparation room 87
preventive measures 18
pricking out 20, 41
primary culture 86
pro-embryo 86
propagation experiments 93
propagator 4
propagules 85, 86
Prosopis africana 50
Prosopis juliflora 34
protocol 14
Prunus africana 32, 50, 99
Prunus amygdalina 63
psyllids 35
Pterocarpus erinaceus 50
pyralidae 35
pyrethrum 39

Q

quality seedlings 19

.....

R

raised beds 21
 raised frames 21
 randomization 96
 recalcitrant 14
 record keeping 28
 regeneration 86
 rejuvenation 60
 replication 96
 Rhizobia 23
Rhizobium 19
 root 43
 root deformities 20, 41
 root elongation 47
 root primordia 47
 root pruning 21
 root trainers 22
 root-knot nematode 32
 rooting process 43, 45
 rooting substrate 46
 rootstock 57
 rubber 85, 92

S

Samanea saman 34
 sawflies 35
 scion 57
 seedlings 86
Senna siamea 34
Sesamum indicum 35
Sesbania sesban 35, 95
Sesbania spp. 32, 34, 36
 shading 21
 shoot apical meristem culture 89
 shoot elongation 7
 simple layering 75, 79
 somatic embryogenesis 84, 91
 somatic embryos 89
 sooty mould 32
 splice graft 56, 65
 split-plot 100
Spodoptera spp. 35
 steam pasteurized 26
 stem cuttings 43, 45
 stem elongation 46
 stock plants 44, 48

stooling 75, 76, 79
 subculture 86
 substrate 17, 22
 suckers 57
 sulphur 24
 synthetic auxins 7

T

T- and patch budding 56
Tagetes spp. 37
Tamarindus indica 50
Taphrina spp. 32
Tectona grandis 34
Tephrosia spp. 34
 thrips 35
 tissue 83
 tissue maturity 8
 tobacco 39
 top-grafting 60
 top-wedge grafting 56
 trait 9
 transfer 87
 treatments 96
 tree nurseries 17
Triplochiton scleroxylon 48

V

vascular cambium 57, 60
 vascular tissue formation 61
Vicia faba 64
 virus 10
 virus detection 60
 virus-free plant material 83
 viruses 34

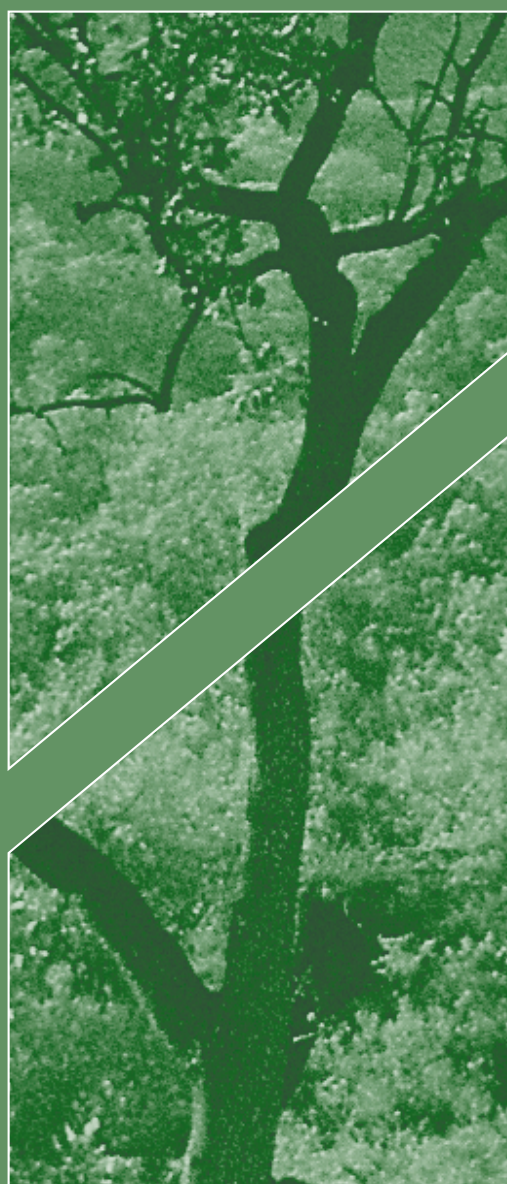
W

water-holding capacity 23
 waterlogging 23
 whip and tongue graf 56, 65
 wound healing 47
 wound healing response 61

Z

zeatin 7

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