Introduction to Vegetative Propagation of Tropical Trees

Roger R.B. Leakey

Agroforestry and Novel Crops Unit

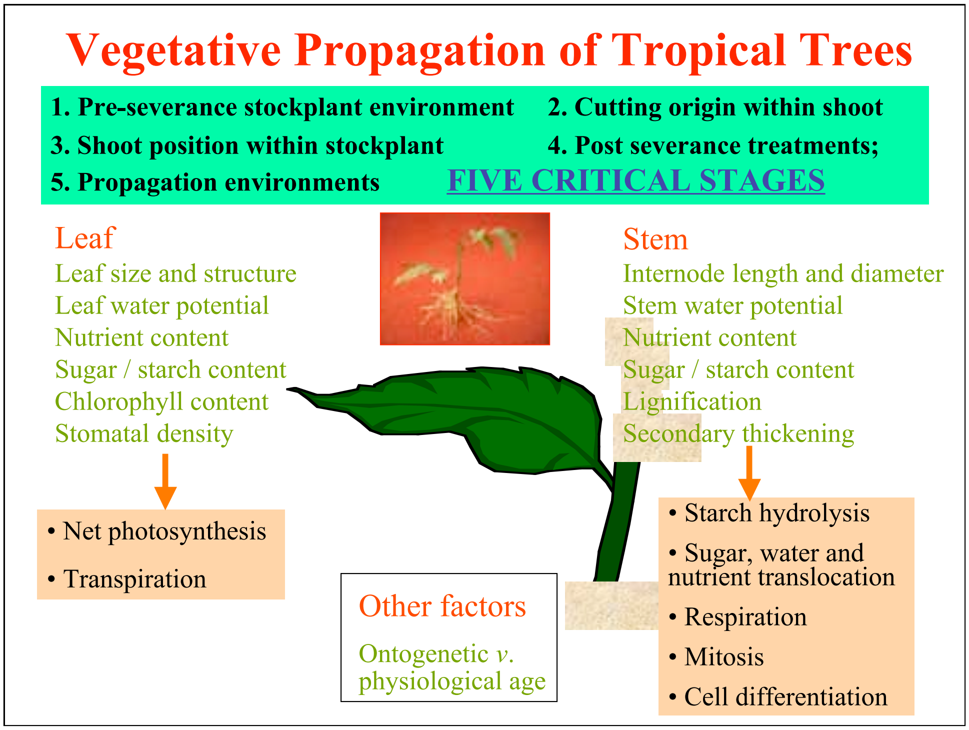
School of Tropical Biology

James Cook University

Cairns, Australia

Training Manual and Video Programmes

In this Course we are going to do ‘theory’ in the morning and ‘practical’ in the afternoon.



Vegetative Propagation of Tropical Trees

1. Pre-severance stockplant environment
2. Cutting origin within shoot
3. Shoot position within stockplant
4. Post severance treatments;
5. Propagation environments

FIVE CRITICAL STAGES

Leaf

Leaf size and structure
Leaf water potential
Nutrient content
Sugar / starch content
Chlorophyll content
Stomatal density

- Net photosynthesis
- Transpiration

Stem

Internode length and diameter
Stem water potential
Nutrient content
Sugar / starch content
Lignification
Secondary thickening

- Starch hydrolysis
- Sugar, water and nutrient translocation
- Respiration
- Mitosis
- Cell differentiation

Other factors

Ontogenetic *v.* physiological age

Propagation Systems

Mist propagation

Non-mist propagation

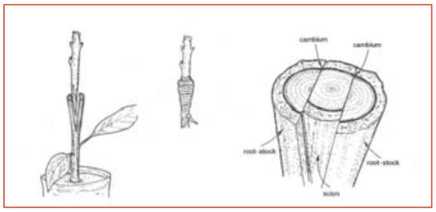
Marcotting (Air Layering)

A tool for capturing mature genotypes

Our understanding of marcott physiology is very poor

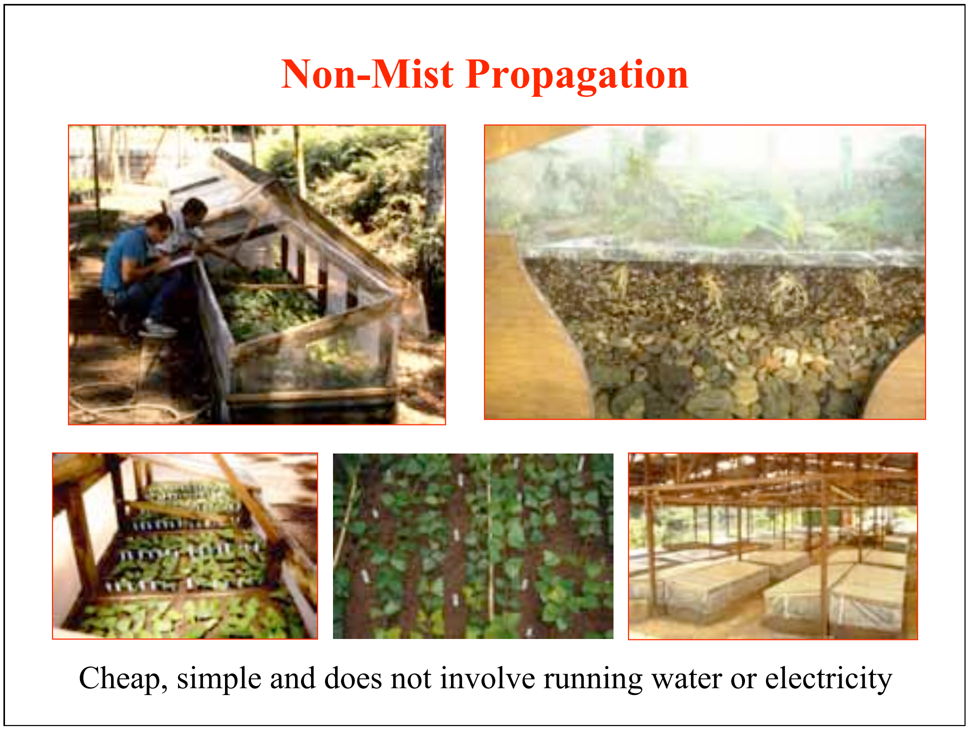
Grafting

is used to propagate and capture the genotype of mature trees



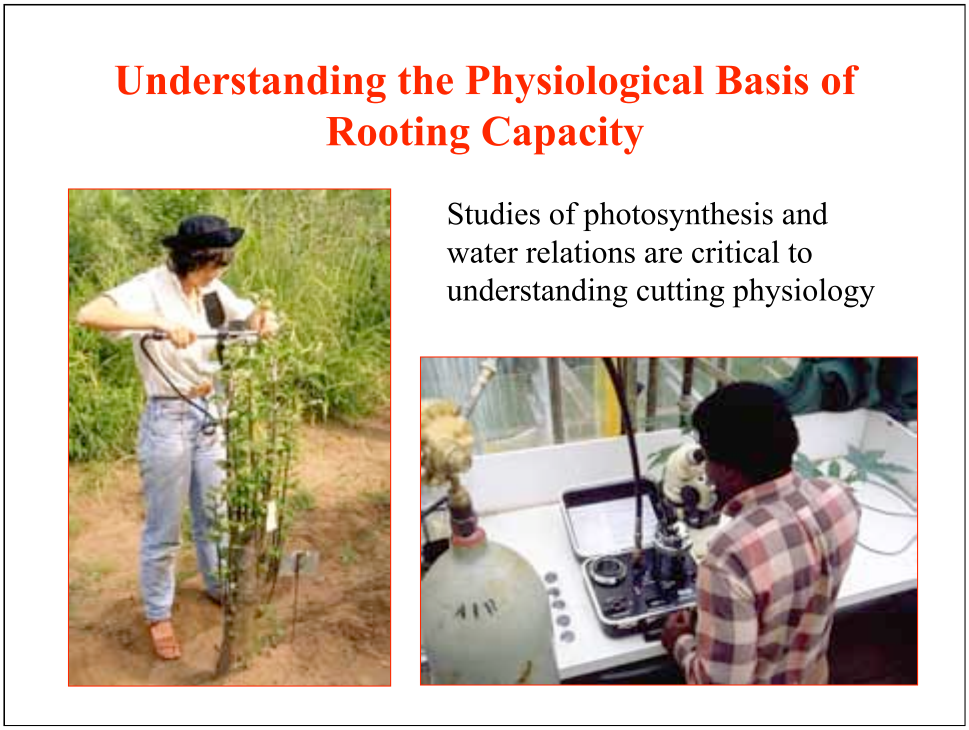
In vitro Micropropagation

is potentially a means to propagate plants in large numbers



Non-Mist Propagation

Cheap, simple and does not involve running water or electricity

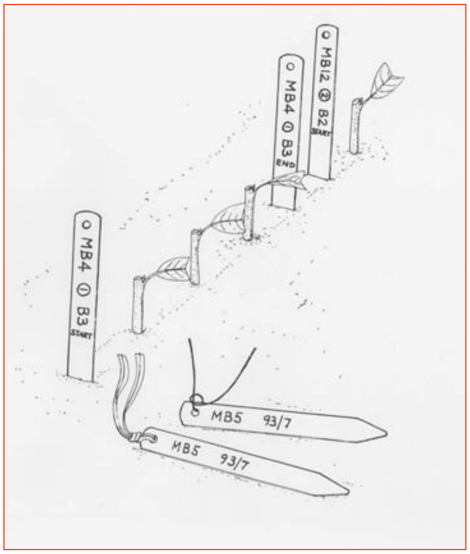


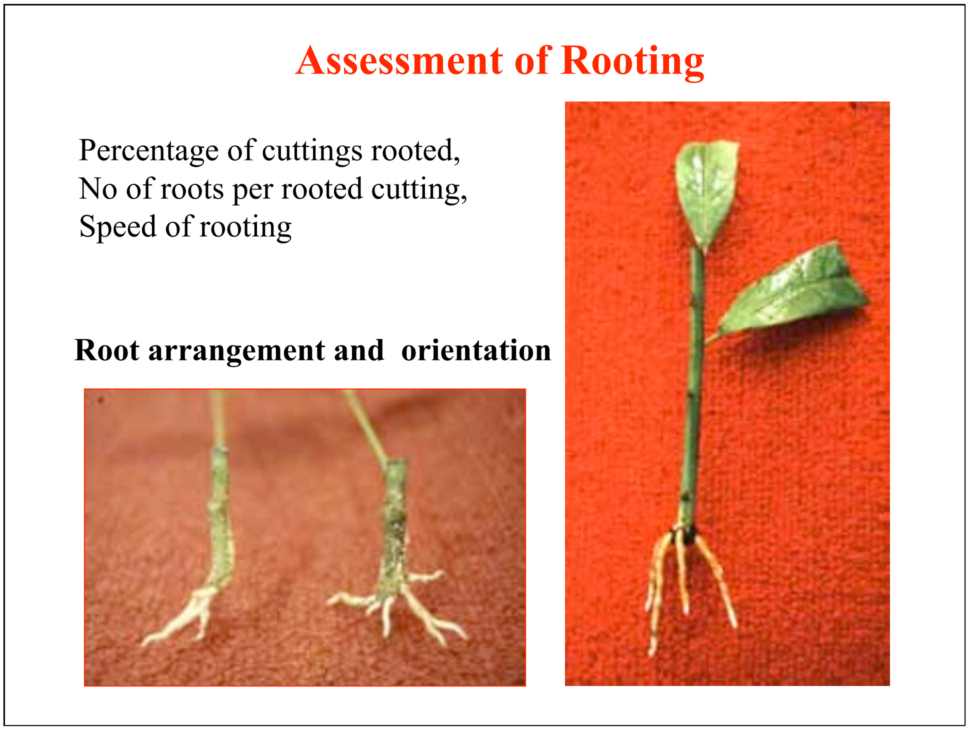
Understanding the Physiological Basis of Rooting Capacity

Studies of photosynthesis and water relations are critical to understanding cutting physiology

Cutting Identification

It is very important to keep good records and to know the identity of every cutting



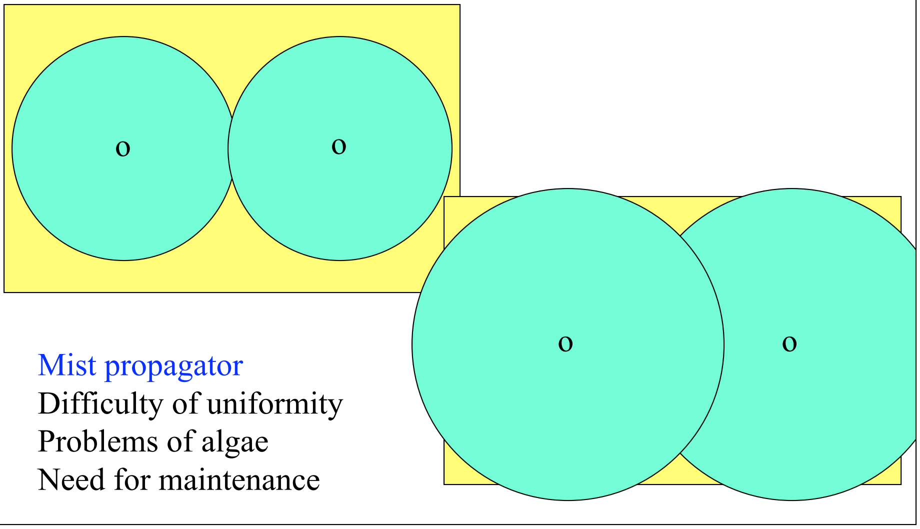


Assessment of Rooting

Percentage of cuttings rooted,
No of roots per rooted cutting,
Speed of rooting

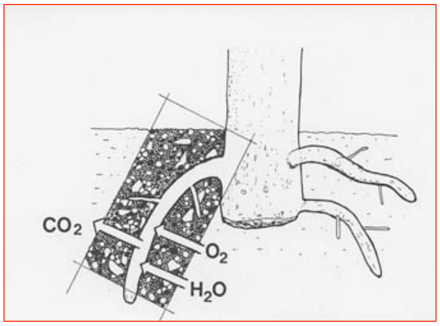
Root arrangement and orientation

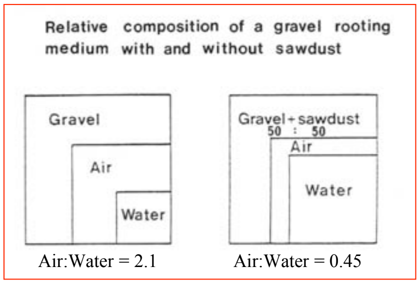
CRITICAL STAGE 5
The Propagation Environment



Mist propagator
Difficulty of uniformity
Problems of algae
Need for maintenance

Rooting Medium



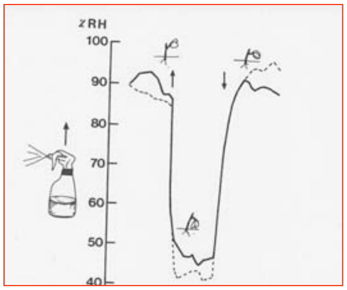

Relative composition of a gravel rooting medium with and without sawdust

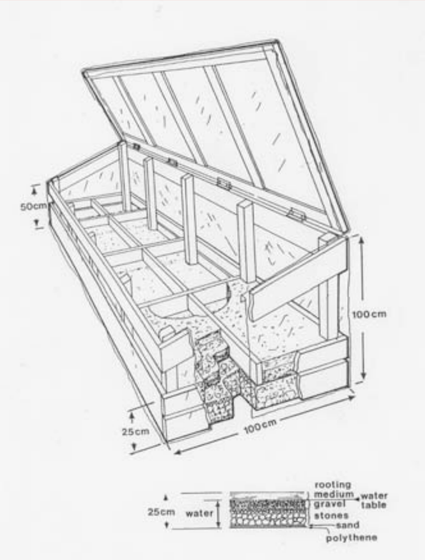
Air:Water = 2.1

Air:Water = 0.45

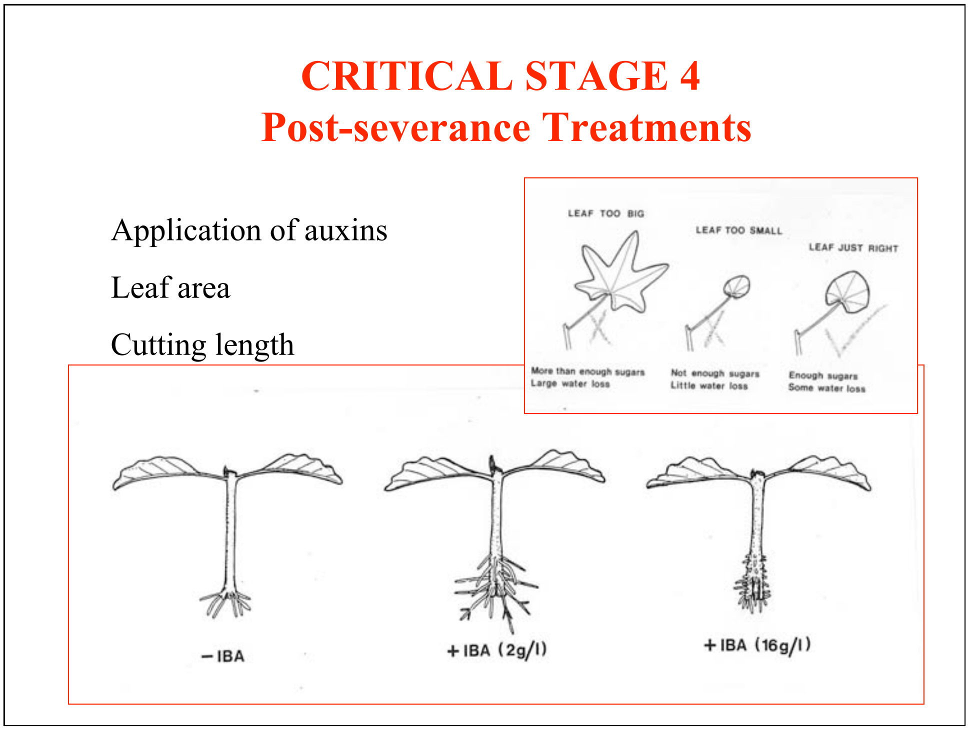
Rooting requires both water and air in the medium. The air:water ratio tends to be species specific.

Non-mist Propagator





This propagator provides a very uniform and humid environment if the box it air-tight and water-tight. When the lid is opened the cuttings have to be sprayed.



CRITICAL STAGE 4
Post-severance Treatments

Application of auxins

Leaf area

Cutting length

Auxin Concentration

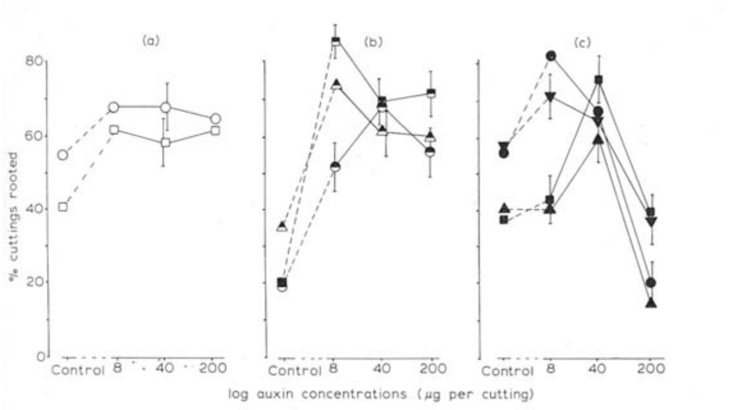
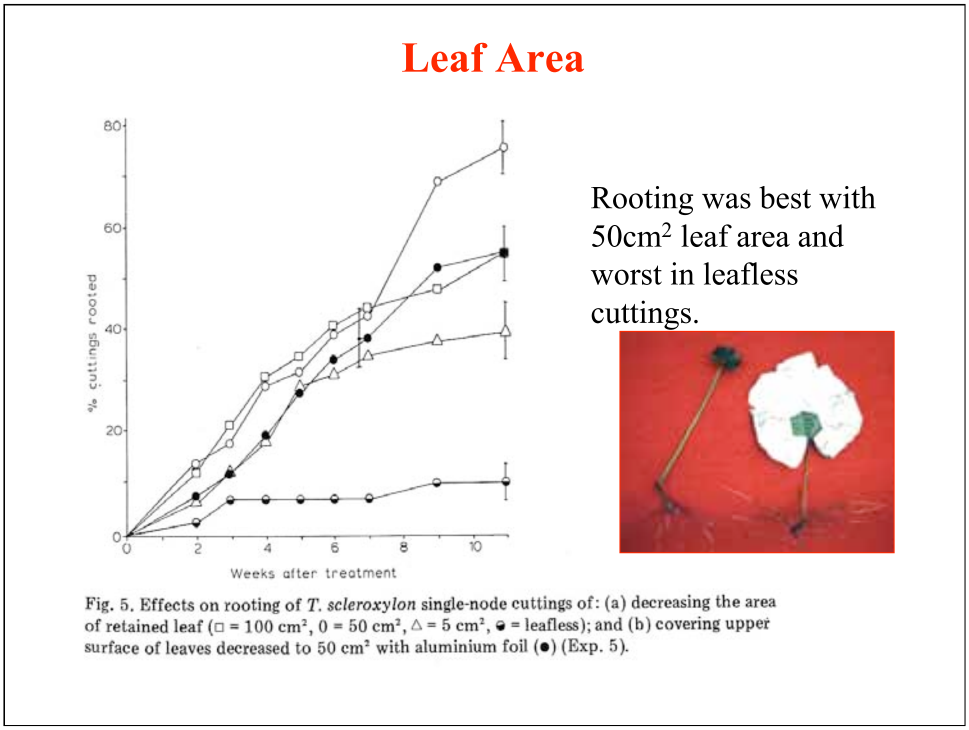


Fig. 2. Effects, after 12 weeks, of applying different amounts of 50 : 50, IBA : NAA mixtures on rooting of leafy single-node cuttings from a range of *T. scleroxylon* clones (Exp. 2).
(Clones: ○ = 8038, □ = 8021, ◒ = 8035, △ = 8028, ⬓ = 8019, ● = 8036, ▲ = 8032, = 8020, ■ = 8034).

Clones showed three different response types to different auxin concentrations



Leaf Area

Rooting was best with 50cm^2 leaf area and worst in leafless cuttings.

Fig. 5. Effects on rooting of *T. scleroxylon* single-node cuttings of: (a) decreasing the area of retained leaf (□ = 100 cm^2, 0 = 50 cm^2, △ = 5 cm^2, ◒ = leafless); and (b) covering upper surface of leaves decreased to 50 cm^2 with aluminium foil (●) (Exp. 5).

Leaf Area and Water Stress

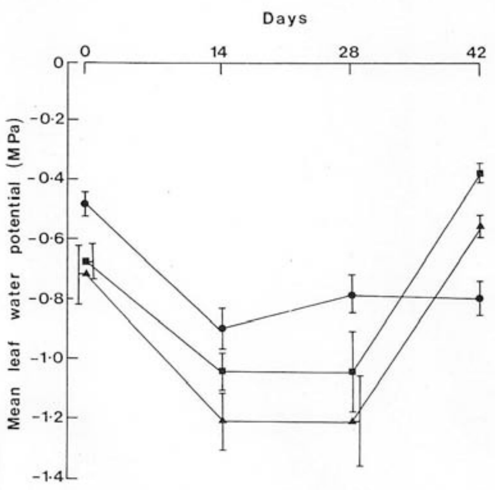


Figure 4. Effects of lamina area (● = 10 cm^2, ■ = 50 cm^2, ▲ = 100 cm^2) on the leaf water potential of single-node *T. scleroxylon* cuttings during 6 weeks of propagation under intermittent mist.

Large leaved cuttings suffered water stress

Leaf Area and Carbohydrates

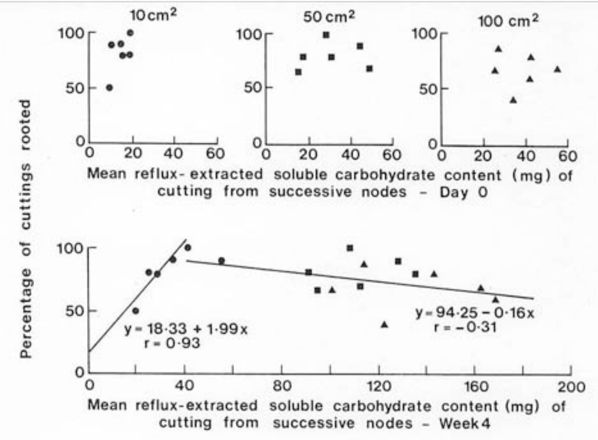
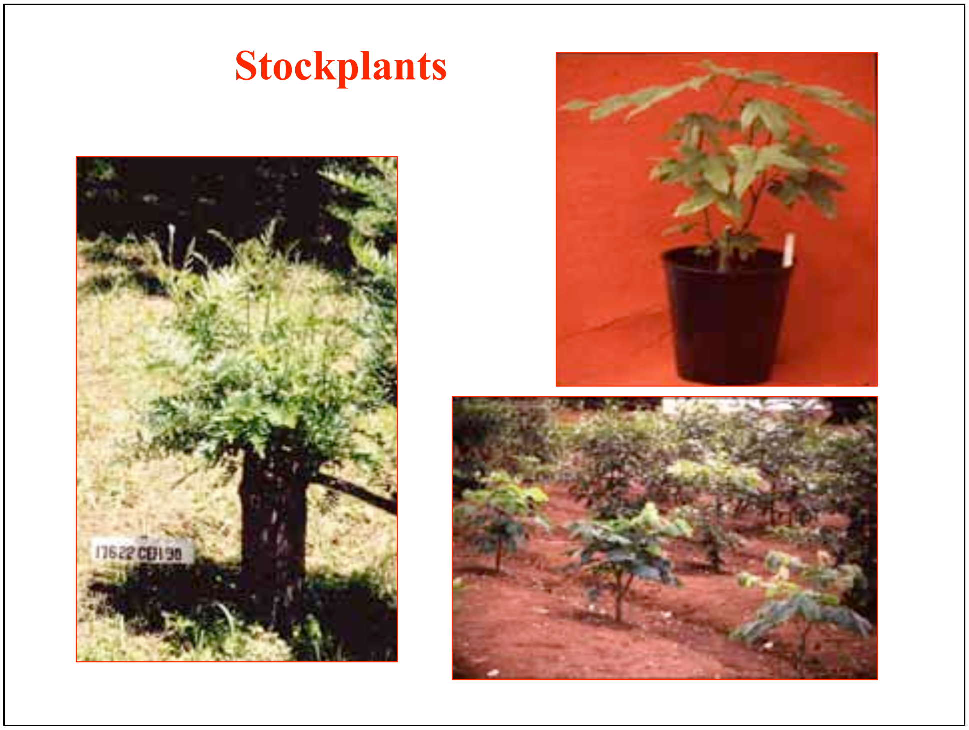
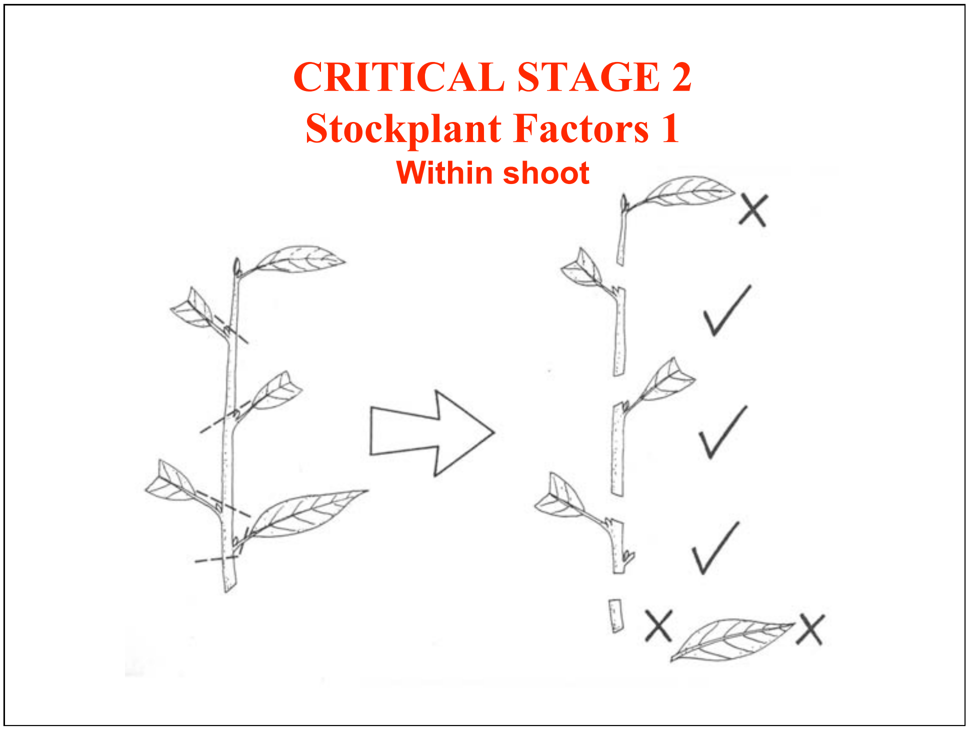


Fig. 8. Relationships between rooting ability (week 6) and mean reflux-extracted soluble carbohydrates at 0 and 28 days after taking *T. scleroxylon* cuttings with different lamina areas (● = 10, ■ = 50, ▲ = 100 cm²). Each point represents the mean value for a different node position.

There was no relationship between soluble carbohydrate and rooting on Day 0, but by Day 28 a relationship had developed.



Stockplants



CRITICAL STAGE 2
Stockplant Factors 1
Within shoot

Node Position

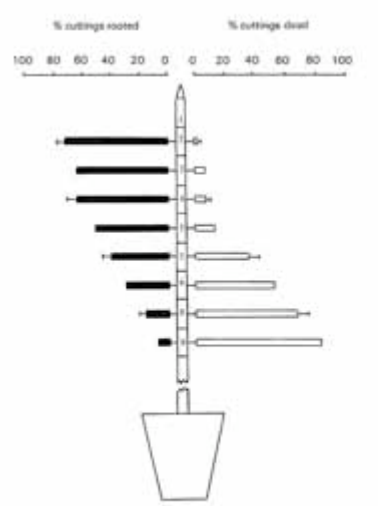


Fig. 1
Mean effects, after 10 weeks, of node positions on rooting and death of single-node leafy mainstem cuttings from undecapitated stockplants of three *T. scleroxylon* clones. Bar = + SE.

Within a stem there are strong gradients in rooting ability and cutting mortality

Cutting Length

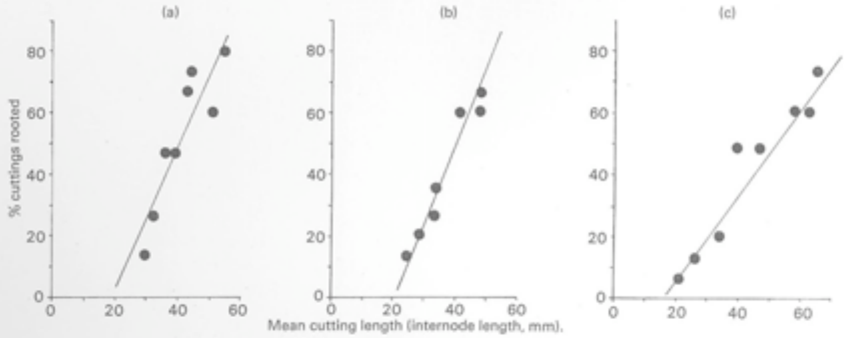
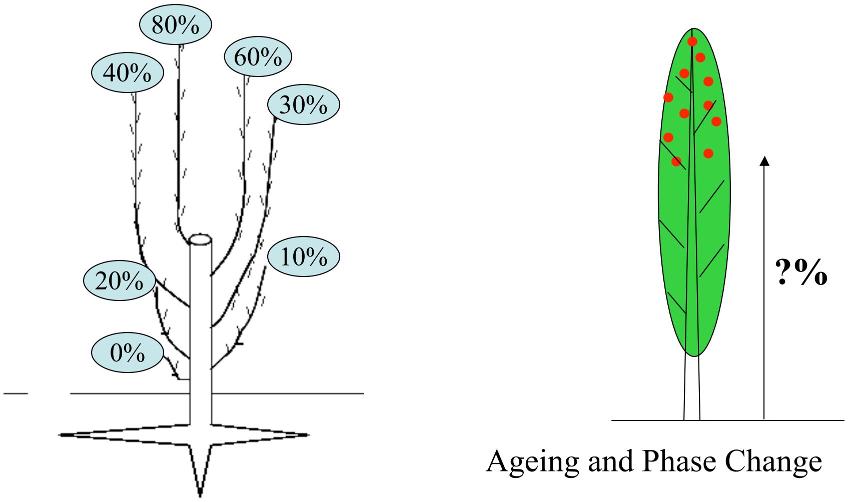


FIG. 10
Relationships between the % of cuttings rooted (week 10) and the mean length of single node cuttings from undecapitated, unbranched plants of *T. scleroxylon* with:
(a) 21 nodes ($y = 42.3 + 2.28x$. $r = +0.88$)
(b) 26 nodes ($y = 53.8 + 2.52x$. $r = +0.97$)
(c) 31 nodes ($y = 21.8 + 1.43x$. $r = +0.97$)

There is a gradient in internode length and a strong relationship between cutting length and rooting ability

CRITICAL STAGE 3
Stockplant Factors 2
Between shoots



Ageing and Phase Change

Plagiotropism

Non-erect shoots have a greater tendency to plagiotropism.
Species vary in the strength of plagiotropism.

Stockplant Height and the Number of Branches

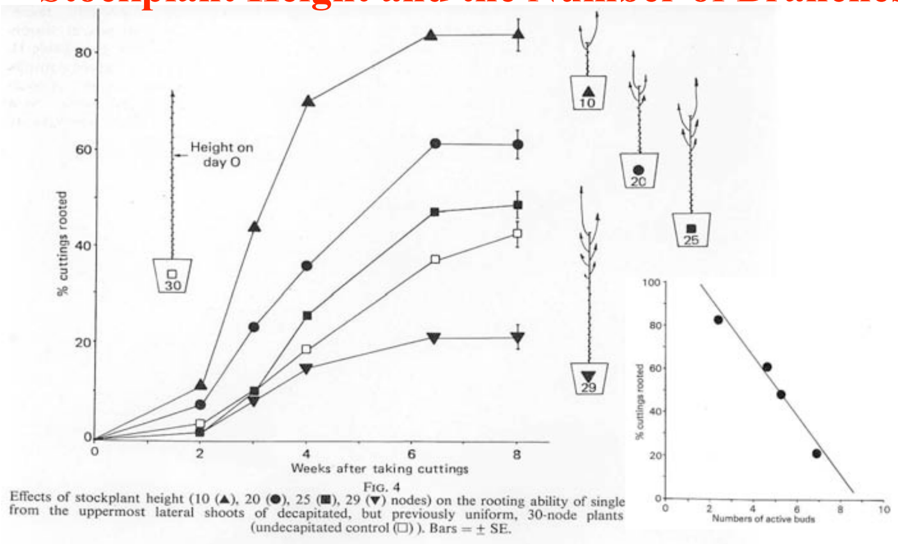
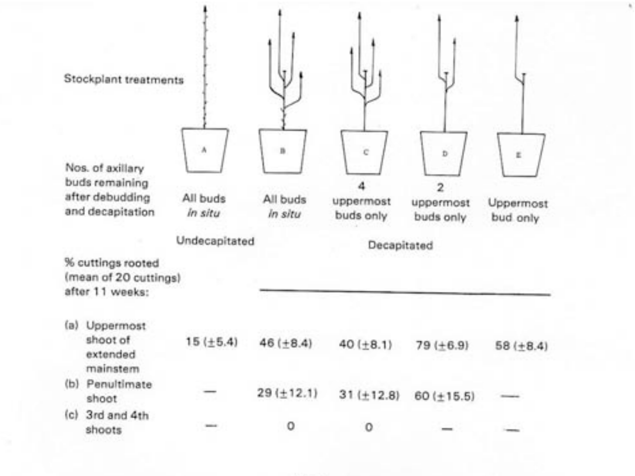


FIG. 4

Effects of stockplant height (10 (▲), 20 (●), 25 (■), 29 (▼) nodes) on the rooting ability of single from the uppermost lateral shoots of decapitated, but previously uniform, 30-node plants (undecapitated control (□)). Bars = ± SE.

Rooting ability is affected by the number of shoots on the stockplant

Number of Shoots

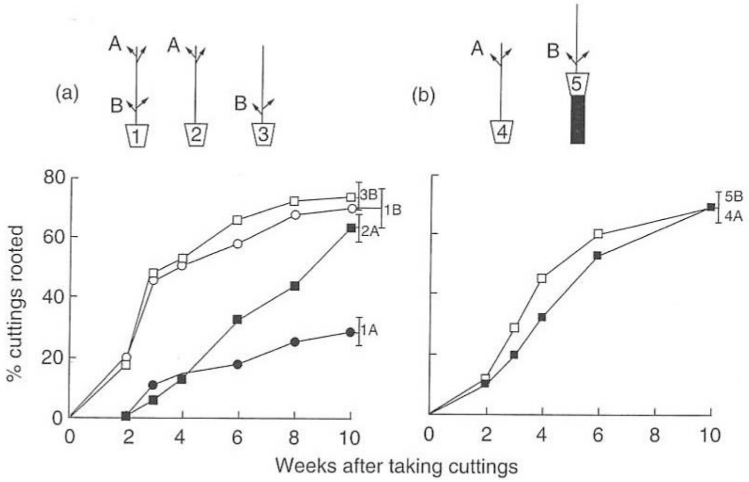


Stockplant treatments	A	B	C	D	E
Nos. of axillary buds remaining after debudding and decapitation	All buds *in situ*	All buds *in situ*	4 uppermost buds only	2 uppermost buds only	Uppermost bud only
	Undecapitated	Decapitated			
% cuttings rooted (mean of 20 cuttings) after 11 weeks:					
(a) Uppermost shoot of extended mainstem	15 (±5.4)	46 (±8.4)	40 (±8.1)	79 (±6.9)	58 (±8.4)
(b) Penultimate shoot	—	29 (±12.1)	31 (±12.8)	60 (±15.5)	—
(c) 3rd and 4th shoots	—	0	0	—	—

Fig. 6
Effects of the number of lateral shoots per stockplant on % rooting (± SE) of leafy, single-node cuttings of *T. scleroxylon*.

Two-shoot stockplants had the highest rooting ability.
The upper shoot is best

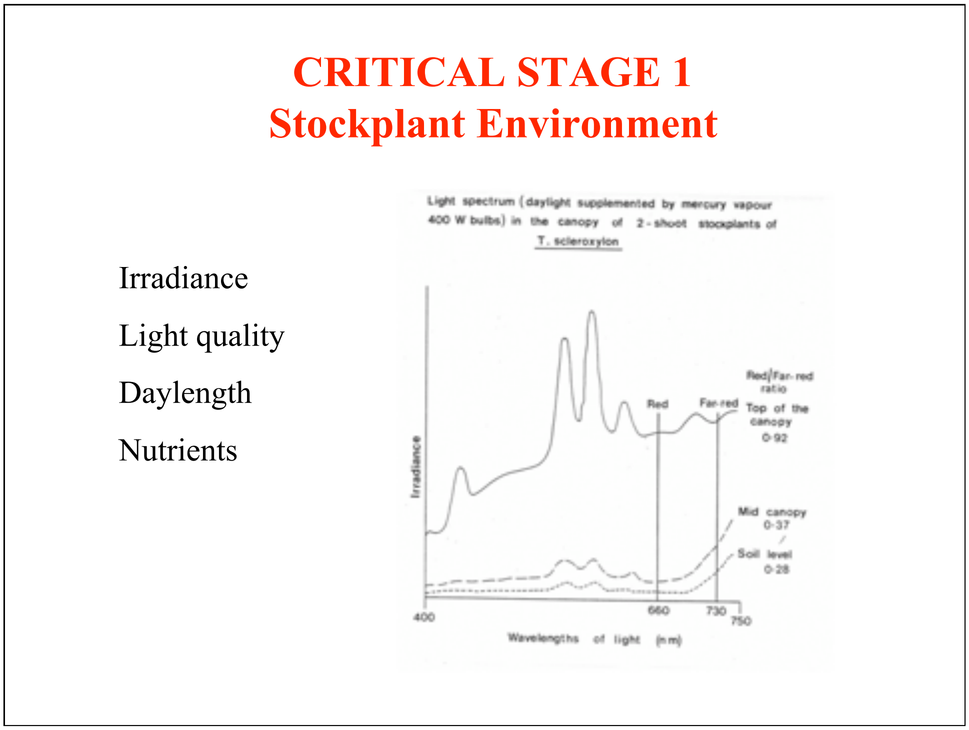
Effects of Shoot Number and Position on Rooting



2 shoots better than 4 shoots.

Basal shoots better than apical shoots.

Apical and basal shoots equally good if in same light environment.



CRITICAL STAGE 1
Stockplant Environment

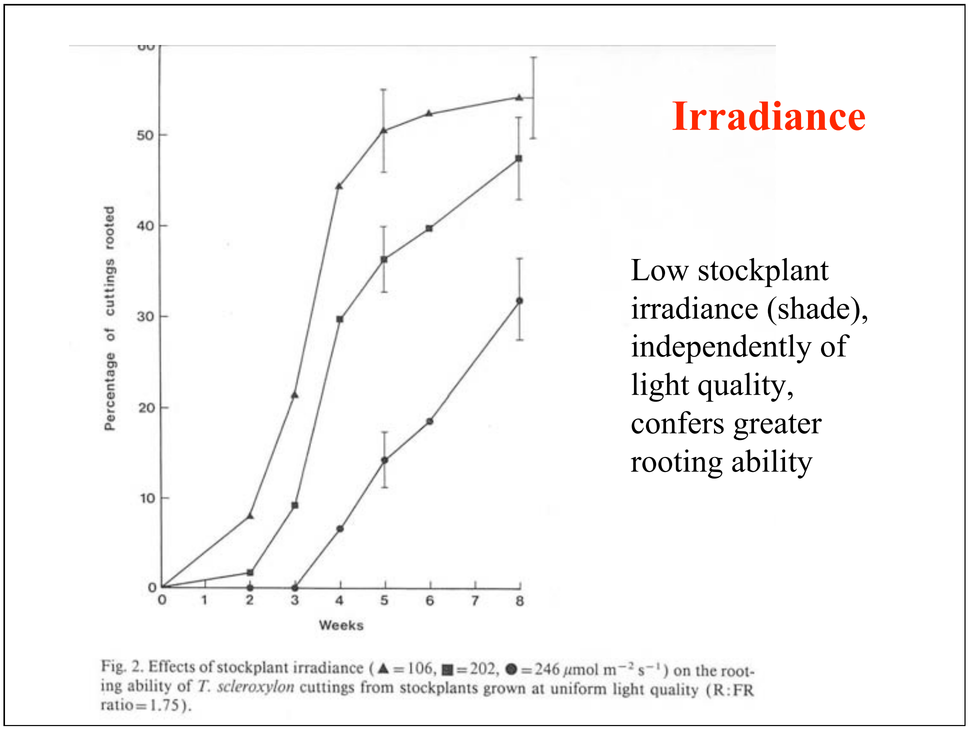
Irradiance

Light quality

Daylength

Nutrients

Light spectrum (daylight supplemented by mercury vapour 400 W bulbs) in the canopy of 2-shoot stockplants of *T. scleroxylon*



Irradiance

Low stockplant irradiance (shade), independently of light quality, confers greater rooting ability

Fig. 2. Effects of stockplant irradiance (▲ = 106, ■ = 202, ● = 246 μmol m^{-2} s^{-1}) on the rooting ability of *T. scleroxylon* cuttings from stockplants grown at uniform light quality (R:FR ratio = 1.75).

Irradiance

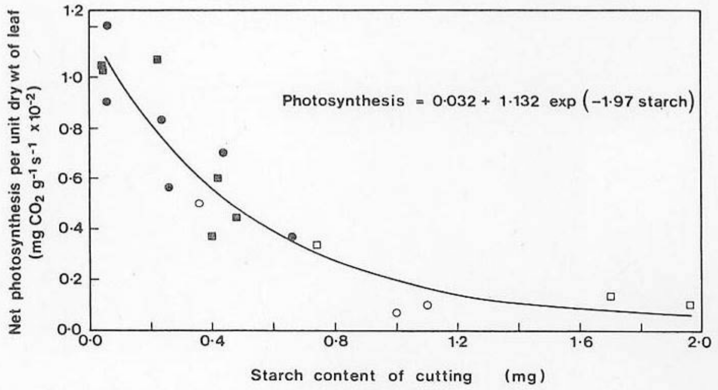


Fig. 7. Relationship in *T. scleroxylon* stockplants between net photosynthesis per unit dry weight of leaf and the starch content (mg per cutting) of the stem portion of cuttings when stockplants were grown at different levels of irradiance (●, ▨ = 250, ○, □ = 650 μmol m^{-2} s^{-1}) and fertilizer (○, ●, with; and □, ▨, without 0.2% SS20 liquid feed) at a uniform light quality (R:FR ratio = 6.3).

Cuttings with high starch content are associated with low rates of photosynthesis. High irradiance with low nutrients resulted in high starch content.

Light Quality

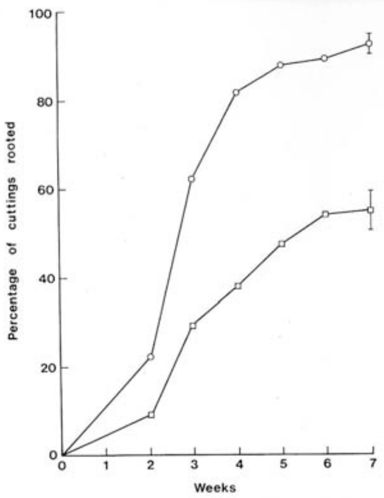


Fig. 3. Effects of light quality (○ = 1.6, □ = 6.3 R:FR ratios) as a stockplant pretreatment on rooting ability of single-node *T. scleroxylon* cuttings grown on uniform irradiance (294 μmol m^{-2} s^{-1}).

Independent of irradiance, stockplants under light with lower far-red content (shade), have the highest rooting ability

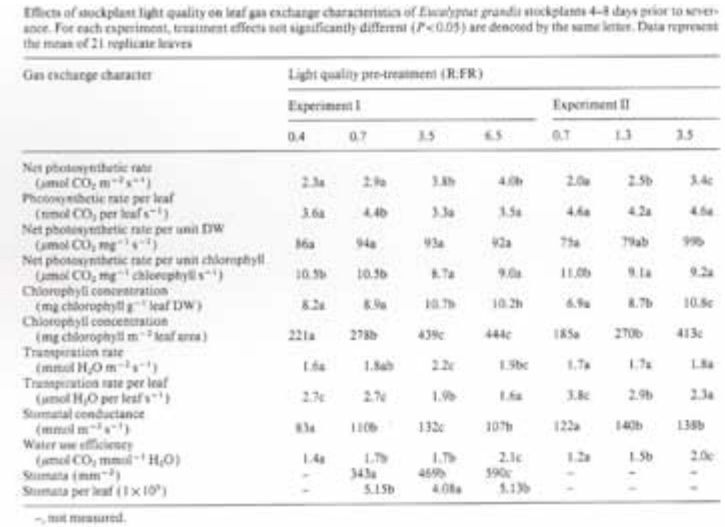
Light Quality

Table 1 Effects in *Eucalyptus grandis* of stockplant light quality on morphological characteristics of two-node (two half-leaf) cuttings at severance. (A) experiment I and (B) experiment II. For each experiment, treatment effects not significantly different ($P < 0.05$) are denoted by the same letter. Data represent the mean of 15 two-node cuttings

Growth character	**Light quality Pre-treatment (R:FR ratio)**		
Pre-severance	**0.7**	**1.3**	**3.5**
Leaf dry weight (mg)	61.3c	54.2b	46.6a
Stem dry weight (mg)	173c	130b	96a
Leaf area (cm^2)	22.7b	17.3a	13.3a
Specific leaf area (cm^2 mg^{-1})	0.37b	0.32a	0.28a
Stem length (cm)	5.9c	4.9b	3.8a
Stem diameter (cm)	0.33a	0.34a	0.31a
Stem volume (cm^3)	0.51c	0.44b	0.31a
Post-severance (Day 35)			
Root weight (mg)	18.0c	11.3b	8.4
New leaf dry weight (mg)	21.4c	13.8	6.3a

In shade light: leaf wt., stem wt., leaf area, specific leaf area, stem length, stem volume were all greater

Light Quality



Effects of stockplant light quality on leaf gas exchange characteristics of *Eucalyptus grandis* stockplants 4–8 days prior to severance. For each experiment, treatment effects not significantly different ($P<0.05$) are denoted by the same letter. Data represent the mean of 21 replicate leaves

Gas exchange character	Light quality pre-treatment (R:FR)						
	Experiment I				Experiment II		
	0.4	0.7	3.5	6.5	0.7	1.3	3.5
Net photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	2.3a	2.9a	3.8b	4.0b	2.0a	2.5b	3.4c
Photosynthetic rate per leaf (nmol CO_2 per leaf s^{-1})	3.6a	4.4b	3.3a	3.5a	4.6a	4.2a	4.6a
Net photosynthetic rate per unit DW (μmol CO_2 mg^{-1} s^{-1})	86a	94a	93a	92a	75a	79ab	99b
Net photosynthetic rate per unit chlorophyll (μmol CO_2 mg^{-1} chlorophyll s^{-1})	10.5b	10.5b	8.7a	9.0a	11.0b	9.1a	9.2a
Chlorophyll concentration (mg chlorophyll g^{-1} leaf DW)	8.2a	8.9a	10.7b	10.2b	6.9a	8.7b	10.8c
Chlorophyll concentration (mg chlorophyll m^{-2} leaf area)	221a	278b	439c	444c	185a	270b	413c
Transpiration rate (mmol H_2O m^{-2} s^{-1})	1.6a	1.8ab	2.2c	1.9bc	1.7a	1.7a	1.8a
Transpiration rate per leaf (μmol H_2O per leaf s^{-1})	2.7c	2.7c	1.9b	1.6a	3.8c	2.9b	2.3a
Stomatal conductance (mmol m^{-2} s^{-1})	83a	110b	132c	107b	122a	140b	138b
Water use efficiency (μmol CO_2 $mmol^{-1}$ H_2O)	1.4a	1.7b	1.7b	2.1c	1.2a	1.5b	2.0c
Stomata (mm^{-2})	–	343a	469b	590c	–	–	–
Stomata per leaf (1×10^5)	–	5.15b	4.08a	5.13b	–	–	–

–, not measured.

In addition to stem morphology, shade light affects the physiological processes of the leaf

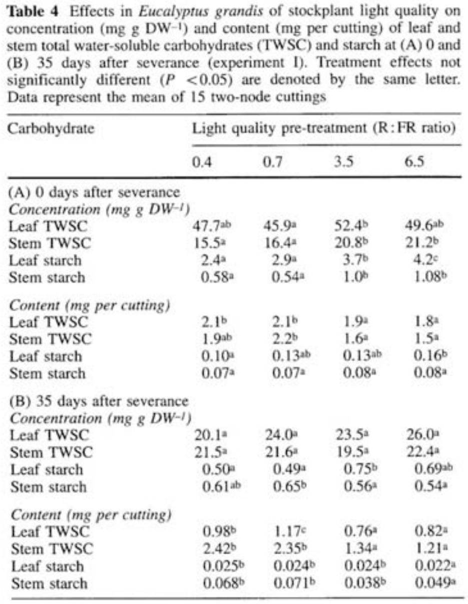


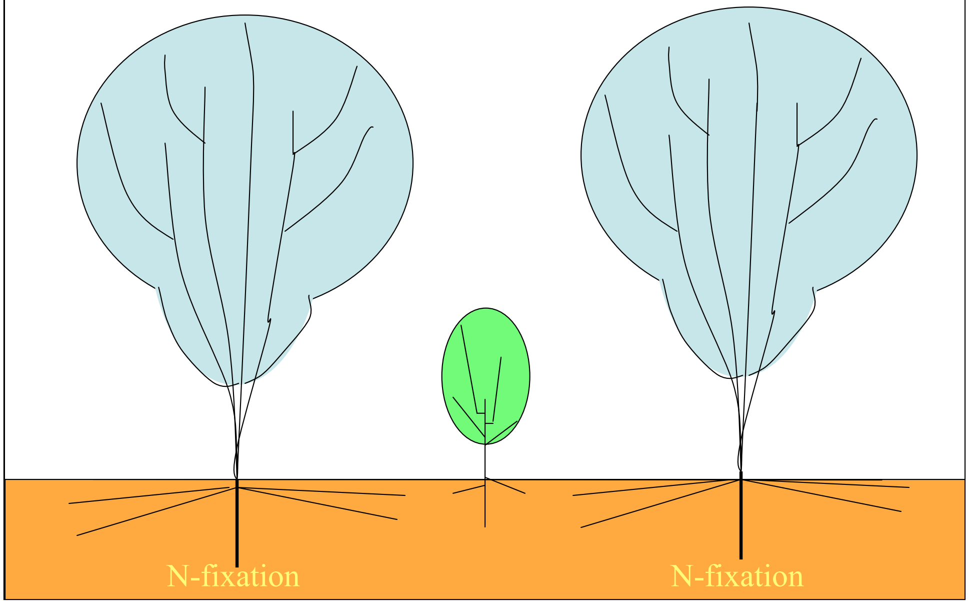
Table 4 Effects in *Eucalyptus grandis* of stockplant light quality on concentration (mg g DW^{-1}) and content (mg per cutting) of leaf and stem total water-soluble carbohydrates (TWSC) and starch at (A) 0 and (B) 35 days after severance (experiment I). Treatment effects not significantly different ($P < 0.05$) are denoted by the same letter. Data represent the mean of 15 two-node cuttings

Carbohydrate	Light quality pre-treatment (R:FR ratio)			
	0.4	0.7	3.5	6.5
(A) 0 days after severance				
Concentration (mg g DW^{-1})				
Leaf TWSC	47.7^{ab}	45.9^{a}	52.4^{b}	49.6^{ab}
Stem TWSC	15.5^{a}	16.4^{a}	20.8^{b}	21.2^{b}
Leaf starch	2.4^{a}	2.9^{a}	3.7^{b}	4.2^{c}
Stem starch	0.58^{a}	0.54^{a}	1.0^{b}	1.08^{b}
Content (mg per cutting)				
Leaf TWSC	2.1^{b}	2.1^{b}	1.9^{a}	1.8^{a}
Stem TWSC	1.9^{ab}	2.2^{b}	1.6^{a}	1.5^{a}
Leaf starch	0.10^{a}	0.13^{ab}	0.13^{ab}	0.16^{b}
Stem starch	0.07^{a}	0.07^{a}	0.08^{a}	0.08^{a}
(B) 35 days after severance				
Concentration (mg g DW^{-1})				
Leaf TWSC	20.1^{a}	24.0^{a}	23.5^{a}	26.0^{a}
Stem TWSC	21.5^{a}	21.6^{a}	19.5^{a}	22.4^{a}
Leaf starch	0.50^{a}	0.49^{a}	0.75^{b}	0.69^{ab}
Stem starch	0.61^{ab}	0.65^{b}	0.56^{a}	0.54^{a}
Content (mg per cutting)				
Leaf TWSC	0.98^{b}	1.17^{c}	0.76^{a}	0.82^{a}
Stem TWSC	2.42^{b}	2.35^{b}	1.34^{a}	1.21^{a}
Leaf starch	0.025^{b}	0.024^{b}	0.024^{b}	0.022^{a}
Stem starch	0.068^{b}	0.071^{b}	0.038^{b}	0.049^{a}

Light Quality

The consequence of the effects on leaf physiology is variation in carbohydrate contents (sugars and starch), especially of stem starch concentration

Control of Light in Practice



Genetic Variation in Rooting Ability?

Rooting ability is strongly determined by the morphology and physiological state of cuttings pre- and post-severance.

Clones vary morphologically and physiologically. Is this the reason for clonal variation in rooting ability, or is it genetically controlled?

This data finds that leaf loss, cutting length, treatments, new shoot growth, leaf area and node position are all more important determinants of rooting than clone.

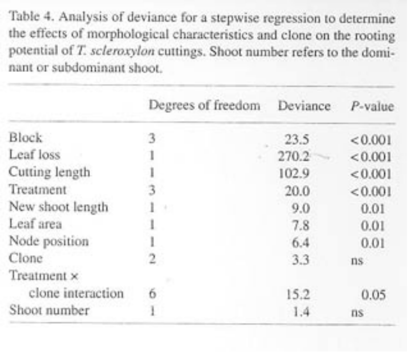


Table 4. Analysis of deviance for a stepwise regression to determine the effects of morphological characteristics and clone on the rooting potential of *T. scleroxylon* cuttings. Shoot number refers to the dominant or subdominant shoot.

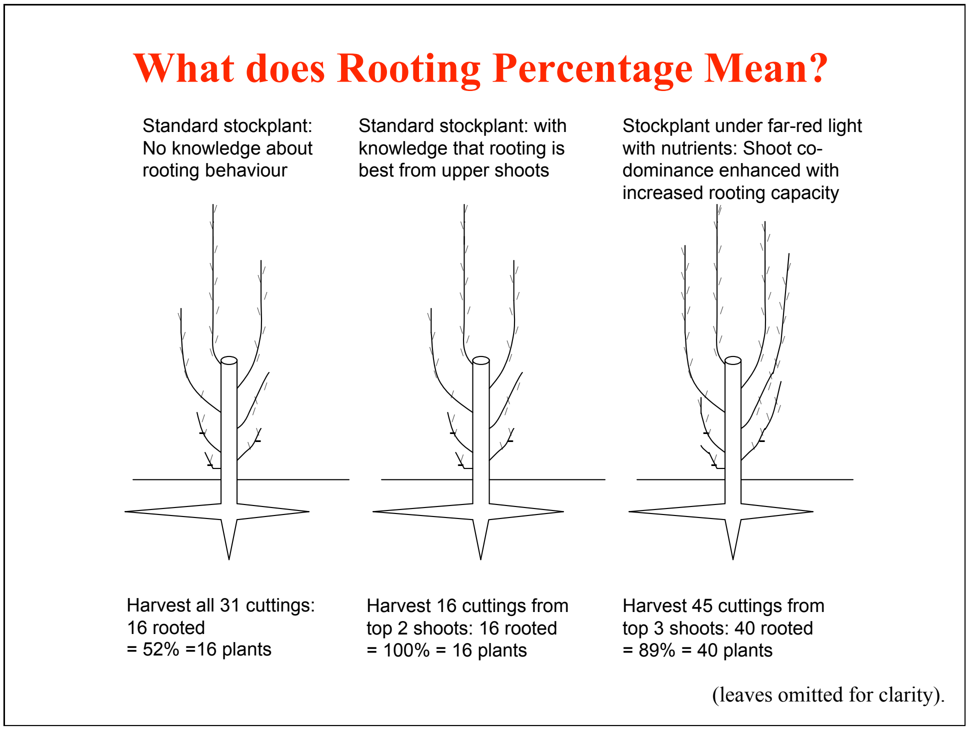
	Degrees of freedom	Deviance	*P*-value
Block	3	23.5	<0.001
Leaf loss	1	270.2	<0.001
Cutting length	1	102.9	<0.001
Treatment	3	20.0	<0.001
New shoot length	1	9.0	0.01
Leaf area	1	7.8	0.01
Node position	1	6.4	0.01
Clone	2	3.3	ns
Treatment × clone interaction	6	15.2	0.05
Shoot number	1	1.4	ns

The Apparent Lack of Principles

An examination of the literature on the rooting of stem cuttings indicates that there have been very many studies made, but it is very difficult to identify any common principles between different species and different studies. This has led to the conclusion that there are none, but is this true?

To some extent it seems to be a result of people doing different things under different conditions and not recording them. For example, very few authors characterize the stockplant or propagator environment, or give details about the origin of the cutting within and between stems, or define the size of the cutting and its leaf.

We have seen that these make big differences to the rooting capacity.



What does Rooting Percentage Mean?

Standard stockplant:
No knowledge about
rooting behaviour

Harvest all 31 cuttings:
16 rooted
= 52% =16 plants

Standard stockplant: with
knowledge that rooting is
best from upper shoots

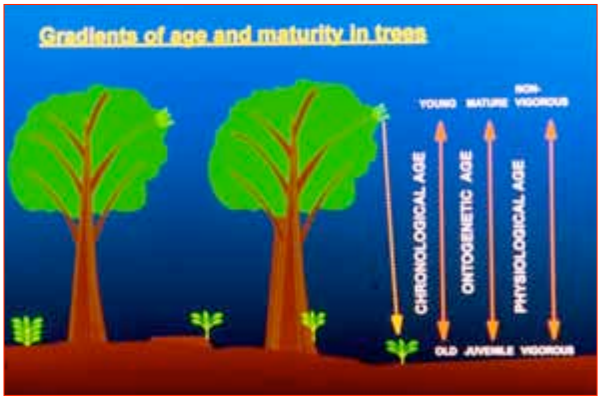
Harvest 16 cuttings from
top 2 shoots: 16 rooted
= 100% = 16 plants

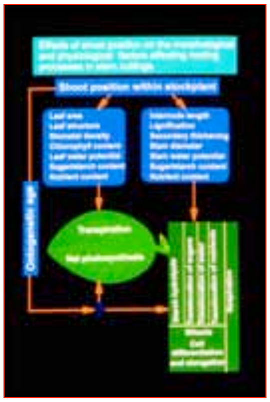
Stockplant under far-red light
with nutrients: Shoot co-dominance enhanced with
increased rooting capacity

Harvest 45 cuttings from
top 3 shoots: 40 rooted
= 89% = 40 plants

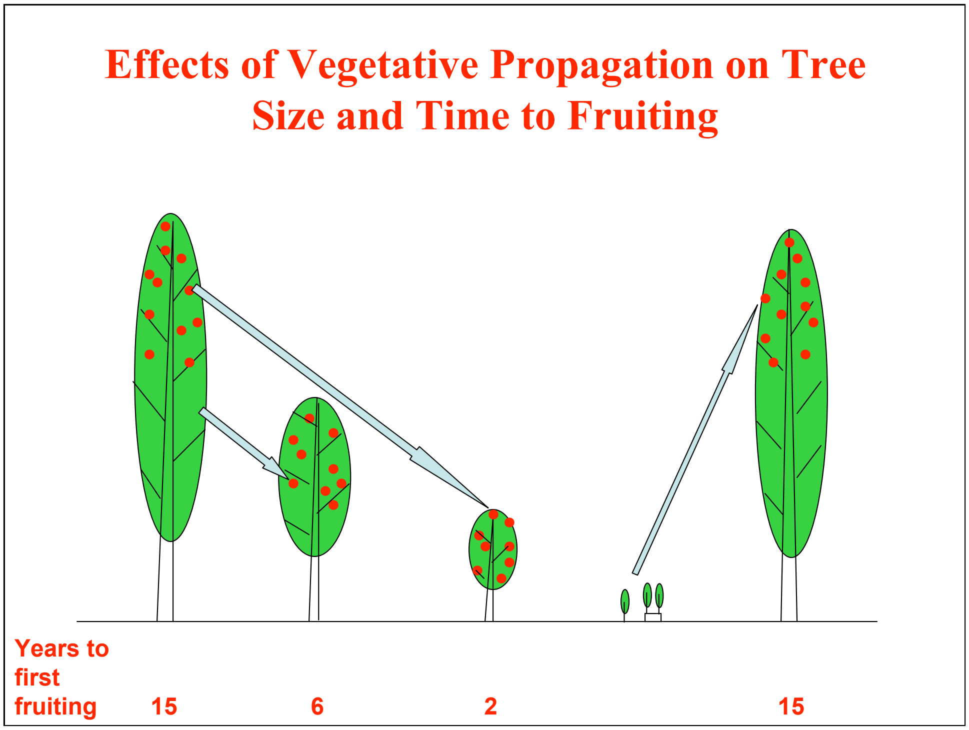
(leaves omitted for clarity).

Phase Change

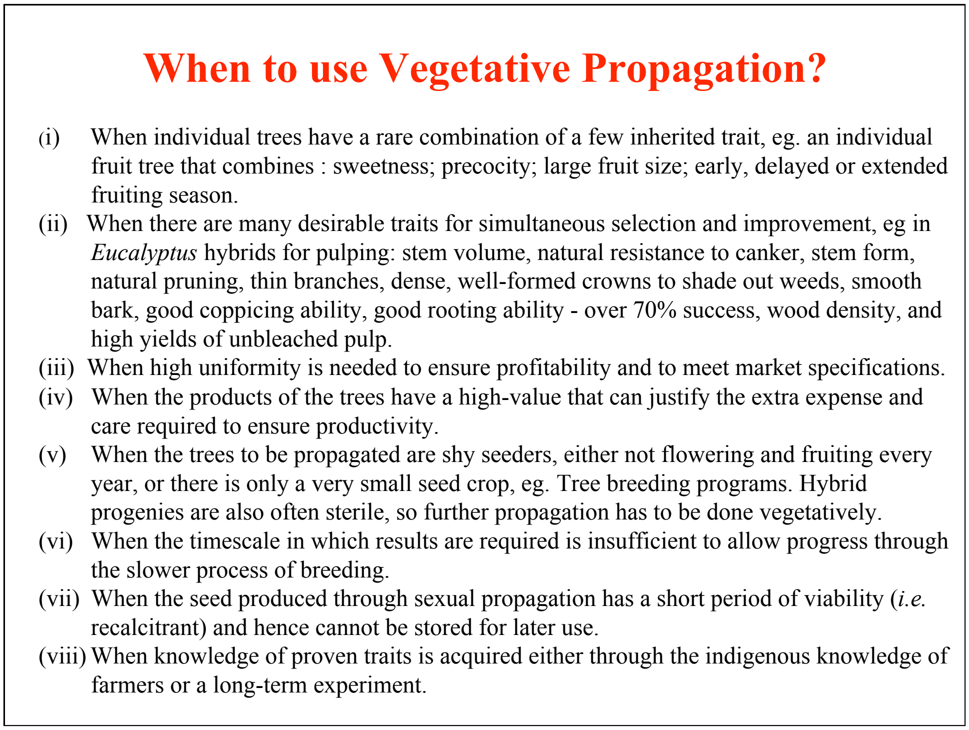




It is very difficult to root cuttings from the crown of a mature tree. Why?



Effects of Vegetative Propagation on Tree Size and Time to Fruiting



When to use Vegetative Propagation?

(i) When individual trees have a rare combination of a few inherited trait, eg. an individual fruit tree that combines : sweetness; precocity; large fruit size; early, delayed or extended fruiting season.

(ii) When there are many desirable traits for simultaneous selection and improvement, eg in *Eucalyptus* hybrids for pulping: stem volume, natural resistance to canker, stem form, natural pruning, thin branches, dense, well-formed crowns to shade out weeds, smooth bark, good coppicing ability, good rooting ability - over 70% success, wood density, and high yields of unbleached pulp.

(iii) When high uniformity is needed to ensure profitability and to meet market specifications.

(iv) When the products of the trees have a high-value that can justify the extra expense and care required to ensure productivity.

(v) When the trees to be propagated are shy seeders, either not flowering and fruiting every year, or there is only a very small seed crop, eg. Tree breeding programs. Hybrid progenies are also often sterile, so further propagation has to be done vegetatively.

(vi) When the timescale in which results are required is insufficient to allow progress through the slower process of breeding.

(vii) When the seed produced through sexual propagation has a short period of viability (*i.e.* recalcitrant) and hence cannot be stored for later use.

(viii) When knowledge of proven traits is acquired either through the indigenous knowledge of farmers or a long-term experiment.

Participatory Domestication

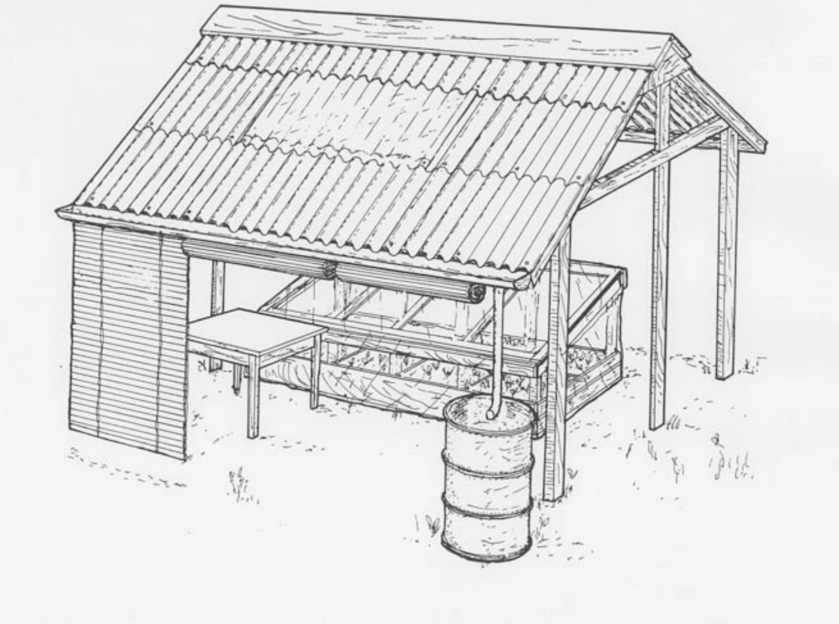
Establishment of village nurseries

Stockplant garden

Propagation facilities

In accord with the Convention on Biological Diversity

Establishment of Village Nurseries



Tree Improvement Program

Vegetative propagation is used to capture genetic variation and to mass produce genetic copies of selected individuals for planting in clonal agroforestry or plantation systems