

REPRODUCTION OF *EUCALYPTUS DEGLUPTA* BY CUTTINGS

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ABSTRACT

The effects on rooting ability of cuttings of *Eucalyptus deglupta* Blume of position in the seedling stem and ontogenetic age are described. In cuttings taken from 3-months-old seedlings position of the cutting on the shoot system had no effect on rooting or subsequent growth. Cuttings rooted very freely when taken from upper parts of trees up to 12 months old and they appeared to grow exactly like seedlings. Corresponding material from trees aged 5 years and more completely failed to root. Stem cuttings of *E. deglupta* were used as bioassay materials to test extracts from tissues of various ontogenetic ages. Responses were clear-cut, and indicate that the failure of cuttings from older trees was due to a rooting inhibitor.

A technique for rooting large numbers of cuttings is described. Almost 100% success in rooting is achieved after 8 weeks in a misting cabinet.

INTRODUCTION

Various workers have found leafy cuttings taken from very young seedlings or shoots from lignotubers of *Eucalyptus* easy to root (e.g., Giordano, 1961; Pryor, 1957, 1961; Pryor and Willing, 1963). This genus appears to be a further example of the close association which exists generally between juvenility and rooting ability (Schaffalitzky de Muckadell, 1959; Paton *et al.*, 1970), as the capacity to strike from cuttings is almost nonexistent at the fifteenth leaf pair stage in nearly all species (Pryor and Willing, 1963; Paton *et al.*, 1970).

Many trials have been made with species from all taxonomic groups and, except for attempts with some apparently intractable species (e.g., *E. calophylla*, Rudman *et al.*, 1969), rooting of cuttings takes place in 2-5 weeks using sand and peat mixtures with bottom heat under polythene.

The value of propagating cuttings from a seedling whose adult characteristics are necessarily unknown is restricted. However, cuttings from juvenile material can be useful for experimental work, especially in tree breeding and genetics.

Propagation from older trees by cuttings, should it become feasible and sufficiently cheap to be economic, may allow marked gains in genetic quality. There are various indications that successful propagation of adult material will be achieved. These come from recent fundamental research (e.g., Paton *et al.*, 1970), from observation that roots have formed on adult *Eucalyptus* stems following flooding or natural layering (Jacobs,

1955), from experimental air-layering (Pryor, 1957; Pryor and Willing, 1963) and from the formation of aerial roots on plantation trees of *E. robusta* in Hawaii (Pryor and Willing, 1963) and of *E. deglupta* in Papua New Guinea (Fig. 1).



FIG. 1—Aerial roots on the base of the stem of *E. deglupta*.

Cuttings of juvenile *E. deglupta* were first propagated in Papua New Guinea at Keravat by the author in 1967 without mist spray or added hormone assistance (Fig. 2a). Subsequently, cuttings have been rooted in water (Fig. 2b) and in artificial media. Hormones and mist sprays have been used in attempts to strike cuttings from material of various physiological ages.

Part I of this paper reports experiments on the physiological factors affecting the rooting of cuttings of *E. deglupta*. Part II describes a technique for rooting large numbers of seedling cuttings for research purposes.

PART I

In some preliminary experiments with *E. deglupta*, roots were successfully grown from vein tissue in portions of leaves (Fig. 2c) and various types of cuttings, classified according to the portion of seedling stem or branches included, were tried (Figs 2d, e, f, g). Generally, *E. deglupta* is capable of rooting successfully from stem segments less than 2 years old. Root formation occurs in over 90% of cases. The addition of hormones, such as indole-acetic acid (IAA), indole-butyric acid (IBA) or naphthalene-acetic acid (NAA) in concentrations of 10-30 ppm raises success rate to over 99%. Root formation on untreated cuttings occurs in 5 to 9 days, and on treated cuttings in about 5 days.

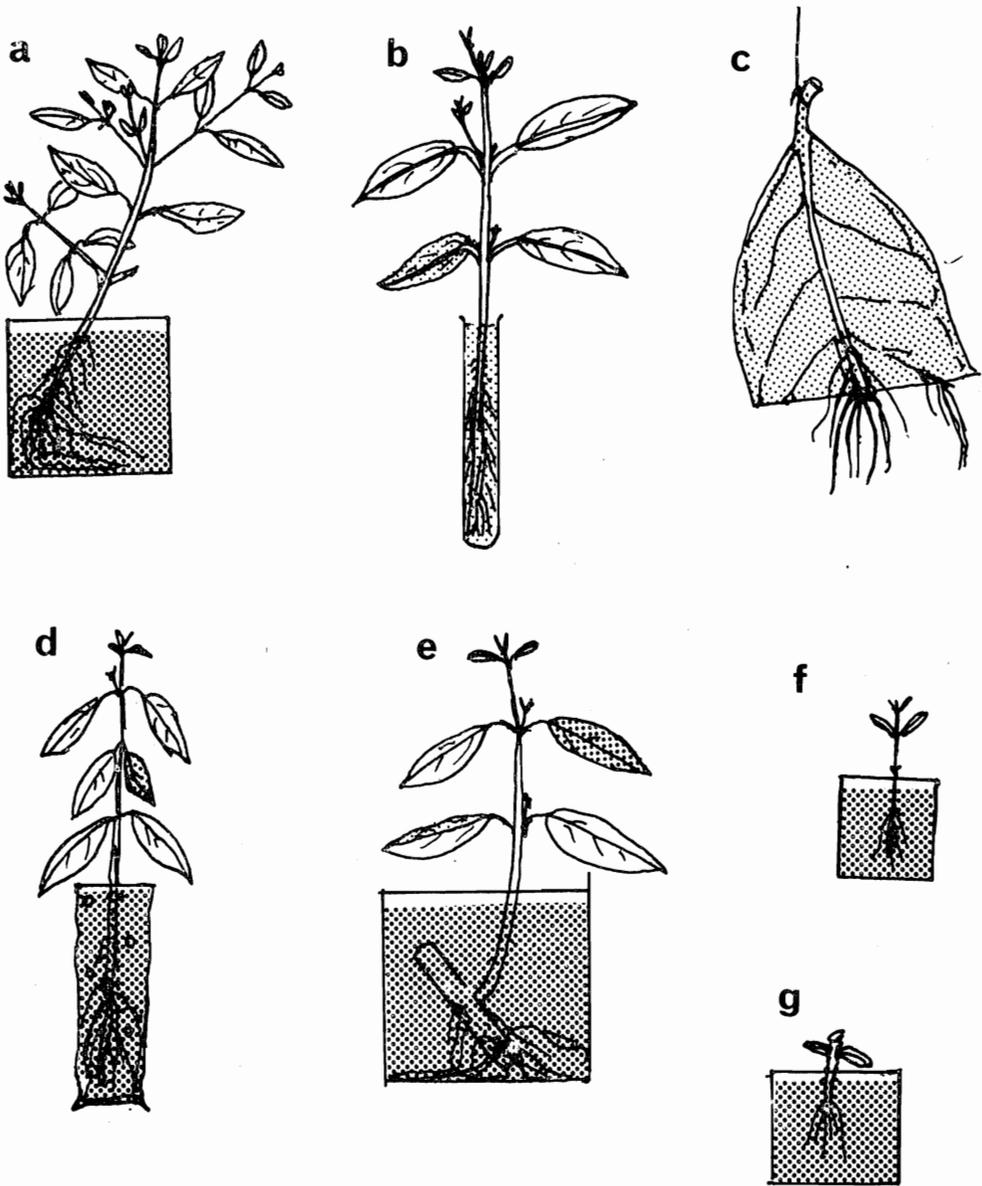


FIG. 2—*a*. The first cuttings of *E. deglupta* consisting of the upper half of three-month-old seedling stems pushed into sandy loam, kept in shade and watered twice daily. *b*. Two-month-old seedling stem rooting in water. *c*. Roots developing from a seedling leaf suspended under mist spray. *d*. Entire cutting severed at cotyledonary node rooted in a plastic tube containing peat and sand. *e*. Branch cutting attached to portion of seedling stem rooted in peat and vermiculite. Growth is orthotropic. *f*. Tip-cutting of two-month-old seedling. *g*. Basal cutting consisting of cotyledonary node and the node above.

This time compares with 10-14 days for seedling cuttings of other *Eucalyptus* species (Paton *et al.*, 1970). Seedling cuttings of *E. deglupta* can also root successfully in water or when suspended in air under intermittent mist spray.

1. EFFECT OF SHOOT POSITION ON CUTTINGS FROM JUVENILE MATERIAL

i. *Materials and Methods*

Cuttings were prepared from five *E. deglupta* seedlings approximately 3 months old (one metre high and consisting of 25-30 stem nodes) in the manner depicted in Fig. 3a. Some 50-70 propagules were obtained from each seedling, each being identified by the numbering system shown in Fig. 3a.

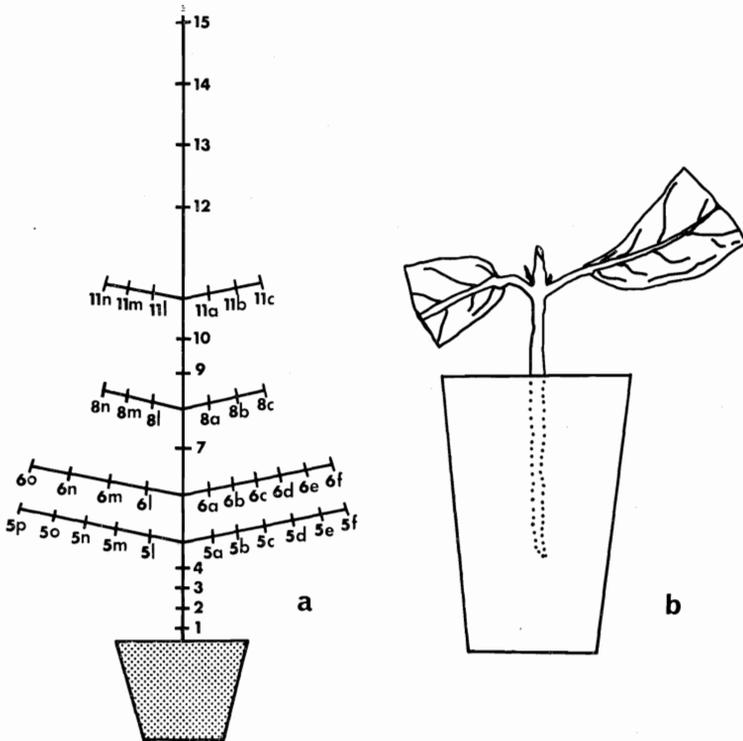


FIG 3—a. Method of designating position in seedling parent of segmental cuttings. b. A single segmental cutting, consisting of one node and portions of the adjoining internodes, potted in a paper cup.

The individual segment cuttings, consisting of one node and portion of the subtending internode were potted into labelled paper cups containing a 1 : 1 : 1 mixture of heat-sterilised river sand, vermiculite and shredded peat moss (Fig. 3b). No hormone treatment was used. To prevent drying out, mist spray was applied intermittently and as an additional precaution, half the lamina of each large leaf was removed.

ii. *Results*

Ninety-eight % of cuttings rooted and all these survived planting out into pots. There was no effect of position in the stem on subsequent growth habit of cuttings.

There was no evidence of plagiotropism in the leading shoots. No difference was apparent between cuttings at 3 months after striking and 4-month-old seedlings.

Some cuttings were later planted out in the field at Keravat, New Britain. After 1 year of growth trees of normal appearance had been produced similar in habit and rate of growth to routine plantings nearby of *E. deglupta*.

2. ROOTING AND ONTOGENETIC AGE

A. SEGMENTAL CUTTINGS FROM TREES OF SEVERAL AGES

i. *Materials and Methods*

Twenty segmental cuttings were prepared from the upper portion of trees in each of the following age groups: 1 month (20), 3 months (2), 6 months (1), 12 months (1), 5 years (1), 10 years (1), 15 years (1) and 20 years (1). The figures in brackets refer to the number of trees sampled. The samples from the upper crowns of large trees (40-50 m tall) were collected by shooting with a rifle.

The basal ends of cuttings were dipped in a weak sucrose and water solution, then into 'Seradix'* powder.

Each prepared cutting was then placed in a paper cup filled with a 2 : 1 mixture of vermiculite and peat.

The cuttings were kept moist with intermittent mist spray.

ii. *Results*

Percentages of cuttings in each age class that rooted and the number of roots per cutting are shown in Table 1. No rooting occurred when the source material was more than 12 months old.

TABLE 1—Rooting percentage of cuttings of different ages

Ontogenetic age of material	Percent cuttings rooted	Number of roots per cutting	
		Range	Mean
1 month	100	10-13	11
3 months	100	10-15	12
6 months	95	11-15	12
12 months	95	9-14	12
5 years	0	—	0
10 years	0	—	0
15 years	0	—	0
20 years	0	—	0

* Seradix is a trademark of May and Baker Ltd, England and New Zealand, the active ingredient being 0.3% IBA.

B. BIOLOGICAL TESTING OF CRUDE EXTRACTS OF CUTTINGS FROM TREES OF SEVERAL AGES

a. *Cress Seed Bioassay*

Cress seed germination has been used as a bioassay in many experiments concerned with presence or absence of promotive or inhibitory substances, and especially for detection of substances active in root formation. Experiments involving detection of substances concerned with root formation began at Keravat in 1970.

i. *Materials and Methods*

Cuttings consisting of three opposite leaf pairs and supporting stem tissue were removed from the apical portion of small plants or the apices of branches at the top of the crowns of large trees. The same age series as in Section 2Ai was used. The material in each age treatment was macerated with a mortar and pestle and extracted with 10 ml of 17% methanol for 12 hours at approximately 25°C. The mixture was filtered, yielding approximately 9 ml of pale brown liquid.

Discs of filter paper were placed in five Petri dishes for each treatment. Into each Petri dish 0.2 ml of crude extract was pipetted. The filter paper was then saturated with distilled water. Fifty cress seeds were placed on the filter paper in each dish and the lids placed on. Control treatments were prepared similarly with 0.2 ml of 17% methanol substituted for the abstract. Very little moisture loss occurred: any tendency for the paper to dry out was corrected by the addition of more distilled water.

Counts of seeds which had germinated were made at weekly intervals for 4 weeks.

ii. *Results*

Germination results are presented in Table 2. Germination of cress seeds was retarded in tissue extract from 12-months-old plants and almost completely suppressed in extracts from material aged 5 years and older. Some substance in the adult tissue was apparently a germination inhibitor and so possibly a rooting inhibitor too.

TABLE 2—Germination percentage of cress seeds in extracts from tissues of different ages

Ontogenetic age of material extracted	Percentage germination*	
	Range (5 replications)	Mean
1 month	96-100	98
3 months	95-100	97
6 months	98-100	100
12 months	56-88	75
5 years	2-4	2
10 years	—	0
15 years	0-2	0
20 years	—	0

* Germination of appropriate controls ranged from 95-100%.

b. Seedling Cutting Bioassay

Preliminary trials by the author from 1967-69 had shown that seedling cuttings of *E. deglupta* would root in water with better than 95% success at any time. The possibility was realised (cf. Paton *et al.*, 1970) of using these cuttings as a bioassay of substances which could be inhibiting root formation on cuttings taken from adult tissue.

i. Experimental Material

Crude extracts were prepared from the same trees as described in Section 2Ai and 2Bai above.

The bioassay cuttings were prepared in the following manner. Entire 3-month-old seedlings of *E. deglupta* growing in tubes in the Keravat nursery were decapitated at the cotyledonary node. Each cutting bore five or six pairs of leaves on the main stem. The lowest three or four pairs of leaves (three or four nodes as the leaves of *E. deglupta* are oppositely arranged) were stripped from the stem, leaving two pairs of leaves on the apical portion. The larger ones of these were reduced in area.

Twenty plastic drink cups approx. 10 cm high with top diam. 7 cm, with aluminium foil covers, were prepared for each treatment as shown in Fig. 4, and 0.3 ml of crude

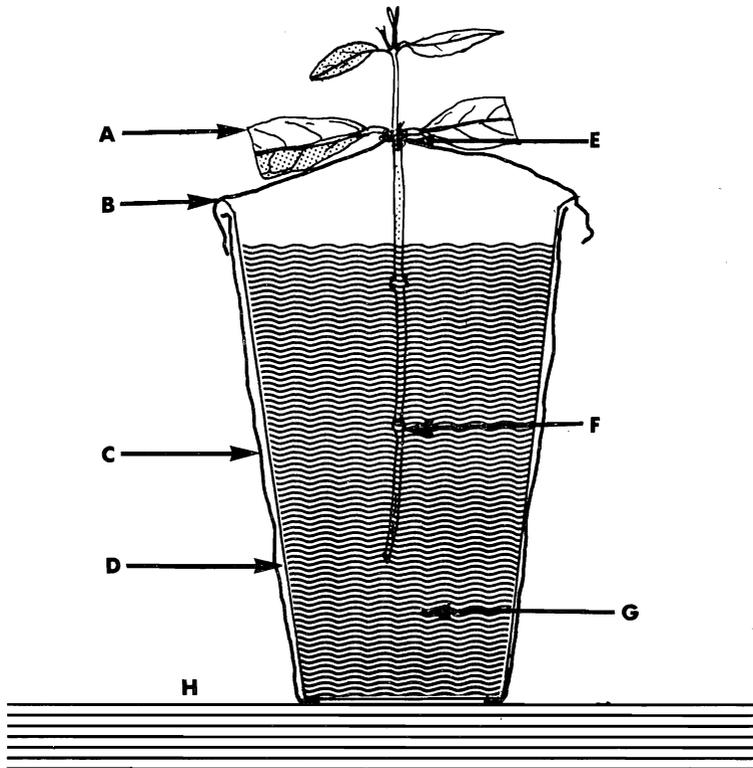


FIG. 4—To test crude extracts, seedling cuttings of *E. deglupta* are used as bioassay material. A, seedling cutting consisting of 5-6 nodes with large leaves reduced in area; B, aluminium foil cover; C, aluminium foil outer cover to prevent entry of light; D, transparent plastic drink cup; E, waterproof seal of grafting mastic between cutting and aluminium cover; F, node with leaves removed; G, tissue extract solution; H, bench top.

extract was pipetted into each and 100 ml of distilled water added. In addition 20 replicates to serve as a control were prepared similarly with the addition of only water and 0.3 ml of 17% methanol mixture. One cutting was assigned to each cup.

The basal end of each prepared cutting was passed through an aluminium foil cover and allowed to rest on the petioles of the lower leaf pair. Any opening between the seedlings and cover was sealed with grafting mastic.

The containers with cuttings were placed under mist spray. The liquid level in the cups was maintained by occasional additions of distilled water.

ii. Results

All cuttings rooted in the control and in extracts derived from material aged 12 months or less, each producing 8 to 17 roots with a mean of 14. Numbers of roots did not differ between these treatments.

Cuttings failed to root in extracts derived from material of age 5 years or greater.

DISCUSSION

High rooting ability was demonstrated by the segmental cuttings taken from seedlings about one metre high. This ability to root was irrespective of position in the stem or branches from which the cutting was derived and irrespective of the presence or absence of an apical bud. The absence of a requirement of bud tissue for rooting is supported by the ease with which roots can be grown from parts of leaves containing no bud tissue. However, aerial shoots did not form on these rooted leaf cuttings. At least some leaf tissue is required; if all leaf material is removed from a cutting, roots will not develop unless fresh shoots arise from axillary positions before the cutting dies.

Although no effect of ontogenetic age was evident in cuttings from plants consisting of 25-30 nodes, unsuccessful attempts at rooting material from the crowns of 5 to 20-year-old trees indicate that inhibition does occur in this material, despite the statement by Paton *et al.* (1970) that "This species is capable of rooting successfully from stem cuttings taken from adult trees". However, Paton and co-workers do not specify the age of their "adult" material of *E. deglupta*. I have obtained rooted cuttings using 2-year-old material and by certain criteria this could be called "adult". Certainly *E. deglupta* can be rooted at a greater ontogenetic age than other *Eucalyptus* cuttings (Paton *et al.*, 1970; Cresswell, 1971). The ability of some mature *Eucalyptus* to form aerial roots and successfully respond to air-layering suggests some natural mechanism overcomes inhibition in these cases. However, air-layering has not yet been attempted on *E. deglupta*.

The bioassays based on inhibition of rooting of young *E. deglupta* seedling cuttings and on inhibition of germination of cress seeds had similar sensitivity to the same set of crude tissue extracts.

The relationship between the level of root-inhibiting substances and rooting ability suggests that the observed decrease in rooting ability with ontogenetic ageing in *E. deglupta* is probably related to increased inhibitor content in a causal and quantitative fashion.

The ontogenetic age at which sufficient inhibitor is produced to prevent rooting of *E. deglupta* cuttings is uncertain, as the experiments to date have not included age classes between the ages of 1 and 5 years. Also, in age classes of 6 months or more only one tree has been repeatedly sampled and there may be differences between trees in rooting ability. These aspects remain to be tested.

The rooting inhibitor postulated by Paton *et al.* (1970) would not appear to be translocated, as rooting ability was consistently high in older stem cuttings of *E. grandis*, provided the base of the cutting was at or below the third node above the cotyledons. This could be examined for *E. deglupta* by taking a small patch of phloem from the base of a young seedling and grafting this on to an adult twig by patch grafting (see Davidson, 1974). The twig could be severed later, treated as a cutting and the place of origin of roots observed. If roots were not obtained from the juvenile patch this would indicate that the inhibitor might have been translocated.

Research in the Department of Botany at the Australian National University has resulted in the identification of the substance inhibiting the formation of roots by stem cuttings taken from adult *Eucalyptus grandis*. The substance has been successfully synthesised in the Department of Chemistry of the same University. If the inhibitor is the only mechanism preventing rooting of adult eucalypt cuttings, means might be found to overcome its effects to allow rooting of adult cuttings.

There are prospects for continued improvement in the techniques of propagating cuttings of *E. deglupta*. Results to date for older material of this species are inconclusive (cf. Paton *et al.*, 1970). However, ultimate success in raising plants from cuttings of all physiological ages seems assured.

I foresee no problems in economic mass production of cuttings of *E. deglupta* for plantation establishment. If, in the future, clones could be built up from a fairly large number of individuals, 200-300, the prospects for improvement are very great. In the plantations of *E. deglupta* already established at Keravat, New Britain, there is considerable variation (Davidson, 1972) and the selection and use of superior clones should greatly increase productivity.

Various insect pests have been reported as attacking *E. deglupta* (Browne, 1968). The insects can probably be controlled by silvicultural practices, by insecticides, or by biological control. However, the use of genetic resistance to insect attack is promising. Plantations contain individuals which are not attacked and clonal material from a number of these individuals (at least 30-50) could be the starting point for raising stocks with a high level of resistance.

Another problem is heartwood decay in young fast-grown plantations of *E. deglupta* (Davidson, 1973). There is considerable variation in decay between individual trees. Severity of decay appears to be associated with site, vigour, wood density and moisture content, and self-pruning ability. Again, it is feasible that a stock of resistant trees could be built up by cloning resistant individuals.

PART II

INTRODUCTION

Large numbers of seedling cuttings are produced for research purposes at Bulolo, Papua New Guinea by the following routine technique.

METHOD

Preparation of Cuttings

Seedlings are grown in large, black, plastic tubes until about 1-1.5 m in height (4 months old). During the last month, weekly applications of 'Aquasol*', a complete

liquid fertiliser, are made using the recommended strength to force vigorous growth.

Segmental cuttings are made by cutting the stem and branches a little distance above each node (Fig. 5a). Sharp implements are required to prevent bruising of the tissues.

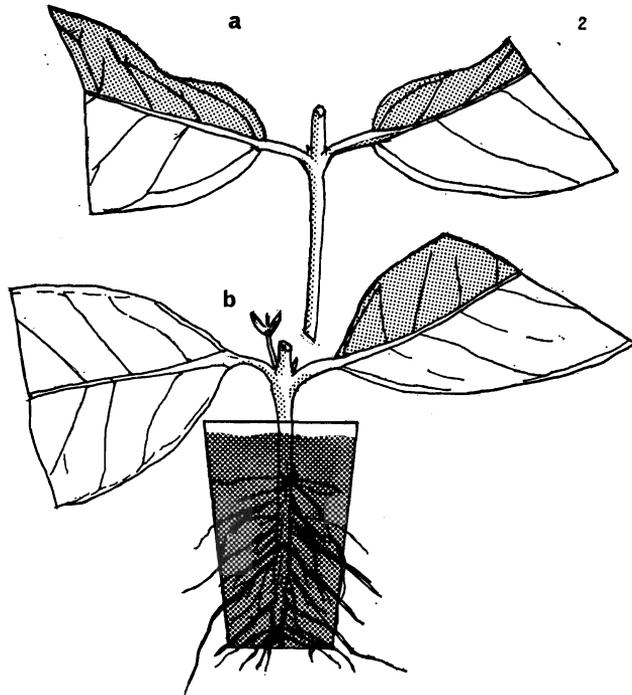


FIG. 5—*a*. A segmental cutting of *E. deglupta*. The leaves are arranged in opposite pairs so a single cutting consists of one node. *b*. A segmental cutting of *E. deglupta*, one month after placing in a small peat pot under mist spray. A naked axillary bud has developed to form a shoot.

Hormone Treatment

The basal ends of the cuttings are dipped first into a weak sucrose solution, then into 'Seradix'*. Any excess powder is shaken from the cutting. The sucrose solution is used only to bind the hormone powder to the stem.

Containers and Rooting Medium

The following media are suitable for rooting cuttings of *E. deglupta*:

- 1 : 1 : 1 shredded peat, vermiculite, coarse sand
- 2 : 1 vermiculite, shredded peat
- 1 : 1 coarse sand, vermiculite
- 1 : 1 vermiculite, perlite

These ingredients are heat-sterilised or fumigated with methyl bromide before use.

The rooting medium is placed in peat pot strips, each consisting of 24 tapered pots about 40 mm square at the top and 50 mm deep.

The cuttings are pushed into the medium until either the base of the cutting touches the bottom of the pot or the petioles touch the surface of the mixture.

Misting Equipment

The 'Humex' equipment used at Bulolo is illustrated in Fig. 6. This equipment is capable of producing 5,000 cuttings on a 2-month cycle.

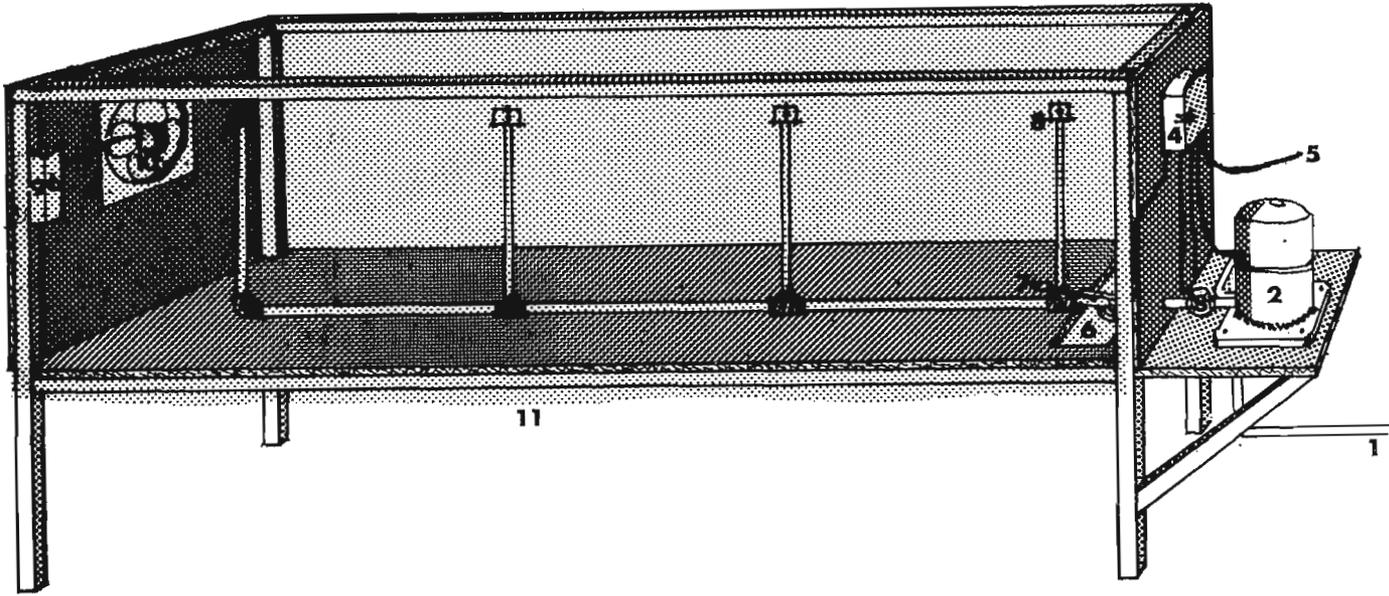


FIG. 6—Layout of misting equipment used at Bulolo for propagation of cuttings of *E. deglupta*. The wooden bench covered with plastic was constructed locally but the hardware and 'Humex'* controls were purchased as components. 1, water supply; 2, pressure pump; 3, solenoid-controlled valve; 4, mist control unit; 5, power supply; 6, ventilation intake; 7, remote moisture-sensing switch; 8, adjustable mist nozzle; 9, thermostat and fan speed control; 10, ventilation exhaust fan; 11, clear plastic cover. The equipment is generally operated under high shade resulting from 30% cover of sarlon cloth.

Subsequent Growth and Planting Out

For the first 3 weeks the 'lean' switch is in the 'lean' position, causing the mist spray to operate more frequently because of the evaporative action of the heating coil under the foam pad (Fig. 6).

At the end of 3 weeks the 'lean' switch is placed in the 'normal' position for a further 4 weeks. During this period the interval timing and the duration of misting are controlled by the natural evaporation from the foam pad, the size and thickness of which is previously adjusted so that the mist spray is triggered just as the surfaces of leaves become dry. Roots penetrate the pots profusely by the end of the first month (Fig. 5b). At this stage many of the petioles absciss.

During the last week, the mist spray is switched off and the pots and cuttings are saturated twice daily with a weak 'Aquasol' solution (1 g/l of water). In this period the cuttings 'harden off' and can be planted out into larger pots under high shade resulting from 30% cover of sarlon cloth. They are then raised in the same manner as potted seedlings.

Often, naked or dormant axillary buds develop in both opposite axils on the one cutting. The smaller is removed to ensure that one becomes dominant. No plagiotropism occurs in cuttings taken from seedlings of *E. deglupta*.

After 3 to 4 months the cuttings can be planted in the field. They grow into trees which are indistinguishable from those of seedling origin.

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TRADE NAMES

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