VEGETATIVE PROPAGATION OF *LOVOA TRICHILIOIDES*: EFFECTS OF PROVENANCE, SUBSTRATE, AUXINS AND LEAF AREA

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TCHOUNDJEU, Z. & LEAKEY, R. R. B. 2001. Vegetative propagation of *Lovoa trichilioides*: effects of provenance, substrate, auxins and leaf area. *Lovoa trichilioides* (African walnut, dibetou or bibolo) is an important commercial timber species indigenous to West and Central Africa. However, the lack of seeds and the destruction of young seedlings by shoot-borers have hampered large-scale regeneration of this species. As vegetative propagation is an effective means of multiplying selected trees within a tree improvement programme, this study focused on the main factors affecting the rooting ability of leafy stem cuttings. When testing different rooting substrates, single-node, leafy stem cuttings rooted significantly better in coarse gravel than in a forest topsoil–gravel mixture. The application of indole-3-butyric acid (IBA) had no clear effect on mean rooting perentage, although three out of four treatments with differing concentrations of IBA gave final results that were significantly better than the control. However, 50μg IBA can be considered to be an appropriate concentration to promote their rooting. Rooting ability was also affected by the node position. Cuttings from the apical nodes rooted significantly better than those from basal nodes of the same stem. Cuttings with large leaf areas (50–200 cm^2) rooted better than those with smaller trimmed leaves (60% in 9 weeks versus 0 and 37% for those with 0 and 25 cm^2 respectively). The optimum of 200 cm^2 is higher than that for other tropical tree species such as *Triplochiton scleroxylon* and *Khaya ivorensis*.

Key words: Rooting - stem cuttings - non-mist propagator - media

TCHOUNDJEU, Z. & LEAKEY, R. R. B. 2001. Pembiakan tampang *Lovoa trichilioides*: kesan provenans, substrat, auksin dan luas kawasan daun. *Lovoa trichilioides* (walnut Afrika, dibetou atau bibolo) ialah spesies balak komersial asli yang penting bagi Afrika Tengah dan Barat. Bagaimanapun, kekurangan biji benih dan pemusnahan anak-anak benih muda oleh penggerek pucuk telah menghalang pemulihan besar-besaran bagi spesies ini. Memandangkan pembiakan tampang merupakan cara yang berkesan untuk membiakkan pokok yang terpilih dalam rancangan pembaikan pokok, kajian ini memfokuskan kepada faktor-faktor utama yang mempengaruhi keupayaan pengakaran bagi keratan batang berdaun. Apabila menguji substrat pengakaran yang berbeza, buku tunggal, keratan batang berdaun mengakar dengan lebih baik dengan bererti di dalam kerikil besar berbanding dengan di hutan campuran tanah atas dan kerikil kasar. Penggunaan asid indola-3-butirik (IBA) tidak mempunyai kesan yang jelas terhadap min peratus pengakaran, walaupun tiga daripada empat rawatan dengan kepekatan IBA yang berbeza memberikan keputusan akhir yang lebih baik dengan bererti berbanding dengan kawalan. Bagaimanapun, 50 µg IBA bolehlah dianggap sebagai kepekatan yang sesuai untuk menggalakkan pengakarannya. keupayaan pengakaran juga dipengaruhi oleh kedudukan buku. Keratan daripada buku apeks mengakar dengan lebih baik dengan bererti daripada keratan dari pangkal buku daripada batang yang sama. Keratan dengan luas daun yang besar (50–200 cm^2) mengakar lebih baik daripada keratan dengan daun yang dikerat lebih kecil (60% dalam masa 9 minggu berbanding dengan 0 dan 37% bagi keratan masing-masing dengan 0 dan 25 cm^2). Optimum sebanyak 200 cm^2 adalah lebih tinggi daripada optimum untuk spesies pokok tropika yang lain seperti *Triplochiton scleroxylon* dan *Khaya ivorensis*.

Introduction

Lovoa trichilioides Harms. (African walnut, dibetou or bibolo) is an important commercial hardwood timber tree of the Meliaceae found throughout the humid forest zone of Cameroon. Its natural distribution extends across the evergreen forest from Sierra Leone in West Africa to Angola in Central Africa (Figure 1). *Lovoa trichilioides* is a large or emergent forest tree, often reaching a height of 45 m or more, with a diameter at breast height exceeding 1 m. The bole is sometimes straight and cylindrical to a height of 25 m where the first branches occur. More commonly, however, the bole is crooked and heavily forked illustrating the high potential there is for improvement of this species. The crown tends to be heavily branched and fairly open, becoming dome-like on emergent trees.

There is often abundant local regeneration in the high forest and adjacent low bush. The seedlings can tolerate shade for a long period and, when the overhead canopy is opened by a falling tree, they start to grow and form a long slender stem that terminates in a small crown with large leaves. Despite the abundance of seed, many factors hamper regeneration programmes with this species:

- Seed collection is difficult because mast years occur at intervals of 3–4 y, although some flowering trees can be found in most years.
- Seed production is spread over a few weeks and the winged seeds can be carried far from the mother tree.
- Insects and small mammals feed on the seeds reducing the numbers that can germinate.
- Although fresh seeds may initially have a good germination rate, viability decreases rapidly. Consequently, wildings are usually collected from the natural forest for forest regeneration programmes, although their growth in the nursery may be quite slow.

- Like other members of the Meliaceae, *L. trichilioides* is attacked by the mahogany shoot-borer, the larvae of the moth *Hypsipyla robusta* (FAO 1958). The hatching larvae penetrate the young stem tissues, killing the shoot apex and causing considerable damage and often the death of young trees, hence discouraging large-scale planting.

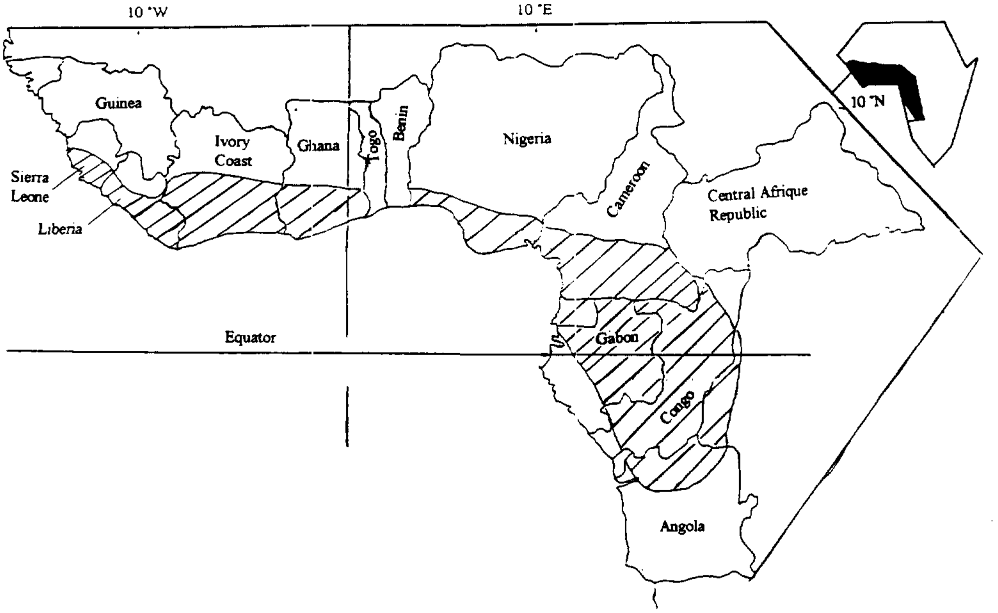

Figure 1. The natural distribution of *Lovoa trichilioides* in West and Central Africa (shaded area) (from Vivien & Faure 1985)

Vegetative propagation is considered because of the above constraints to large-scale regeneration from seed and the opportunity to select and mass propagate superior trees for clonal forestry (Leakey 1991). Additionally, clonal propagation has been recommended as an approach to circumvent the *Hypsipyla* problem (Newton *et al.* 1994), because in other members of the Meliaceae, genetic resistance has been identified at low frequency in natural populations (Newton *et al.* 1999). If amenable to vegetative propagation, such resistant material would be appropriate for forest regeneration and agroforestry (Matos *et al.* in press). This paper reviews the main factors affecting the rooting rates of juvenile material of *L. trichilioides* in a non-mist propagator.

Materials and methods

The vegetative material used in the various experiments of this study was produced from young seedlings of six provenances collected in natural stands in Cameroon. The provenances originated from areas of Cameroon within the natural range of *L. trichilioides* with different climates (Table 1).

Table 1. Some characteristics of different zones of origin of *Lovoa trichilioides* provenances used to establish juvenile stockplants of seedling origin

Provenance	Number of plants of each provenance	Altitude (m)	Latitude	Longitude	Mean rainfall (mm)	Mean temperature (°C)	Mean humidity (%)	Main wet season	Main dry season
Mbalmayo	174	650	3° 05' N	11° 00' E	1640	23	77	Sept–Nov	Dec–Feb
Kumba	57	236	4° 32' N	9° 19' E	2405	29	86	Mar–Oct	Nov–Mar
Makak	34	750	3° 30' N	11° 20' E	1687	23	80	Sept–Oct	Dec–Feb
Loum	54	150	4° 50' N	9° 05' E	2500	26	83	June–Oct	Mar–May
Ottotomo	210	802	3° 36' N	11° 19' E	1750	24	80	Sept–Nov	Dec–Feb
Edea	129	52	3° 46' N	10° 04' E	2750	26	82	Sept–Oct	Dec–Jan

Production of cuttings from seedlings

Prior to their transfer to the nursery of the Office National de Développement des Forêts (ONADEF), wildings of each provenance were collected from the forest and grown for 9–12 months. The plants were then cut back to a height of 10 cm to minimise the physiological stress of transit and transferred to the experimental site in Mbalmayo. Six hundred stockplants of six provenances of *L. trichilioides* (100 per provenance) were planted in the nursery in rows spaced at 0.5 × 1.0 m. Pruning subsequently stimulated sprouting and the production of shoots that formed an appropriate source of future 'juvenile' cutting material. Fifty grams of a compound fertiliser (NPK 20:10:10) were applied to each plant to accelerate their growth (application was repeated after every three months over a year).

Preparation of cuttings

Single-node cuttings were harvested sequentially down the current flush of the top two shoots from managed stockplants, taking the maximum cutting length allowed by the length of the internode. Unless otherwise stated, leaves on these cuttings were trimmed to about 50 cm^2. Auxin (10 µl 0.2% indole-3-butyric acid, IBA), dissolved in 100% ethanol, was applied to the cutting base with a micrometer syringe. The alcohol was evaporated off using a fan, before the cuttings were set in non-mist propagators described by Leakey *et al.* (1990).

Cutting length was assessed while setting up the experiment. Rooting ability was assessed once a week from the fourth week through to the twelfth. This involved recording the number of rooted and dead cuttings and the state of flushing of the axillary buds on the cuttings. Newly-rooted cuttings with one or more roots longer than 1 cm were potted in black polyethylene bags containing a 50:50 mixture of forest topsoil with rotted sawdust. Unrooted cuttings, or those with roots shorter than 1 cm, were returned to the propagation bed. Potted cuttings were hardened off in non-mist propagators under shade for 2–3 weeks before being transferred to the open nursery.

Effects of different media on rooting ability of L. trichilioides stem cuttings under non-mist propagator (Experiment 1)

Two different rooting media were tested: coarse gravel (CG : 3-10 mm diameter) and a 50:50 mixture of coarse gravel and forest topsoil (FT). The coarse gravel was obtained by sieving river sand. After sieving, the gravel was washed to remove weed seeds, organic matter, etc. A total of 300 cuttings (3 cuttings per plant) from managed seedling stockplants of 2 provenances (Edea and Kumba) were set under a non-mist propagator using a 2-factorial design with 2 levels of substrate (CG and FT) by 2 provenances (n=150). There were 25 cuttings in each treatment replicated 3 times (n=75).

Effects of different concentrations of IBA on the rooting of the L. trichilioides stem cuttings (Experiment 2)

Cuttings were collected from five clones (original seedling stockplant and rooted ramets from it) originating from Mbalmayo: (MB4, MB5, MB9, MB11 and MB50). Five concentrations of IBA were chosen for the experiment: 0, 50, 100, 150 and 250 μg in 10μl. Thirty cuttings were used in each treatment and each treatment was replicated three times. In all, 450 cuttings were set under non-mist propagators in a randomised block design. The rooting substrate was a 50:50 mixture of coarse gravel and rotted sawdust.

Effects of different lamina areas on rooting of L. trichilioides stem cuttings (Experiment 3)

Five different lamina areas (0, 25, 50, 100 and 200 cm^2) were tested in this experiment. Each treatment had 30 cuttings and was replicated 3 times. Thus, 450 cuttings were treated with 50 μg IBA before being inserted in randomised block design under a non-mist propagator containing a 50:50 mixture of coarse gravel and rotted sawdust.

Statistical analysis

ANOVA or *t*-test, as appropriate, was done using the Statview 512 (Abacus Concepts, Inc., 24009 Venture Blvd., Calabasas, California, USA) computer package and unless otherwise stated, statistical significance is given at the 5% level ($p<0.05$). Standard errors for percentages of rooted or dead cuttings were calculated using the procedures of Snedecor and Cochrane (1989) for data with binomial distribution.

Results and discussion

Effects of different rooting media on the rooting of L. trichilioides stem cuttings. (Experiment 1)

Rooting started between weeks 3 and 4, and was faster in pure coarse gravel (CG) than in the 50:50 mix with forest topsoil (FT). This difference persisted and at week 7, the percentage of cuttings rooted in CG became significantly greater ($p=0.001$) than in FT. In addition, there were a greater number of roots per rooted cutting in CG than in the FT. Negative relationships were found between % cuttings rooted and numbers of roots per rooted cutting in CG ($y=303.14 - 31.36x$; $r=0.95$) and FT ($y=117.53 - 13.23x$; $r= 0.93$). Cutting mortality in FT was double or more that in CG in cuttings from two provenances (Table 2).

Table 2. Effects of substrate (CG = coarse gravel, FT = forest topsoil) and provenance on the percentages of rooted and dead cuttings of *L. trichilioides*.

	Edea		Kumba		Overall	
Media	CG	FT	CG	FT	CG	FT
Rooting %	78 ± 5.4	45 ± 6.8	61 ± 8.5	50 ± 8.3	72 ± 4.6	46 ± 5.0
Mortality %	15 ± 4.6	47 ± 6.8	24 ± 7.4	33 ± 7.8	20 ± 4.0	40 ± 5.2

In CG, cuttings of Edea provenance rooted better than those from Kumba. In FT there was no significant difference between the two provenances. Although there was no significant difference in survival rates between the two provenances in the CG, the mortality of cuttings from Edea was higher in FT (Table 2). There was, however, no significant difference between the two media for survival rates of cuttings from Kumba.

In recent years there have been a number of comparative studies between different rooting media for tropical tree species (see Leakey *et al.* 1990 and the references therein). While it has been clear that species do root better in certain media, it is not possible to determine very clear trends. Sawdust was the preferred medium for *Milicia excelsa* (Ofori *et al.* 1996), *Irvingia gabonensis* (Shiembo *et al.* 1996a), *Gnetum africanum* (Shiembo *et al.* 1996b) and *Ricinodendron heudelotii* (Shiembo *et al.* 1997), all of which are lowland moist forest species of West Africa, while the central American species *Cordia alliodora* (Mesen *et al.* 1997) and *Albizia guachapele* (Mesen 1993) rooted best in gravel. The former is a moist forest species of the lower slopes of the cordillera, while the latter is from the dry forests of the Pacific coast. *Shorea leprosula*, a hillside species of Southeast Asia, did not root particularly well in either sand or coconut fibre (Aminah *et al.* 1995). These different media have differing air:water ratios (Leakey *et al.* 1990, Mesen 1993). From this limited evidence it seems unlikely that species adapted to wet conditions root better in media with a high water holding capacity. It is perhaps relevant that tropical tree species vary in their foliar relative water content, with dry zone species having lower values than humid zone species (Newton & Jones 1993). However, little is known about the processes involved. It is likely that there is an interaction between the water relations of the medium and those of the cuttings. For example, different rooting media are known to affect water uptake by cuttings (Grange & Loach 1983) and to have effects on photosynthesis and stomatal conductance of cuttings (Mesen *et al.* 1997). The relationships between % rooting and numbers of roots per rooted cutting suggest that in this study there were more water resources available for rooting in cuttings set in the coarse gravel medium.

Effects of different IBA concentrations on the rooting of L. trichilioides stem cuttings (Experiment 2)

Percentage rooting in cuttings treated with 0, 50, 100, or 200 μg IBA per cutting was not significantly different. Surprisingly, those treated with 150μg IBA per cutting were lower than the others, apparently due to higher mortality (26 ± 6.2%).

However, the general lack of an effect of IBA on rooting was further indicated by the lack of significant differences between treatments in the number of roots formed per cutting.

There were, however, significant differences between rooting and cutting mortality between nodes within the same shoot. Significant differences were found in the rooting percentages of cuttings from apical and basal nodes (Figure 2), cutting mortality (Figure 3) and the numbers of roots per cutting (4 ± 0.62 vs. 2 ± 0.29 for node 1 and node 5 respectively). Cuttings from basal nodes were the most susceptible to mortality and they had the greatest proportion that remained unrooted.

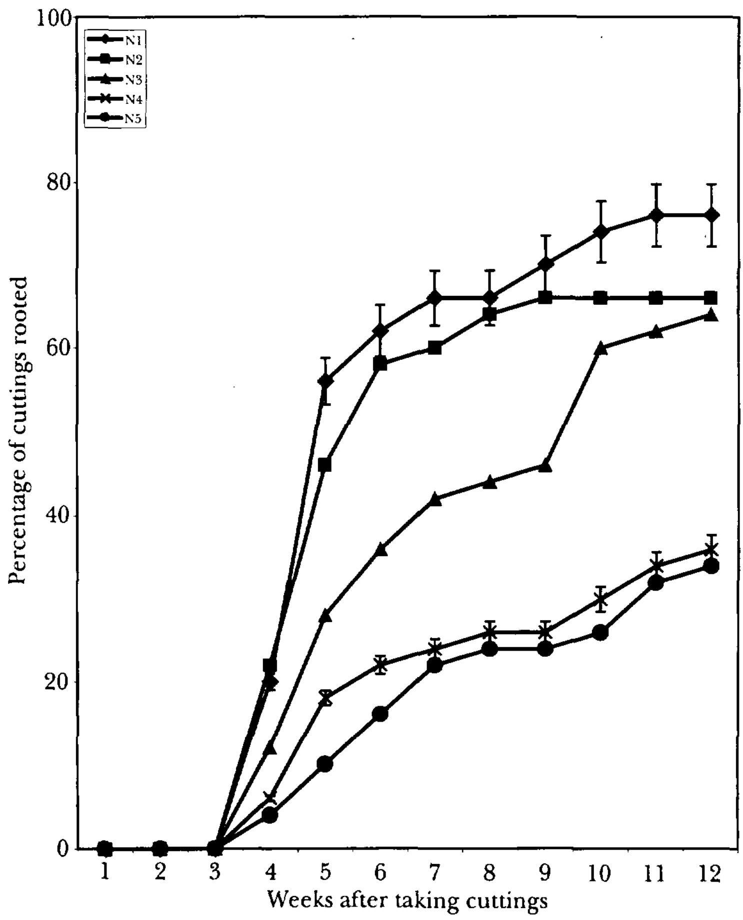

Figure 2. Effects of node position on the rooting percentage of *L. trichilioides* single-node leafy stem cuttings (mean across IBA treatments)

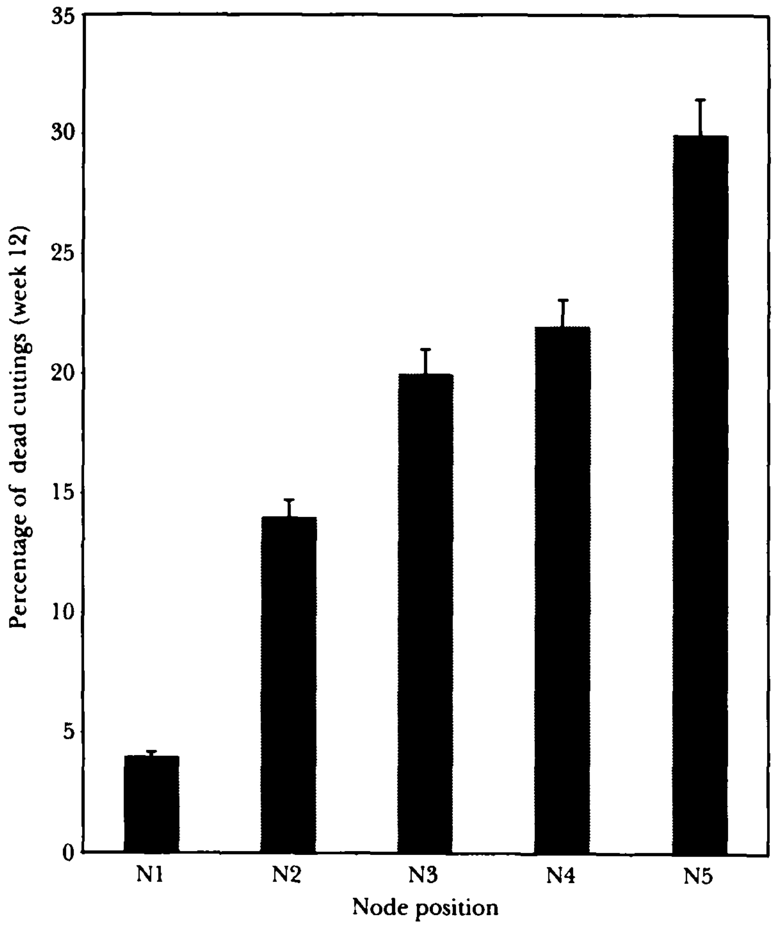

Figure 3. Effects of node position on the percentage mortality of *L. trichilioides* single-node leafy stem cuttings (mean across IBA treatments)

There was some evidence of clonal variation in the rooting of cuttings from seedlings of Mbalmayo provenance, with those of four clones rooting better than MB5, the fifth clone (Figure 4).

Auxins generally enhance rooting ability in cuttings of most plant species, including tropical trees (Leakey *et al.* 1990, 1994). However, the results from this study show that *L. trichilioides* seems to be different from many other tropical trees, as rooting was not increased, hastened or proliferated by a range of auxin concentrations. However, another experiment with higher auxin concentrations (Tchoundjeu 1989) has suggested that to enhance rooting capacity substantially higher concentrations of auxin are required and that, on larger leaved cuttings, are beneficial.

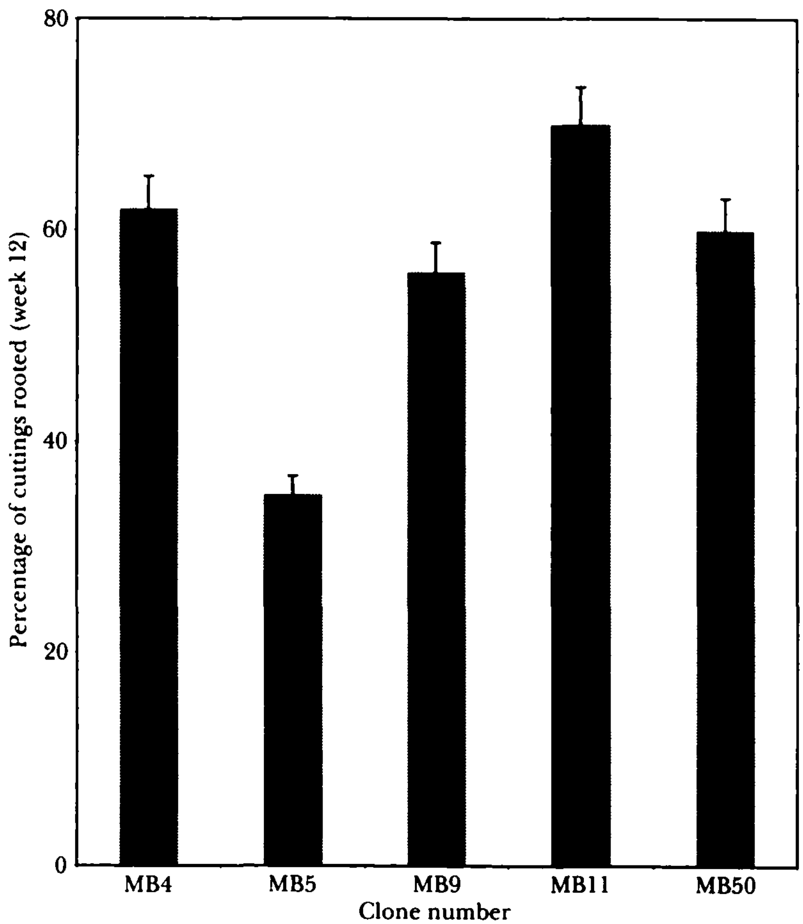

Figure 4. Clonal variation in the rooting percentage of *L. trichilioides* single-node leafy stem cuttings (mean across IBA treatments)

This study observed polarity between cuttings from different nodes in mortality and the lack of rooting. The cause of this mortality is not well known in *L. trichilioides*. However, in *Triplochiton scleroxylon*, a West African hardwood, there was some evidence that rooting was dependant on the volume of the cutting's stem portion and hence in the cutting's capacity to store current assimilates prior to the formation of roots and a new sink for them (Leakey & Mohammed 1985, Leakey *et al.* 1992). In *L. trichilioides* basal cuttings have been found to have short internodes and large diameters, but to be smaller in volume than the more apical nodes. Thus there may be a similarity with the findings in *T. scleroxylon*, although the differences between the species in their growth habit may complicate the relationship: *T. scleroxylon* has free growth, while *L. trichilioides* grows by recurrent flushing. In contrast, basal cuttings of *Khaya ivorensis*, another member of the

Meliaceae, rooted better than apical ones, but long cuttings only rooted better than short cuttings when the cuttings had a large leaf area (Tchoundjeu & Leakey 1996).

Effects of different lamina areas on rooting of L. trichilioides stem cuttings (Experiment 3)

Leafless cuttings did not root and most (90%) had died by week 9. Cuttings with larger leaf areas (50–200 cm^2) rooted significantly better than did cuttings with 25 cm^2, those with 200 cm^2 rooting best. They also had the greatest mean number of roots per rooted cutting (Figure 5). The percentages of dead cuttings decreased with the increasing leaf area, suggesting that cutting survival depended on current photosynthesis and that water stress was not a major problem.

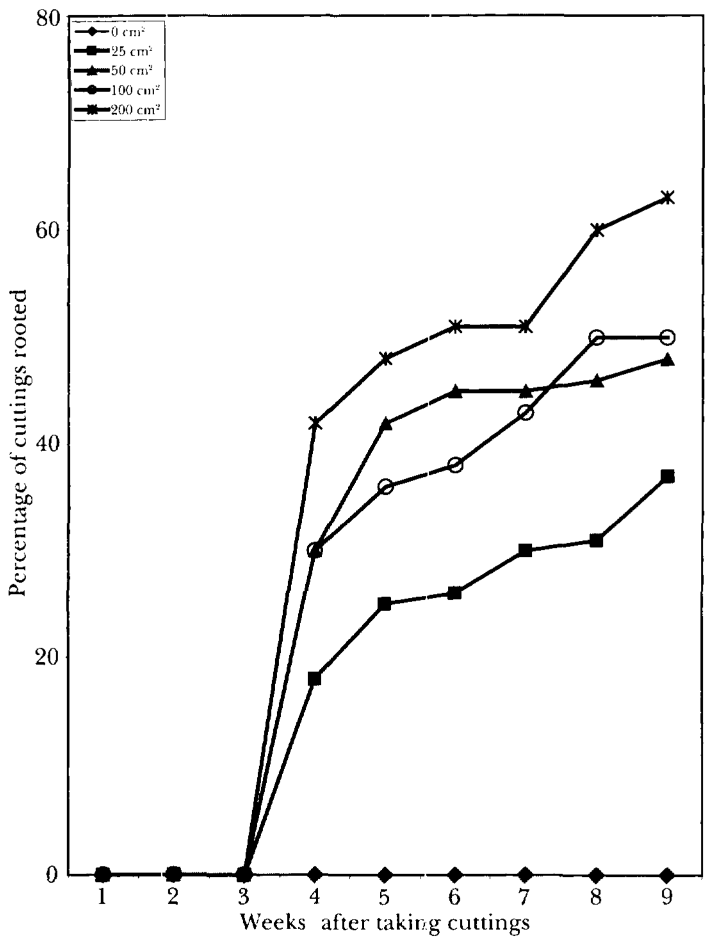

Figure 5. Effects of lamina area on the rooting percentage of auxin-treated, single-node, leafy stem cuttings of *L. trichilioides*

Leaves of *L. trichilioides* differ from those of most of the other West African hardwoods that have been propagated vegetatively: *T.scleroxylon, T. superba* and *Nauclea diderrichii* (Martin & Quillet 1974, Howland 1975, Koyo 1983, Leakey & Coutts 1989). However, like *K. ivorensis, L. trichilioides* has compound leaves with 3–7 opposite pairs of leaflets. Despite this similarity, the two species appear to require a different leaf area for successful rooting. Optimum rooting percentages of *K. ivorensis* under intermittent mist were achieved when leaves were trimmed to 10–50 cm^2 (Tchoundjeu & Leakey 1996). In contrast, *L. trichilioides* cuttings with larger leaf areas (50–200 cm^2) rooted better in the conditions provided by a non-mist propagator. It is not clear whether this is an intrinsic difference between the species, or whether water stress was less marked under the latter system, so reducing transpiration losses. Photosynthetically active larger leaves would produce more assimilates altering the optimum balance between the benefits of photosynthesis and the negative effects of transpiration.

Dick and Dewar (1992) have developed a process-based model for rooting in stem cuttings, based on principles derived from the studies on *T. scleroxylon*. To validate this model with data from tree species with apparently different responses to standard treatments, more physiological studies are needed. Research in the Meliaceae should seek to explain the apparent interactions between cutting volume, leaf area, cutting origin, assimilate storage, and the effects of stockplant environment, especially light and nutrients, in species with compound leaves and growth by recurrent flushing. This would have important implications for the domestication of mahogany and related species (Newton *et al.* 1994), for which vegetative propagation is needed to take advantage of the incidence of a low level of naturally occurring genetic resistance to *Hypsipyla* shoot borers (Newton *et al.* 1999).

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