VEGETATIVE PROPAGATION OF *RICINODENDRON HEUDELOTII*, A WEST AFRICAN FRUIT TREE

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Received May 1995

SHIEMBO, P.N., NEWTON, A.C. & LEAKEY, R.R.B. 1997. Vegetative propagation of *Ricinodendron heudelotii*, a West African fruit tree. Leafy stem cuttings of *Ricinodendron heudelotii* (Baill) Pierre *ex* Pax, a West African fruit tree, were taken from seedlings or coppice shoots and inserted in a low-technology non-mist propagation system in Cameroon. Three separate experiments were tested: (i) six propagation media, namely sawdust (SD), fine sand (FS), medium sand (MS), gravel (G), and 50:50 mixtures of G:SD and MS:SD; (ii) four IBA concentrations, namely 0, 8, 40 and 200 µg IBA dissolved in 10 µl of alcohol; and (iii) four leaf area treatments, namely 0, 25, 50 and 80 cm^2, obtained using paper templates. The overall effect of propagation medium on final rooting percentage was highly significant (p=0.003, ANOVA), highest values being recorded in FS and SD. The number of roots per rooted cutting also differed markedly between treatments, mean values ranging from 3.8 to 7.3 in G and SD respectively. Application of IBA had no significant effect on final rooting percentage, although root number was positively related to IBA concentration, values ranging from 2.7 to 10.5 in 0 and 250 µg respectively. Leaf area had a highly significant effect on rooting percentage and root number ($p < 0.001$, ANOVA), with highest values recorded in the 80 cm^2 treatment. Defoliated cuttings completely failed to root. The maximum rooting percentages exceeding 80% obtained in all three experiments indicate that *R. heudelotii* is amenable to vegetative propagation using these techniques, which should be of value to current domestication efforts.

Key words: Vegetative propagation - fruit tree - low technology - *Ricinodendron heudelotii* - West Africa - domestication

SHIEMBO, P.N., NEWTON, A.C. & LEAKEY, R.R.B. 1997. Pembiakan tampang *Ricinodendron heudelotii*, sejenis pokok buah-buahan Afrika Barat. Keratan batang berdaun *Ricinodendron heudelotii* (Baill) Pierre *ex* Pax, sejenis pokok buah-buahan di Afrika Barat, diambil dari anak benih atau pucuk kopis dan dimasukkan satu sistem pembiakan tidak-berkabus teknologi rendah di Cameroon. Tiga ujikaji yang berbeza diuji: (i) enam bahantara pembiakan iaitu habuk gergaji (SD), pasir halus (FS), pasir

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sederhana (MS), kerikil (G), dan 50:50 campuran G:SD dan MS:SD; (ii) empat pekatan IBA, iaitu 0, 8, 40 dan 200 µg IBA dilarutkan dalam 10 µl alkohol; dan (iii) empat rawatan luas daun iaitu 0, 25, 50 dan 80 cm^2, yang diperoleh menggunakan templat kertas. Kesan keseluruhan bahantara ke atas peratus pengakaran akhir sangat ketara ($p = 0.003$, ANOVA), nilai tertinggi dicatatkan dalam FS dan SD. Bilangan akar bagi setiap keratan akar juga berbeza di antara rawatan, nilai purata berjulat dari 3.8 hingga 7.3 dalam G dan SD masing-masing. Penggunaan IBA tidak mempunyai kesan ketara ke atas peratus pengakar akhir, walaupun bilangan akar berkaitan secara positif dengan pekatan IBA, nilainya berjulat dari 2.7 hingga 10.5 dalam 0 dan 250 µg, masing-masing. Luas daun mempunyai kesan yang sangat ketara ke atas peratus pengakaran dan bilangan akar ($p < 0.001$, ANOVA), dengan nilai tertinggi dicatatkan dalam rawatan 80 cm^2. Keratan ranggas gagal mengakar sepenuhnya. Peratus pengakar maksimum melebihi 80% yang diperoleh dalam ketiga-tiga ujikaji menunjukkan bahawa *R. heudelotii* bergantung kepada pembiakan tampang menggunakan teknik-teknik ini yang sepatutnya bernilai kepada usaha pendomestikan semasa.

Introduction

Ricinodendron heudelotii (Baill) Pierre *ex* Pax (Euphorbiaceae) is a dioecious deciduous tree native to West Africa. The species is characteristic of secondary forest (Thikakul 1985) and has a wide variety of uses. The wood is used for making household implements, drums and carvings (Shiembo 1994) and the bark is used to treat both elephantiasis (Abbiw 1990) and leprosy (Bokemo 1984). In Cameroon, the fruit is traded widely and is a significant source of cash income for many farmers. The seeds are a sought-after ingredient of soups, and used to supplement a wide variety of dishes (Shiembo 1994). The oil obtained from the seeds is also used for making soap and varnish (Abbiw 1990).

Although *R. heudelotii* displays many characteristics which make it suitable for cultivation such as high foliage production for mulching, the species is rarely planted. This primarily reflects the difficulty in obtaining planting stock, as the seeds are difficult to germinate (Shiembo 1994). The difficulty of determining which trees are female at the seedling stage is an additional disincentive to farmers. Vegetative propagation techniques may be of great value in overcoming these problems, by enabling selected genotypes to be multiplied and the availability of planting stock to be increased. In this way, the process of domestication may be significantly encouraged (Leakey & Newton 1994).

Previous research with a range of tropical tree species has identified a wide variety of factors which influence adventitious root development in leafy stem cuttings (Leakey *et al.* 1994). For example, many species display contrasting responses to post-severance treatments such as the propagation medium and the concentration of applied IBA (Leakey *et al.* 1990). In addition, propagation of some species is sensitive to variation in cutting leaf area, although this is by no means universal (Leakey *et al.* 1982, Newton *et al.* 1992). Although some preliminary propagation trials with *R. heudelotii* have been undertaken in the United Kingdom (Leakey *et al.* 1990), the appropriate treatments for optimal rooting of this species have not previously been defined.

The objective of the experiments described here was to investigate the vegetative propagation of *Ricinodendron heudelotii* to determine appropriate treatments for mass production of clonal planting stock. In particular, these experiments were designed to assess the effect of variation in (i) propagation medium, (ii) auxin concentration, and (iii) leaf area on the rooting of leafy stem cuttings. Experiments were also designed to test the suitability of a low technology non-mist propagation system (Leakey *et al.* 1990) for propagation of this species. This system is designed for use in rural tropical areas and has successfully been used for the propagation of a large number of other tropical tree species (Leakey *et al.* 1994).

Materials and methods

This study was carried out at the Southern Bakundu Forest Reserve (4° 12'- 5° 29' N, 9°- 9° 55' E; approximately 230 m above mean sea level) in the Southwest Province of Cameroon (see Richards 1963, Songwe *et al.* 1988). Rainfall ranges from 1800 to 2600 mm y^{-1}. A propagation unit was established at the Forestry Research Station at Kumba, SW Cameroon as described by Shiembo *et al.* (1996). A total of six propagators were constructed following the low-technology non-mist design described by Leakey *et al.* (1990). The propagators were sited in a shade house roofed in transparent plastic sheets, providing an irradiance inside the propagators of 13 - 28% of that received outside the propagation unit.

Experiment 1. Effect of propagation media on rooting

Six different propagation media were prepared as described by Shiembo *et al.* (1996), namely sawdust (SD), fine sand (FS), a 50:50 mixture of gravel and sawdust (G:SD), medium sand (MS), gravel (G) and a 50:50 mixture of medium sand and sawdust (MS:SD). The sawdust was obtained from a local sawmill, and the sand from a nearby river. All media were sieved and cleaned prior to use. The media were sprayed with a systemic fungicide prior to insertion of the cuttings. The six media were randomly assigned to different compartments of the propagator.

Four trees of *R. heudelotii* located either within or nearby the Forest Reserve were felled for the production of coppice shoots. After two months, 90 single-node cuttings, five from each shoot, were taken from each of the 4 clones. Leaves were trimmed to approximately 50 cm^2 using paper templates. The base of each cutting was treated with 40 µg of indole-3-butyric acid (IBA) in a solution of industrial alcohol, applied in a 10 µl drop from a micrometer syringe, then allowed to dry in a current of air from a fan (following Leakey *et al.* 1982). Fifteen cuttings from each clone were inserted according to node position, into each of the six media, giving a total of 60 cuttings in each medium (5 cuttings × 3 shoots per clone × 4 clones). Cutting lengths, measured at time of insertion, varied between 4.5 and 9.5 cm. The cuttings were sprayed with systemic insecticide and fungicide after insertion. During propagation, the cuttings were sprayed with water at 0730 h and 1730 h each day, using a hand-held sprayer. Cuttings were assessed weekly for callus development, the presence and number of roots (≥ 2 mm in length), leaf shedding, cutting mortality and stem diameter.

Experiment 2 - Effect of IBA concentration on rooting

Forty-eight seedlings of *R. heudelotii* were collected from an abandoned farm near to Kumba, Cameroon, then potted into polythene pots (10 cm diameter x 18 cm depth) and raised in the propagation unit under shade cloth (Tildenet 50% transmission; Kerry PAR, Bristol, BS3 2HA). When the seedlings had produced 10 to 14 leaves, 4 single-node cuttings were taken from each one, and randomly allocated to one of four IBA treatments, namely 0 (control), 8, 40 and 200 µg IBA, applied as described in Experiment 1. The control treatment was 10 µl of industrial alcohol only. The leaves of each cutting were trimmed to approximately 50 cm^2 using paper templates. Cuttings were randomly allocated to one of ten blocks of sixteen cuttings (4 treatments × 4 cuttings) and inserted in the sawdust rooting medium.

Experiment 3 - Effect of leaf area on rooting

Seedlings of *R. heudelotii* were collected from the Forest Reserve, potted in polythene pots (10 cm diameter x 18 cm depth), and raised in the propagation unit at Kumba. When the seedlings had produced 8 to 12 leaves, 4 single-node cuttings were taken from each one, and randomly allocated to one of four leaf area treatments, namely 0, 25, 50 and 80 cm^2, obtained using paper templates. After applying 40 µg of IBA to the base of each cutting, as described earlier, the cuttings were inserted in the sawdust rooting medium. Cuttings were randomly allocated to one of thirteen blocks of sixteen cuttings (4 treatments × 4 cuttings).

In each experiment, results were subjected to analysis of variance using SAS (1980) to determine treatment effects. Percentage data were arcsin transformed prior to analysis. Standard errors and confidence limits of percentages were calculated following the procedure described by Snedecor and Cochran (1980) for binomial data. Analysis of deviance by stepwise regression in GENSTAT 5 (Payne *et al.* 1987) was applied to assess the effect of treatment and non-treatment variables on final rooting percentages.

Results

Experiment 1

Rooting commenced in week 2 in all treatments, percentage rooting increasing markedly between weeks 2 and 3. From week 2 onwards, highest rooting percentages were recorded in SD, although values in this treatment were not significantly different from FS at the end of the experiment (82% and 73% respectively). Although rooting percentages in the other four media increased progressively

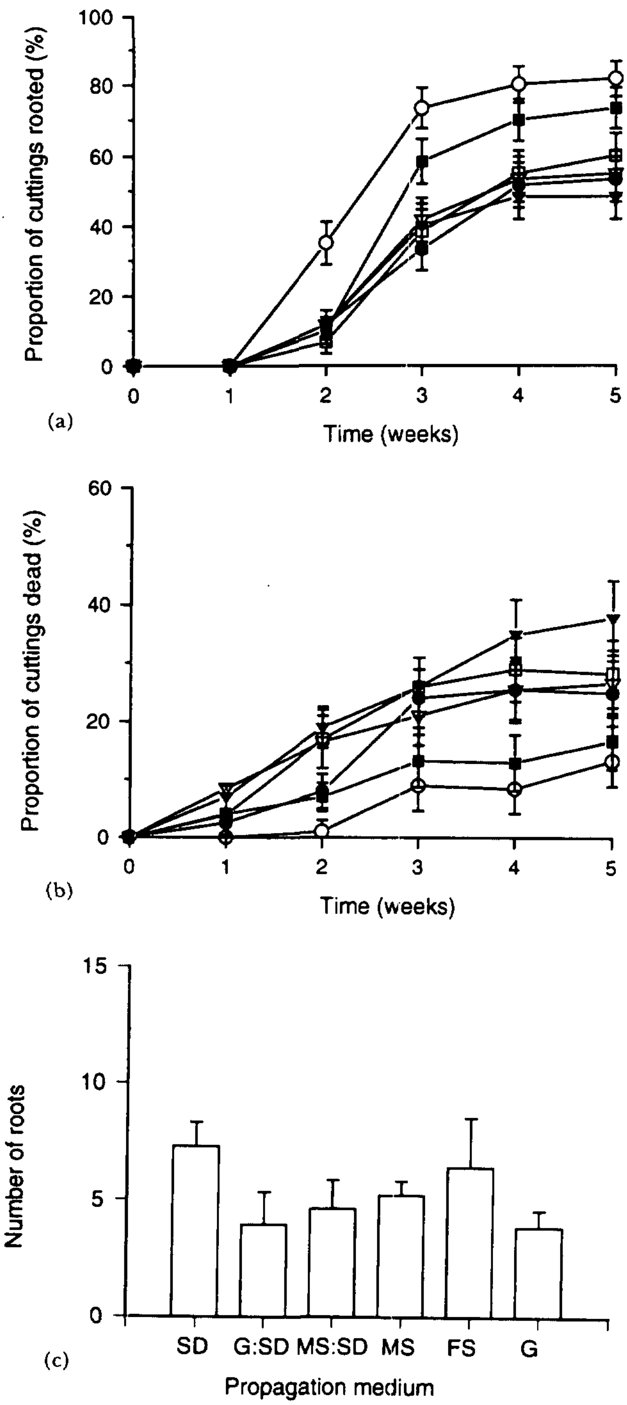

Figure 1. Effect of different propagation media on (a) rooting percentage, (b) cutting mortality and (c) number of roots per rooted cutting of leafy stem cuttings of *Ricinodendron heudelotii* in a non-mist propagation system in Cameroon. Values presented are means (n = 55); vertical bars, s.e.m. Symbols: ○ : sawdust; ■ : fine sand; □ : medium sand; ▽: mixture (50 : 50) of medium sand and sawdust; ● : gravel; ▼: mixture (50 : 50) of gravel and sawdust.

from weeks 2 to 4, there were no significant differences between them ($p > 0.05$; Figure la). The overall effect of propagation medium on final rooting percentage was highly significant ($p = 0.003$, ANOVA). Analysis of deviance indicated that node position also had a significant effect on rooting ($p < 0.01$), with lower rooting percentages recorded lower down the stem, although stem length and diameter had no significant effect. Five weeks after insertion, the highest mean (7.3) number of roots per cutting was recorded in SD, while the lowest (3.8) was recorded in G (Figure lc).

Highest percentage mortalities were recorded in G:SD from weeks 2 to 5, when values ranged between 13 and 38% in SD and G:SD respectively (Figure lb). Highest values of percentage leaf abscission were also recorded in the G:SD treatment, values ranging from 15 to 38% in FS and G:SD respectively at week 5. Most of the cuttings that abscissed their leaves subsequently died.

Experiment 2

When rooting commenced at the second week after insertion, rooting percentages ranged from 30% in the 0 µg treatment to 45% in the 8 µg treatment. By week 3, rooting percentage in the 8 µg treatment was significantly ($p < 0.05$) higher than in the other treatments, which were not significantly different from each other. At week 4, rooting percentage in the 8 µg treatment was still relatively high (88%), with the lowest percentage (60%) recorded in the 200 µg treatment (Figure 2a). However, the overall effect of IBA on final rooting percentage was not significant ($p > 0.05$; ANOVA). Analysis of deviance indicated that rooting was significantly influenced by cutting basal diameter ($p < 0.001$) and node position ($p < 0.05$), but not by treatment. The number of roots per rooted cutting at week 4 was positively related to IBA treatment, values ranging from 2.7 in the control (0 µg) treatment to 10.5 in 200 µg (Figure 2c).

Cutting mortality remained low in the 8 µg and control treatments, values not exceeding 5 and 8% respectively throughout the experiment. Higher values were recorded in the 200 µg treatment than in the others from week 2 to week 4 (Figure 2b). By the end of the experiment, percentage leaf abscission ranged from 48 to 60% in the 8 and 40 µg treatments respectively. However, at this time, there were no significant ($p > 0.05$) differences between the four treatments with respect to leaf abscission.

Experiment 3

The cuttings that were initially leafless did not root, most of them rotting during the first week of the experiment and subsequently dying. From week 2, when rooting commenced in all leafy treatments, rooting percentage increased with increasing leaf area. Although this pattern was maintained throughout the experiment, the differences between the leafy treatments became less pronounced with time (Figure 3a). The overall effect of leaf area on final rooting percentage was

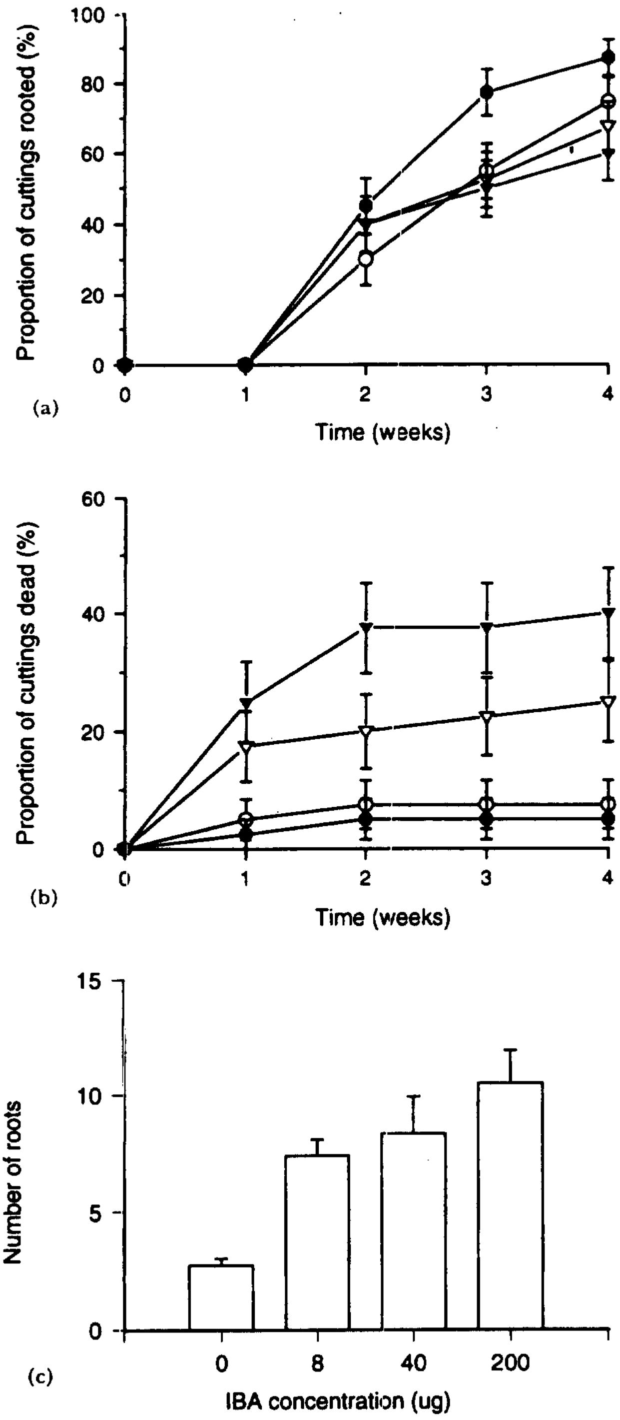

Figure 2. Effect of different IBA concentrations on (a) rooting percentage, (b) cutting mortality and (c) number of roots per rooted cutting of leafy stem cuttings of *Ricinodendron heudelotii* in a non-mist propagation system in Cameroon. Values presented are means (n = 40); vertical bars, s.e.m. Symbols: O : 0 μg; ● : 8 μg; ▽: 40 μg; ▼: 200 μg.

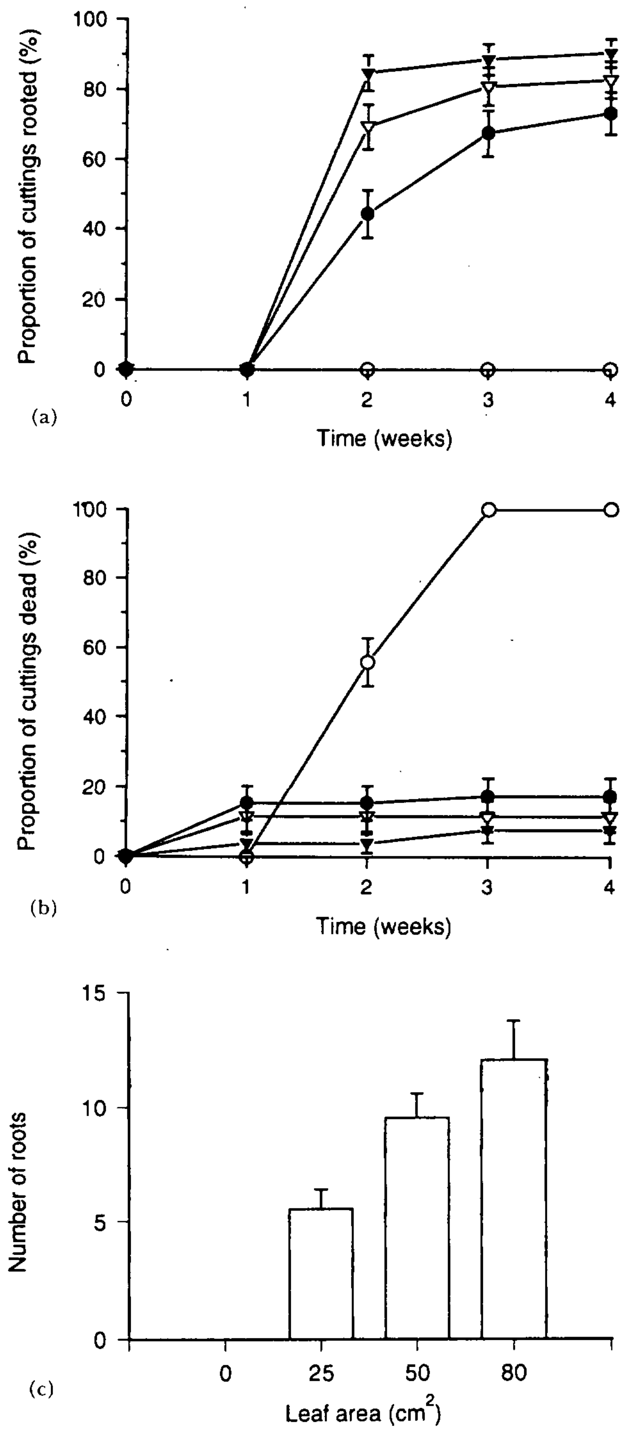

Figure 3. Effect of different leaf areas on (a) rooting percentage, (b) cutting mortality and (c) number of roots per rooted cutting of leafy stem cuttings of *Ricinodendron heudelotii* in a non-mist propagation system in Cameroon. Values presented are means (n = 52); vertical bars, s.e.m. Symbols: O : 0 cm^2; ● : 25 cm^2; ▽: 50 cm^2; ▼ : 80 cm^2.

highly significant ($p<0.001$, ANOVA). Analysis of deviance indicated that rooting percentage was highly affected by node position ($p< 0.001$) and stem diameter ($p< 0.05$) as well as by leaf area, but was not affected by stem length.

At the end of the experiment, the number of roots per rooted cutting was positively related to leaf area, values ranging from 5.6 to 11.9 in the 25 cm^2 and 80 cm^2 treatments respectively, with no roots produced by any of the cuttings in the 0 cm^2 treatment (Figure 3c). By week 2, 55% of the leafless cuttings had died, and by the third week, mortality in this treatment was 100%. In terms of percentage mortality, the other three treatments did not differ significantly from each other throughout the experiment (Figure 3b). Similarly, percentage leaf abscission did not differ significantly between treatments, values ranging from 25 to 40% in the 80 and 50 cm^2 treatments respectively.

Discussion

These results indicate that *Ricinodendron heudelotii* may be successfully propagated vegetatively using the non-mist propagation system, confirming previous preliminary results (Leakey *et al.* 1990). The fact that maximum rooting percentages of over 80% were achieved in all three experiments suggests that the species could be multiplied on an operation scale using these techniques, as has been achieved with *Triplochiton scleroxylon* in Cameroon (Ladipo *et al.* 1994). However, the pronounced variation in rooting percentage recorded in the different experimental treatments emphasises the need to adopt appropriate post-severance protocols, if rooting percentages are to be optimised.

With respect to propagation medium, *R. heudelotii* displayed similar responses to a number of other tropical tree species such as *Gnetum africanum* (Shiembo *et al.* 1996), *Irvingia gabonensis* (Shiembo 1994) and *Milicia excelsa* (Ofori 1994), all of which displayed highest rooting percentages in sawdust. This contrasts with results from a number of other species such as *Cordia alliodora, Gmelina arborea, Vochysia hondurensis* and *Albizia guachapele,* where the highest rooting percentages were respectively recorded in gravel (Mesen 1993), fine sand, gravel, and a 50:50 mixture of fine sand and sawdust (Leakey *et al.* 1990). The reasons why different species display contrasting rooting percentages in different rooting media are not well understood (Leakey *et al.* 1990) but may be attributed to variation in the oxygen, water content, pH and porosity of the media. These factors may affect tissue respiration and cell dedifferentiation at the base of the cutting, and subsequent root development (Loach 1985, Loach 1988, Leakey *et al.* 1994). In the experiment described here, the relatively high rooting percentage recorded in sawdust may be attributed to its relatively high air:water ratio and water content (Shiembo *et al.* 1996).

The lack of any effect of IBA on final rooting percentage, and the positive relationship between IBA concentration and number of roots per rooted cutting, precisely parallel results obtained with *Gnetum africanum* (Shiembo *et al.* 1996). Similarly, Leakey *et al.* (1982) demonstrated that the number of roots per rooted cutting of *Triplochiton scleroxylon* increased with increasing auxin concentration, and Bhatt and Todaria (1993) observed the same tendency in cuttings of *Debregeasia salicifolia* and *Boehmeria rugulosa*. However, percentage rooting of a number of other tropical tree species has been shown to be relatively insensitive to applied IBA concentration, for example *Lovoa trichilioides* (Tchoundjeu 1989), *Vochysia hondurensis* (Leakey *et al.* 1990), *Shorea macrophylla* (Lo 1985) and *Hopea odorata* (Aminah 1992). Variation in rooting requirements with respect to IBA may reflect variation in the endogenous IBA content of cuttings prior to severance. The influence of IBA on adventitious root development in cuttings has been attributed to its effect on the mobilisation of carbohydrate reserves by enhancing the activity of hydrolytic enzymes (Middleton *et al.* 1980). Haissig (1986) suggested that the role of auxin in increasing stem respiration rate in cuttings also has a promotive effect on rooting.

A decline in rooting percentage and an increase in cutting mortality were recorded in this investigation at IBA concentrations above 8 µg. Similar deleterious effects of applied IBA at high concentrations have been noted with other species (Middleton *et al.* 1980, Haissig 1986, Leakey *et al.* 1982). For example, mortality was highest in *Shorea macrophylla* cuttings treated with the highest (10 800 ppm) concentration of IBA, which also resulted in a reduction in the number of roots per rooted cutting (Lo 1985). The contrasting effects of increasing IBA concentration on root number and rooting percentage may reflect differential effects of applied auxin on the processes of root development and initiation (cf. Leakey *et al.* 1994).

The fact that none of the leafless cuttings rooted concurs with results from studies with *Terminalia spinosa* (Newton *et al.* 1992), *Triplochiton scleroxylon* (Leakey *et al.* 1982) and *Hopea odorata* (Aminah 1992), where rooting percentages were recorded of 0, 9 and 2% respectively in leafless cuttings. The positive relationships recorded here between leaf area, rooting percentage and root number further illustrate the influence of the leaf on adventitious root development, which may be attributed to its role in the production of carbohydrates and auxins. These results suggest that the optimum leaf area for propagation of *R. heudelotii* is > 80 cm^2, which compares with values of 50 and 10-30 cm^2 in *Triplochiton scleroxylon* (Leakey *et al.* 1982) and *Khaya ivorensis* (Asanga 1989, Tchoundjeu 1989) respectively. The tendency of some species to demonstrate an optimum leaf area for rooting reflects a trade-off between the effects of photosynthesis and transpiration, larger-leaved cuttings being more susceptible to water deficits which may hinder carbon fixation and therefore root development (Leakey & Coutts 1989, Newton *et al.* 1992).

The main cause of cutting mortality in these experiments was stem rotting, following leaf abscission. Rotting at the cutting base may be attributed to waterlogging of the tissues which is particularly prevalent after leaf abscission, as transpiration is arrested. Mechanical blockage of the xylem may also induce cutting death (Mujib 1993). To reduce cutting mortality, careful handling of the cuttings and appropriate management of propagator microclimate may be required to reduce the occurrence of water deficits (Newton & Jones 1993). Other practical implications of these results are that an organic propagation medium such as sawdust should be used, together with a cutting leaf area of at least 80 cm^2. Although IBA concentration had little effect on rooting percentage in the current experiment, addition of at least 40 µg IBA per cutting is recommended to ensure a high number of roots per cutting. Use of these treatments in combination should enable consistently high rooting percentages to be achieved, enabling mass propagation of clonal planting stock to be a viable option in domestication programmes for this species. However, further research is required to identify the extent of genotypic variation in rooting response, which has been found to be significant in other tropical tree species (Leakey *et al.* 1994).

Acknowledgements

Steve Newman and Joanne Wainright of Fountain Renewable Resources Ltd. assisted with implementation of this project. Financial support was provided by the EC, the British Council and the UK Darwin Initiative.

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