

Rooting of Leafy Stem Cuttings of *Baillonella toxisperma*

Marie Ngo Mpeck and Alain Atangana

Abstract: Rooting ability in Moabi was examined using 972 single-node leafy stem cuttings set in each of three blocks of a split-split plot experimental design in nonmist propagators. Each main plot contained three rooting media (sand, sawdust, and a 50:50 mixture of sand and sawdust), whereas three auxin types (indole-3-acetic acid [IAA], indole-3-butyric acid [IBA], and 1-naphthalene acetic acid [NAA]) and a control (alcohol) were tested at the subplot level. At the sub-subplot level, three leaf areas (25, 50, and 75 cm²) were investigated. Significant ($P = 0.05$) and nonsignificant substrate \times hormone \times leaf area interactions on rooting percentage were found at week 8 and during the rest of experiment, respectively. Significant sand versus sawdust \times IBA versus IAA and NAA \times quadratic components of leaf area interaction on rooting percentage was identified from week 10 to week 14 ($P = 0.0462$ – 0.038), and the highest rooting percentage ($85.2 \pm 7.24\%$) was found in 75 cm² \times IBA \times sand-treated cuttings at week 14. Significant substrate \times leaf area interaction on rooting percentage was observed throughout the experiment. Interacting substrate \times leaf area significantly influenced the number of roots per rooted cutting at week 22. This study indicated that Moabi is amenable to vegetative propagation within 14 weeks. FOR. SCI. 53(5):571–579.

Keywords: auxins, leaf areas, Moabi, rooting media, vegetative propagation.

PARTICIPATORY domestication of high-value indigenous fruit, nut, and medicinal trees using agroforestry techniques has been implemented as a farmer-driven and market-led process since 1998 in the humid tropics of West and Central Africa (Simons and Leakey 2004, Tchoundjeu et al. 2006). It uses the variability within species, by selecting trees with desirable traits (Atangana et al. 2002) and propagating them asexually, as a clonal approach aimed to develop cultivars (Tchoundjeu et al. 2006). This technique has involved rooting leafy stem cuttings using inexpensive and low-technology nonmist propagators (Leakey et al. 1990).

Morphological and physiological pre- and postseverance factors affecting rooting of leafy stem cuttings have been reviewed by Leakey (2004). These include the propagation environment, auxin application, leaf area, cutting length, diameter, origin and environment, stockplant management, and genetic origin (clone) attributed to genetic differences in morphology and physiology of cuttings (Dick et al. 2004). To develop a practical rooting protocol for leafy stem cuttings of a previously unstudied species, it is first desirable to determine experimentally the optimal auxin application, optimal leaf area, and optimal cutting length (Tchoundjeu and Leakey 1996) and investigate their interactions within a propagation environment. Although considerable experimental work has been done to identify factors influencing the ability of cuttings to root, few studies have investigated the interaction of these factors (Khasa et al. 1995, Atangana et al. 2006).

Dick and Dewar (1992) have developed a mechanistic

model showing the dependency of root development on leaf area, cutting length, and initial carbohydrate content. The behavior of the model was compared with published data for cuttings of Ayous (*Triplochiton scleroxylon* K. Schum [Sterculiaceae]), and although the simulated starch and sugar dynamics during root growth were in qualitative agreement with observations, a further parameterization was recommended before the model is used as a predictive tool. Other studies have described the rooting process using models built on factors affecting rooting (Khasa et al. 1995, Kubota and Kozai 2001). Good rates of rooting can be achieved when factors affecting rooting are optimized (Atangana et al. 2006), as they hasten the development of protocols for vegetative propagation of an unstudied species.

Moabi (*Baillonella toxisperma* Pierre [Sapotaceae]) is an important commercial forest tree distributed in Africa from Nigeria to Cabinda (Vivien and Faure 1996, pp. 315–316). The monotypic genus *Baillonella* is endemic to the Guineo-Congolian region (White 1983). *B. toxisperma* is limited to the dense primary evergreen rain forests. Moabi timber is used for furniture, cabinet work, decorative flooring, turnery and carving, decorative veneers, joinery, and stove fittings. Timber exploitation of *B. toxisperma* in Cameroon has doubled since 1989/1990 (Schneemann 1995). Moabi is heavily exploited from the wild in West Africa, and the primary threat to the species survival is logging. The minimum exploitable diameter of Moabi in Cameroon is 1 m and in both Gabon and Congo, the minimum exploitable

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diameter is decreed to be 0.8 m (United Nations Environment Program [UNEP]–World Conservation Monitoring Center [WCMC] 2006). To satisfy the strong demand for Moabi timber that comes mostly from Southern Europe (Schneemann 1995), trees are felled before reaching the required diameter. Moreover, the species is further threatened because it requires shade for regeneration to occur (UNEP-WCMC 2006). It takes between 50 and 70 years before *B. toxisperma* starts to flower, and regular fruit production does not occur until the tree is 90–100 years old (Schneemann 1995). The species is considered vulnerable according to the 1994 International Union for the Conservation of Nature and Natural Resources (IUCN) threats categories (IUCN 2006).

Moabi is also valued for the edible oil extracted from the seeds and the medicinal properties of its bark. The oil (locally known as “huile de karité”) is of great importance to local people because it is used as a substitute for palm oil in cooking and for the revenues derived from its sales. For instance, the seed oil can be worth as much as US\$12/liter (Schneemann 1995) in local markets in Cameroon, which is higher than the price for palm oil (US\$0.8–1/liter). The pulp of the fruit is eaten, and the bark used for medicinal and ethnobotanical purposes (Schneemann 1995).

Despite its importance and the threat represented by logging, *B. toxisperma* has no domestication history. The objective of this study was to investigate the interaction of two important postseverance factors (auxin application and leaf area) with rooting media (or substrate) on the rooting of leafy stem cuttings in Moabi for the development of a protocol for vegetative propagation of this species as a contribution to its domestication.

Materials and Methods

Stockplants

Stockplants were raised from seeds originating from three sites in the Haut-Nyong Division (East province) in Cameroon: (1) Abakoum (altitude: 634.7 m, 3°13'N 13°36'E); (1) Djébé (altitude: 693.6 m, 3°15'N 13°36'E); and (3) Djenou (altitude: 683.6 m, 3°16'N 13°36'E). Mature and healthy fruits were collected in September 2004, bulked and brought to and kept for 1 week in the World Agroforestry Centre nursery in Yaoundé (altitude: 700 m, 3°51'N 11°25'E), Cameroon, under ambient temperature (temperature varies between 23 and 25°C and relative humidity generally is between 73 and 85%) to enable decomposition of the mesocarp before seed extraction. Seeds were sown on a seedbed consisting of a 2:1 mixture of forest soil taken from the forest zone where the seeds were collected, and river sand in a wooden frame measuring 2 m × 1 m and enclosed in a double sheet of polythene such that the base was completely watertight. Nine hundred seeds were sown at 3- to 4-cm depth and were watered once a day in the morning using a watering can. Seedlings with at least two completely opened leaves were removed from the seedbed and potted in 2 or 4 dm³ black polythene bags filled with a 2:1 mixture of forest soil and sand. During 2 months, the plants were watered once a day to saturation. Seedlings within polythene bags were then taken out of the shade house and raised under natural shade provided by calliandra (*Calliandra calothyrsus* Meissn. [Fabaceae]) 3-year old plants that were pruned once in 3 months. No nutrients or fertilizer supply was provided for plant growth.

Table 1. Type III tests of fixed effects and contrasts of rooting percentage of Moabi leafy stem cuttings in a substrate × hormone × leaf area split-split plot experiment, at different weeks after inserting cuttings in nonmist propagators

| Effect | DF | Week 8 | | Week 10 | | Week 12 | | Week 14 | | Week 16 | | Week 22 | |
|------------------------|-----|--------|-------|---------|-------|---------|--------|---------|--------|---------|-------|---------|-------|
| | | F | P | F | P | F | P | F | P | F | P | F | P |
| St | 2 | 10.1 | 0.027 | 16 | 0.012 | 49.3 | 0.0015 | 51.7 | 0.001 | 47.65 | 0.006 | 18.8 | 0.009 |
| H | 3 | 4.68 | 0.052 | 4.37 | 0.059 | 7.87 | 0.02 | 5.6 | 0.036 | 2.53 | 0.154 | 2.13 | 0.198 |
| St × H | 6 | 1.63 | 0.22 | 1.25 | 0.35 | 2.78 | 0.06 | 2.17 | 0.12 | 1.21 | 0.51 | 1.3 | 0.34 |
| La | 2 | 8.84 | 0.034 | 13.3 | 0.017 | 19.7 | 0.008 | 24 | 0.006 | 23.19 | 0.005 | 17.7 | 0.01 |
| H × La | 6 | 0.43 | 0.85 | 0.25 | 0.95 | 0.58 | 0.74 | 1.21 | 0.37 | 1.29 | 0.26 | 1.12 | 0.41 |
| St × La | 4 | 6.94 | 0.01 | 5.26 | 0.022 | 6.82 | 0.01 | 8.92 | 0.005 | 6.78 | 0.01 | 5.1 | 0.02 |
| St × H × La | 12 | 2.19 | 0.05 | 1.78 | 0.11 | 1.35 | 0.26 | 0.93 | 0.53 | 0.68 | 0.46 | 1.11 | 0.4 |
| Contrasts | | | | | | | | | | | | | |
| La lin | (1) | 16.4 | 0.015 | 24.2 | 0.008 | 31.6 | 0.005 | 33.8 | 0.004 | 34.79 | 0.003 | 27.4 | 0.006 |
| La quad | (1) | 1.29 | 0.32 | 2.32 | 0.2 | 7.83 | 0.049 | 14.3 | 0.019 | 11.59 | 0.026 | 7.9 | 0.05 |
| Mx vs. all | (1) | 1.47 | 0.29 | 2.46 | 0.19 | 10.5 | 0.032 | 11.7 | 0.027 | 8.95 | 0.23 | 1.92 | 0.24 |
| Sa vs. Saw | (1) | 18.7 | 0.012 | 29.5 | 0.006 | 88.6 | 0.0007 | 92.1 | 0.0007 | 86.34 | 0.002 | 35.7 | 0.004 |
| Mx vs. all and La lin | (1) | 1.81 | 0.215 | 3.23 | 0.11 | 2.32 | 0.17 | 3.54 | 0.096 | 1.14 | 0.26 | 0.83 | 0.39 |
| Mx vs. all and La quad | (1) | 1.80 | 0.216 | 0.16 | 0.7 | 0 | 0.97 | 0.11 | 0.75 | 0.38 | 0.46 | 0.08 | 0.79 |
| Sa vs. Sw and La lin | (1) | 23.8 | 0.001 | 17.6 | 0.003 | 23.5 | 0.001 | 30.5 | 0.0006 | 22.95 | 0.002 | 18.1 | 0.003 |
| Sa vs. Sw and La quad | (1) | 0.38 | 0.557 | 0.06 | 0.82 | 1.45 | 0.26 | 1.52 | 0.25 | 2.66 | 0.21 | 1.43 | 0.27 |

DF, degrees of freedom; St, substrate; H, hormone; La, leaf area; St × H, substrate × hormone interaction; H × La, hormone × leaf area interaction; St × La, substrate × leaf area interaction; St × H × La, substrate × hormone × leaf area interaction; La lin, leaf area linear; La quad, leaf area quadratic; Mx, 50:50 mixture of sand and sawdust; Mx vs. all, 50:50 mixture of sand and sawdust versus sand and sawdust; Sa, sand; Sw, sawdust; Sa vs. Sw, sand versus sawdust; Mx vs. all and La lin, 50:50 mixture of sand and sawdust versus sand and sawdust × leaf area linear; Mx vs. all and La quad, 50:50 mixture of sand and sawdust versus sand and sawdust × leaf area quadratic; Sa vs. Sw and La lin, sand versus sawdust × leaf area linear; Sa vs. Sw and La quad, sand versus sawdust × leaf area quadratic.

Propagation of Plant Material

After 3 months, cuttings were harvested for vegetative propagation. Young shoots of the current growth were cut off, misted with water from a knapsack sprayer, and placed in sealed polythene bags to prevent drying. To avoid physiological stress, the bags were rushed to the nursery where experiments were carried out.

As *B. toxisperma* stems rapidly lignify, bending the stem through 60–90° was used as a method to test the suitability of the material to be propagated. If it sprang back to its original position, it was suitable, but if it broke or remained limp, it was not used. Using a sharp blade, single node cuttings of 3- to 4-cm length were cut. Each cutting consisted of one single node with one fully expanded leaf, plus the full internode underneath, which was cut directly below a node. The numbers of cuttings taken per coppice shoot varied, depending on the number of nodes present and the juvenility of the vegetative material (Tchoundjeu and Leakey 1996, Mesén et al. 1997a). Cuttings were inserted in nonmist poly propagator beds using a wooden dipper to save the base of the cutting from any injury.

The nonmist poly propagators were constructed following a design based on that of Howland (1975), modified by Leakey et al. (1990). The propagators consisted of a wooden frame enclosed in clear polythene sheet so that the base of the propagator was watertight (3 × 1 × 1 m). Access to the propagator was provided by a closely fitting lid, which is also clad in polyethylene and is airtight. The base of the propagator was covered with a thin layer of fine river sand to prevent the polythene from being punctured by the stones followed by successive layers of small stones and gravel overlaid by the rooting medium (to a depth of 10 cm on top of gravel).

Assessments of rooting success were done weekly after the first 2 weeks by lifting the cuttings from the rooting medium. Each cutting was assessed weekly for rooting, number of roots, and cutting death until week 22 after insertion in the propagator. The proportion of rooted cuttings in a plot was calculated as the percentage of cuttings with a root ≥ 10 mm length, and the number of roots per cutting rooted were recorded. This experiment was carried out within 22 weeks.

Experimental Design

To evaluate the amenability of *B. toxisperma* to rooting, a total of 972 cuttings were harvested and set in three blocks of a split-split plot experimental design. Each main plot contained three rooting media (river sand, sawdust, and a 50:50 mixture of sand and sawdust), whereas four different hormone treatments (indole-3-acetic acid [IAA], indole-3-butyric acid [IBA], 1-naphthalene acetic acid [NAA] and control [alcohol]) were tested at the subplot level. At the sub-subplot level, three leaf areas (25, 50, and 75 cm²) were investigated. At each level, treatments were assigned at random to experimental units so as to have 3 rooting media × 4 hormones × 3 leaf areas × 3 blocks × 9 cuttings. Leaf area was determined using a set of templates cut from graph paper. After trimming the leaf to the correct size, auxins were applied to the cutting base as a 10-μl droplet containing 50 μg per cutting of IAA, IBA, or NAA dissolved in ethanol at 95% concentration. The control treatment was 10 μl of ethanol only. Immediately after the solution was applied, ethanol was quickly evaporated in a stream of cold air before insertion of cuttings in rooting media.

Statistical Analysis

Data were submitted to analysis of variance using the Statistical Analysis System (SAS Institute 2006). Dependent variables were rooting percentage and number of roots per rooted cutting. Treatment effects were considered fixed and block effects were assumed to be random, making the model mixed. Before statistical analysis, the homogeneity of residual variances was tested graphically using proc plot in SAS. The normality of experimental errors was tested using proc univariate in SAS. Because split-split plot experiments are expanded split plot experiments, analysis of variance was performed using the mixed procedure in SAS for modified split plot designs (Littell et al. 1996, p. 42) following the mathematical model,

$$Y_{ijkl} = \alpha + \mu_i + \pi_j + (\pi\mu)_{ij} + \delta_k + (\pi\delta)_{jk} + (\mu\delta)_{ki} + (\mu\pi\delta)_{ijk} + \gamma_l + (\pi\gamma)_{jl} + (\delta\gamma)_{lk}$$

Table 2. Effects of rooting medium × leaf area interaction on rooting percentages of Moabi leafy stem cuttings 22 weeks after inserting cuttings in nonmist propagators

| Treatments | DF | Estimate ± SE* | t value | Pr > t |
|--|----|----------------|---------|---------|
| Mixture × 25 cm ² leaf area interaction | 8 | 17.58 ± 6.890 | 2.55 | 0.0341 |
| Mixture × 50 cm ² leaf area interaction | 8 | 17.58 ± 6.890 | 2.55 | 0.0341 |
| Mixture × 75 cm ² leaf area interaction | 8 | 39.83 ± 6.890 | 5.78 | 0.0004 |
| Sand × 25 cm ² leaf area interaction | 8 | 31.48 ± 6.890 | 4.57 | 0.0018 |
| Sand × 50 cm ² leaf area interaction | 8 | 37.03 ± 6.890 | 5.37 | 0.0007 |
| Sand × 75 cm ² leaf area interaction | 8 | 75.94 ± 6.890 | 11.02 | <0.0001 |
| Sawdust × 25 cm ² leaf area interaction | 8 | 12.03 ± 6.890 | 1.75 | 0.1191 |
| Sawdust × 50 cm ² leaf area interaction | 8 | 9.25 ± 6.890 | 1.34 | 0.2163 |
| Sawdust × 75 cm ² leaf area interaction | 8 | 24.06 ± 6.890 | 3.49 | 0.0082 |

DF, degrees of freedom.

* Least square means.

$$\begin{aligned}
& + (\pi\delta\gamma)_{jkl} + (\mu\gamma)_{il} + (\mu\pi\gamma)_{ijl} + (\mu\delta\gamma)_{ikl} \\
& + e_{ijkl},
\end{aligned}
\tag{1}$$

where Y_{ijkl} is the average value of the dependent variable for the i th block, j th rooting medium, k th hormone, and l th leaf area; α is the overall mean; μ_i , π_j , and $(\pi\mu)_{ij}$ collectively represent main plot effects and correspond respectively to block, rooting medium, and main plot error; δ_k , $(\pi\delta)_{jk}$, $(\mu\delta)_{ki}$, and $(\mu\pi\delta)_{ijk}$ collectively represent subplot effects and correspond, respectively, to hormone, hormone \times substrate interaction, block \times hormone interaction, and block \times substrate \times hormone interaction; γ_l , $(\pi\gamma)_{jl}$, $(\delta\gamma)_{lk}$, $(\pi\delta\gamma)_{jkl}$, $(\mu\gamma)_{il}$, $(\mu\pi\gamma)_{ijl}$, $(\mu\delta\gamma)_{ikl}$, and e_{ijkl} collectively represent sub-subplot effects and correspond, respectively, to leaf area, substrate \times leaf area interaction, hormone \times leaf area interaction, substrate \times hormone \times leaf area interaction, block \times leaf area interaction, block \times substrate \times leaf area interaction, block \times hormone \times leaf area interaction, and the random error. Effects of rooting media, hormone, leaf area, and their interactions were assumed fixed, whereas effect of block and its interactions with tested factors yielding error terms at each level were considered random. Contrasts were used at the main plot level and polynomial (linear and quadratic) contrasts at the sub-subplot level for ranking, respectively. Contrasts were also used for ranking of different combinations of tested factors.

Results

Percentage of Cuttings Rooted

Convergence criteria were met for each data set, and type III tests of fixed effects are shown in Table 1. Significant ($P = 0.05$) substrate \times hormone \times leaf area interaction on rooting percentage was found at week 8. From week 10 to the end of the experiment, interaction of the three tested

factors was found to be nonsignificant ($P = 0.11$ – 0.4 at weeks 10 and 22, respectively). Highly significant ($P = 0.005$) and significant ($P = 0.01$ – 0.02) substrate \times leaf area interactions on rooting percentage were observed at week 14 and during the rest of experiment, respectively, with the best rooting percentage at week 22 being found in sand \times 75 cm²-treated leafy cuttings (Table 2). Substrate \times hormone interaction was not found to influence rooting percentage from week 8 ($P = 0.22$) to week 22 ($P = 0.34$). Also, hormone \times leaf area interaction did not increase rooting percentage from week 8 ($P = 0.85$) to week 22 ($P = 0.41$). A highly significant substrate effect on rooting percentage was noted from week 12 ($P = 0.0015$) to week 22 ($P = 0.009$), and a significant effect of the same factor was observed from week 8 ($P = 0.027$) to week 10 ($P = 0.012$). Also, a highly significant effect of leaf area on rooting percentage was observed from week 12 ($P = 0.008$) to week 20 ($P = 0.005$), whereas a significant effect of the same factor was noted at week 8 ($P = 0.034$), week 10 ($P = 0.017$), and week 22 ($P = 0.01$). Effects of hormone on rooting percentage differed throughout the experiment (Figure 1). From week 8 to week 14, hormone had significant ($P = 0.052$ and $P = 0.036$) effects and no significant effects from week 16 ($P = 0.154$) to week 22 ($P = 0.198$) on rooting percentage of Moabi leafy stem cuttings.

Sand versus sawdust contrast \times IBA versus IAA and NAA contrast \times quadratic components of leaf area interaction had significant effects on rooting percentage of Moabi leafy stem cuttings at week 10 ($P = 0.0462$), week 12 ($P = 0.0365$), and week 14 ($P = 0.038$), respectively, and the highest rooting percentage at week 14 ($85.2 \pm 7.24\%$) was identified in 75 cm²-treated cuttings receiving IBA and inserted in sand (Table 3). The interaction between the linear components of leaf area and sand versus sawdust contrast was highly significant (Table 1) throughout the experiment, with sand yielding the best rooting percentage

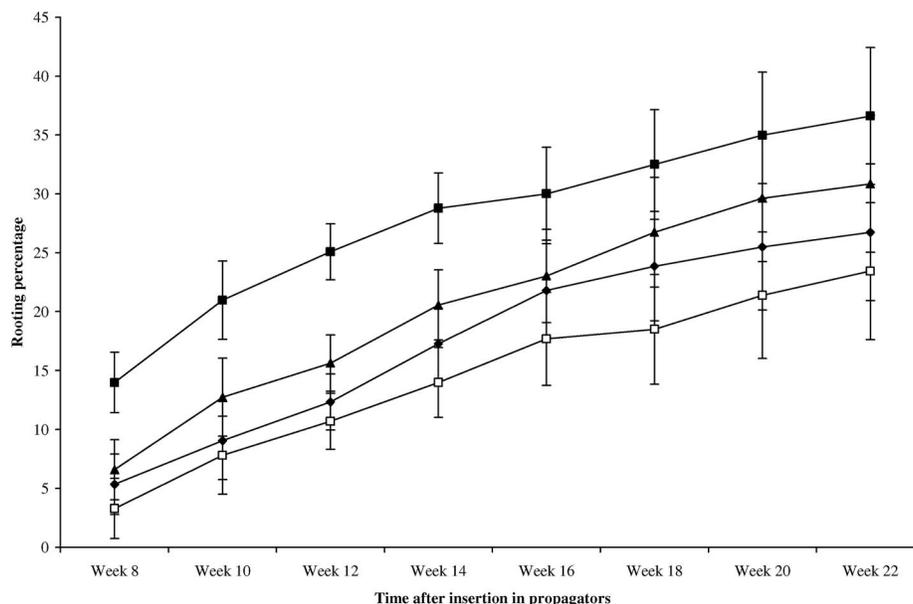


Figure 1. Effects of hormone on rooting percentage of Moabi leafy stem cuttings in nonmist propagators. □, IAA; ■, IBA; ▲, NAA; ◆, alcohol (or control). Error bars indicate ± 1 SE.

Table 3. Effects of sand versus sawdust contrast \times IBA versus IAA and NAA contrast \times leaf area interaction on rooting percentage of Moabi leafy stem cuttings 14 weeks after insertion in nonmist propagators

| Treatments | DF | Estimate \pm SE* | <i>t</i> value | Pr > <i>t</i> |
|--|----|--------------------|----------------|---------------|
| Sand \times IAA \times 25 cm ² leaf area | 24 | 14.8 \pm 7.27 | 2.03 | 0.0531 |
| Sand \times IAA \times 50 cm ² leaf area | 24 | 18.5 \pm 7.27 | 2.54 | 0.0178 |
| Sand \times IAA \times 75 cm ² leaf area | 24 | 44.5 \pm 7.27 | 66.11 | <0.0001 |
| Sand \times IBA \times 25 cm ² leaf area | 24 | 48.1 \pm 7.27 | 6.62 | <0.0001 |
| Sand \times IBA \times 50 cm ² leaf area | 24 | 33.3 \pm 7.27 | 4.58 | 0.0001 |
| Sand \times IBA \times 75 cm ² leaf area | 24 | 85.2 \pm 7.27 | 11.71 | <0.0001 |
| Sand \times NAA \times 25 cm ² leaf area | 24 | 18.50 \pm 7.27 | 2.54 | 0.0178 |
| Sand \times NAA \times 50 cm ² leaf area | 24 | 37 \pm 7.27 | 5.09 | <0.0001 |
| Sand \times NAA \times 75 cm ² leaf area | 24 | 59.3 \pm 7.27 | 8.15 | <0.0001 |
| Sawdust \times IAA \times 25 cm ² leaf area | 24 | 4.7E-14 \pm 7.27 | 0 | 1 |
| Sawdust \times IAA \times 50 cm ² leaf area | 24 | 3.3E-14 \pm 7.27 | 0 | 1 |
| Sawdust \times IAA \times 75 cm ² leaf area | 24 | 11.1 \pm 7.27 | 1.53 | 0.14 |
| Sawdust \times IBA \times 25 cm ² leaf area | 24 | 18.5 \pm 7.27 | 2.54 | 0.0178 |
| Sawdust \times IBA \times 50 cm ² leaf area | 24 | 7.4 \pm 7.27 | 1.02 | 0.3192 |
| Sawdust \times IBA \times 75 cm ² leaf area | 24 | 7.4 \pm 7.27 | 1.02 | 0.3192 |
| Sawdust \times NAA \times 25 cm ² leaf area | 24 | 11.1 \pm 7.27 | 1.53 | 0.14 |
| Sawdust \times NAA \times 50 cm ² leaf area | 24 | 4.4E-14 \pm 7.27 | 0 | 1 |
| Sawdust \times NAA \times 75 cm ² leaf area | 24 | 18.5 \pm 7.27 | 2.54 | 0.0178 |

DF, degrees of freedom.

* Least square means. E, exponential.

when increasing leaf area (Figure 2). Sand versus sawdust contrast and linear and quadratic components of leaf area had highly significant and significant (Table 1) effects on rooting percentage of Moabi leafy stem cuttings at week 22, respectively. Sand versus sawdust contrast \times quadratic components of leaf area interaction did not significantly influence rooting percentage in Moabi leafy stem cuttings throughout the experiment (Table 1), although the overall interaction between rooting medium and leaf area on rooting percentage was significant from week 10 to week 22 (Table 1). Also, the 50:50 mixture of sand and sawdust versus sand and sawdust contrast \times linear components of leaf area and \times quadratic components of leaf area interactions had no effects (Table 1) on rooting percentage of

Moabi leafy stem cuttings, respectively. The 50:50 mixture of sand and sawdust was significantly different from sand and sawdust from week 12 ($P = 0.032$) to week 16 ($P = 0.04$) with regard to rooting percentage of Moabi leafy stem cuttings, and no significant differences (Table 1) were noted between these treatments during the rest of the experiment.

Number of Roots

Convergence criteria were met for each data set, and type III tests of fixed effects are shown in Table 4. Interacting substrate \times leaf area significantly ($P = 0.048$) influenced the number of roots per rooted cutting in Moabi at week 22. Substrate \times hormone \times leaf area and substrate \times hormone

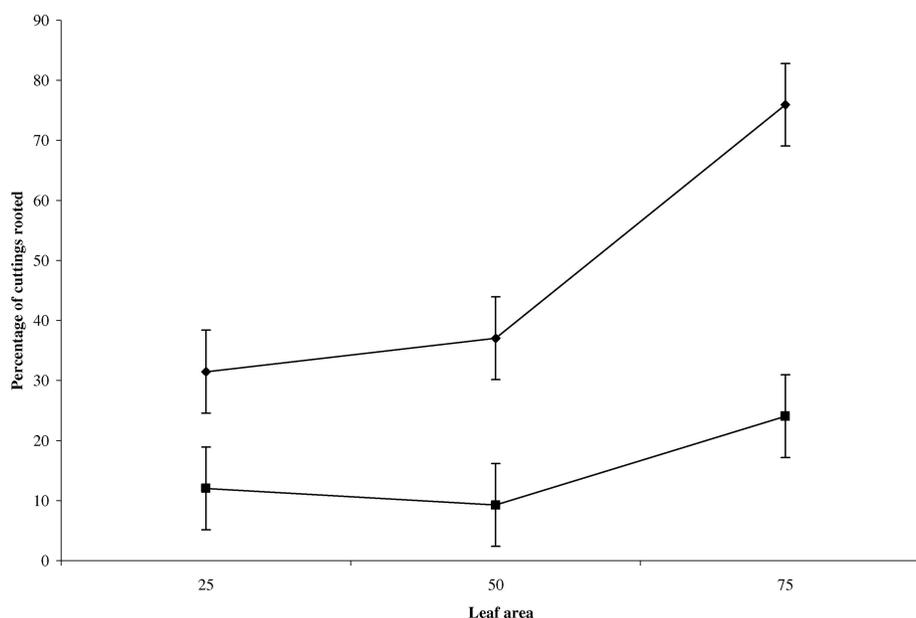


Figure 2. Effects of different rooting media (or substrate) \times leaf area interaction on rooting percentage of Moabi leafy stem cuttings at week 22 after inserting cuttings in the nonmist propagator (leaf areas are in cm²) \blacklozenge , sand; \blacksquare , sawdust. Error bars indicate ± 1 SE.

Table 4. Type III tests of fixed effects and contrasts of number of roots per rooted cutting of Moabi leafy stem cuttings in a substrate × hormone × leaf area split-split plot experiment, at different weeks after inserting cuttings in nonmist propagators

| Effect | DF | Week 8 | | Week 10 | | Week 12 | | Week 14 | | Week 16 | | Week 22 | |
|---------------------------|-----|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| | | <i>F</i> | <i>P</i> |
| St | 2 | 5.3 | 0.074 | 6.1 | 0.060 | 25 | 0.005 | 12.8 | 0.018 | 10.1 | 0.027 | 5.05 | 0.08 |
| H | 3 | 3.6 | 0.085 | 3.5 | 0.088 | 3.6 | 0.086 | 1.2 | 0.4 | 0.8 | 0.54 | 0.74 | 0.56 |
| St × H | 6 | 1.4 | 0.28 | 0.5 | 0.828 | 0.34 | 0.9 | 0.6 | 0.73 | 0.6 | 0.71 | 0.97 | 0.48 |
| La | 2 | 9.5 | 0.03 | 7.3 | 0.046 | 5.9 | 0.064 | 4.8 | 0.086 | 3.3 | 0.14 | 1.86 | 0.27 |
| H × La | 6 | 1.2 | 0.35 | 1.3 | 0.334 | 1.5 | 0.25 | 0.9 | 0.52 | 0.9 | 0.50 | 0.76 | 0.61 |
| St × La | 4 | 1.9 | 0.2 | 0.8 | 0.54 | 0.7 | 0.61 | 1.4 | 0.32 | 1.2 | 0.39 | 3.91 | 0.048 |
| St × H × La | 12 | 1.4 | 0.22 | 1.2 | 0.315 | 0.9 | 0.59 | 0.7 | 0.73 | 0.5 | 0.88 | 1.57 | 0.17 |
| La lin | (1) | 18 | 0.013 | 13 | 0.023 | 8.4 | 0.044 | 6.4 | 0.065 | 5.6 | 0.08 | 2.52 | 0.19 |
| La quad | (1) | 0.6 | 0.47 | 1.7 | 0.27 | 3.3 | 0.14 | 3.2 | 0.15 | 1.1 | 0.36 | 1.2 | 0.33 |
| Mx vs. all | (1) | 2.2 | 0.21 | 2.6 | 0.18 | 12.6 | 0.024 | 4.1 | 0.114 | 2.3 | 0.21 | 0.03 | 0.86 |
| Sa vs. Saw | (1) | 8.5 | 0.044 | 9.6 | 0.04 | 38 | 0.003 | 21.5 | 0.01 | 18 | 0.013 | 10.06 | 0.034 |
| Saw Mx vs. all and La lin | (1) | 1.6 | 0.24 | 1.3 | 0.276 | 0.03 | 0.88 | 0.5 | 0.52 | 0.3 | 0.57 | 4.27 | 0.07 |
| Mx vs. all and La quad | (1) | 0.9 | 0.37 | 0.17 | 0.69 | 0.13 | 0.73 | 0.02 | 0.9 | 0.3 | 0.58 | 3.21 | 0.11 |
| Sa vs. Sw and La lin | (1) | 4.9 | 0.058 | 0.01 | 0.92 | 0.08 | 0.78 | 0.6 | 0.45 | 0.7 | 0.43 | 1.87 | 0.21 |
| Sa vs. all and La quad | (1) | 0.2 | 0.64 | 1.78 | 0.22 | 2.6 | 0.15 | 4.5 | 0.068 | 3.3 | 0.11 | 6.29 | 0.036 |

DF, degrees of freedom; St, substrate (or rooting medium); H, hormone; La, leaf area; St × H, substrate × hormone interaction; H × La, hormone × leaf area interaction; St × La, substrate × leaf area interaction; Sr × H × La, substrate × hormone × leaf area interaction; La lin, leaf area linear; La quad, leaf area quadratic; Mx, 50:50 mixture of sand and sawdust; Mx vs. all, 50:50 mixture of sand and sawdust versus sand and sawdust; Sa, sand; Sw, sawdust; Sa vs. Sw, sand versus sawdust; Mx vs. all and La lin, 50:50 mixture of sand and sawdust versus sand and sawdust × leaf area linear interaction; Mx vs. all and La quad, 50:50 mixture of sand and sawdust versus sand and sawdust × leaf area quadratic interaction; Sa vs. Sw and La lin, sand versus sawdust × leaf area linear interaction; Sa vs. Sw and La quad, sand versus sawdust × leaf area quadratic interaction.

and hormone × leaf area interactions did not significantly influence the number of roots per rooted cutting in Moabi (Table 4), respectively. Also, no substrate × leaf area interaction on number of roots per rooted cutting was identified from week 8 ($P = 0.2$) to week 20 ($P = 0.07$). Rooting medium had highly ($P = 0.005$) significant effects at week 12 and significant effects at week 14 ($P = 0.018$), week 16 ($P = 0.027$), week 18 ($P = 0.04$), and week 20 ($P = 0.049$) on number of roots per rooted cutting in Moabi, respectively. Leaf area significantly increased the number of roots per rooted cutting from week 8 ($P = 0.03$) to week 10 ($P = 0.046$), whereas no effects of this factor on number of roots

were noted from week 12 ($P = 0.064$) to week 22 ($P = 0.27$). Hormone application had no effects (Table 4) on the number of roots per rooted cutting throughout the experiment.

Sand versus sawdust contrast × quadratic components of leaf area interaction had significant effects on the number of roots per rooted cutting in Moabi leafy stem cuttings from week 20 ($P = 0.046$) to week 22 ($P = 0.036$), respectively, and 50 cm²-treated cuttings in sawdust yielded the lowest number of roots per rooted cuttings at week 22 (Figure 3), whereas the highest number of roots per rooted cutting was observed in 75

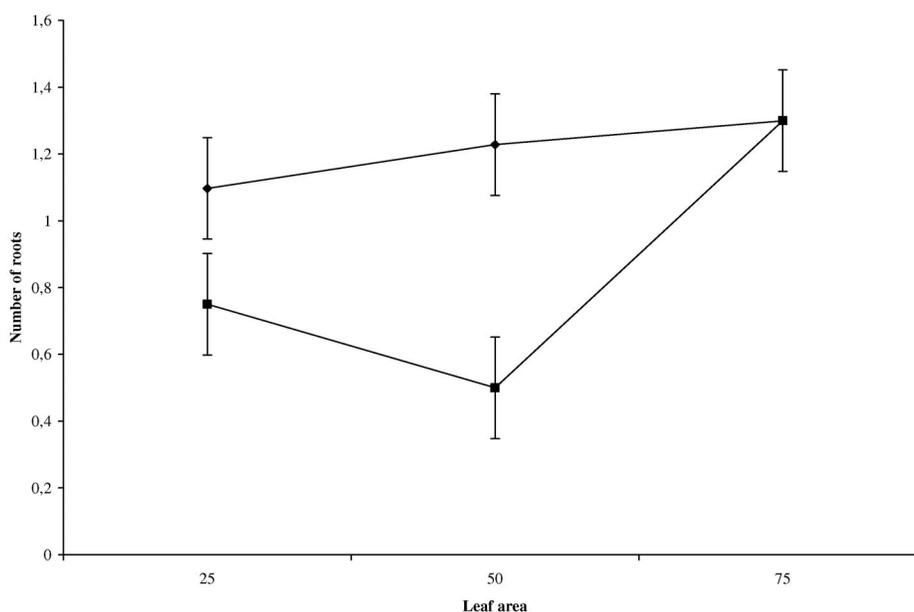


Figure 3. Effects of sand versus sawdust contrast × quadratic component of leaf area on the number of roots of Moabi leafy stem cuttings (leaf areas are in cm²). ♦, sand; ■, sawdust. Error bars indicate ±1 SE.

cm²-treated leafy stem cuttings in sawdust or in sand (Table 5) at week 22. Sand versus sawdust contrast had significant ($P = 0.034$) effects on the number of roots per rooted cutting in Moabi at week 22, with cuttings inserted in sand yielding more roots (1.205 ± 0.099) than those inserted in sawdust (0.85 ± 0.099). Sand versus sawdust contrast \times linear components of leaf area interaction, the 50:50 mixture of sand and sawdust versus sand and sawdust contrast \times linear components of leaf area interaction, and the 50:50 mixture of sand and sawdust versus sand and sawdust contrast \times quadratic components of leaf area interaction had no significant effects ($P = 0.21$, $P = 0.07$, and $P = 0.11$, respectively) on the number of roots per rooted cutting in Moabi at week 22, respectively. Also, the 50:50 mixture of sand and sawdust versus sand and sawdust contrast and linear and quadratic components of leaf area had no significant ($P = 0.86$, $P = 0.19$, and $P = 0.33$, respectively) effects on the number of roots per rooted cutting in Moabi at week 22.

Discussion

Rooting of leafy stem cuttings seems to be affected by a combination of factors affecting root formation (Dick and Dewar 1992, Atangana et al. 2006). In *B. toxisperma*, an increase in rooting percentage and mean number of roots per rooted cutting was observed when combining rooting medium and leaf area. Interacting clone and rooting medium in *Allanblackia* (*Allanblackia floribunda* Oliv. [Clusiaceae]; Atangana et al. 2006), hormone and species, and hormone and provenance within species in acacias (*Racosperma auriculiforme* Cunn ex Benth. [Fabaceae] and *Racosperma mangium* [Willd.] Pedley [Fabaceae]; Khasa et al. 1995) also enhanced rooting percentage in cuttings. Results from this study appear to confirm the hypothesis that higher rooting percentages can be achieved when factors affecting rooting are optimized (Atangana et al. 2006).

Moabi leafy stem cuttings were found to root well as 75 cm²-treated cuttings receiving IBA and inserted in sand. Sand was also identified as the best rooting medium for *Cordia alliodora* (Ruiz & Pavon) Oken (Boraginaceae; Leakey et al. 1990), *Garcinia kola* Heckel (Clusiaceae; Nyansi 2004), and *A. floribunda* (Atangana et al. 2006) leafy stem cuttings. In contrast, high rooting percentages were identified in water-retentive sawdust by Mialoundama et al. (2002) and Tchoundjeu et al. (2002, 2004) for safou

(*Dacryodes edulis* [G. Don] H.J. Lam [Burseraceae]), pygeum (*Prunus africana* [Hook. f.] Kalkman [Rosaceae]), and yohimbe (*Pausinystalia johimbe* [K. Schum] Pierre ex Beille [Rubiaceae]), respectively. Between-species differences in rooting ability are explained by their hydromorphic or xeromorphic status (Loach 1985), and the effects of this on the water relations of the cuttings (Mesén et al. 1997a). An appropriate rooting medium generally has an optimal volume of gas-filled porespace and an oxygen diffusion rate adequate for the needs of respiration (Andersen 1986), and the poor rooting percentage recorded for Moabi cuttings in sawdust may reflect anoxia associated with high water contents.

Softwood cuttings have to produce assimilates faster than they are losing them through respiration to root (Mesén et al. 1997b), explaining the influence of leaf area on rooting of leafy stem cuttings (Shiembo et al. 1996, Tchoundjeu and Leakey 1996, Tchoundjeu et al. 2002, 2004, Nyansi 2004). Cuttings from tropical hardwood species root well at an optimum leaf area (25–50 cm² for *G. kola*, 50 cm² for yohimbe and Ayous, and 200 cm² for bibolo (*Lovoa trichiloides* Harms [Meliaceae]), respectively; Leakey et al. 1982, Tchoundjeu and Leakey 2001, Nyansi 2004, Tchoundjeu et al. 2004). The significant effect of sand versus sawdust contrast and linear components of leaf area on rooting percentage in Moabi indicated that an increase in leaf area might result in an increase in rooting percentage within the tested range of leaf areas, when sand or sawdust is used as the rooting medium. Significant effects of sand versus sawdust contrast \times IBA versus IAA and NAA contrast \times quadratic components of leaf area interaction in rooting percentage and of sand versus sawdust contrast \times quadratic components of leaf area interaction on the number of roots in Moabi leafy stem cuttings found in this study indicated that cuttings from Moabi have a lower rate of rooting at 50 cm², within the range of leaf areas tested. Further studies on the effects of leaf areas combined with other factors affecting rooting in Moabi leafy stem cutting using larger sizes are needed to clarify whether there is an optimum leaf area for rooting in Moabi.

Auxins are reported to play a significant role in stimulating root initiation in stem cuttings of woody plants (Tchoundjeu et al. 2002, 2004). Exogenously applied auxins did not improve rooting in *A. floribunda* cuttings that start

Table 5. Effects of rooting medium \times leaf area interaction on the number of roots of Moabi leafy stem cuttings at 22 weeks after inserting cuttings in nonmist propagators

| Treatments | DF | Estimate \pm SE* | <i>t</i> value | Pr > <i>t</i> |
|---|----|--------------------|----------------|---------------|
| Mixture \times 25 cm ² leaf area interaction | 8 | 1.056 \pm 0.152 | 6.95 | 0.0001 |
| Mixture \times 50 cm ² leaf area interaction | 8 | 1.11 \pm 0.152 | 7.31 | <0.0001 |
| Mixture \times 75 cm ² leaf area interaction | 8 | 0.96 \pm 0.152 | 6.35 | 0.0002 |
| Sand \times 25 cm ² leaf area interaction | 8 | 1.1 \pm 0.152 | 7.22 | <0.0001 |
| Sand \times 50 cm ² leaf area interaction | 8 | 1.233 \pm 0.152 | 8.08 | <0.0001 |
| Sand \times 75 cm ² leaf area interaction | 8 | 1.3 \pm 0.152 | 8.48 | <0.0001 |
| Sawdust \times 25 cm ² leaf area interaction | 8 | 0.75 \pm 0.152 | 4.94 | 0.0011 |
| Sawdust \times 50 cm ² leaf area interaction | 8 | 0.5 \pm 0.152 | 3.29 | 0.011 |
| Sawdust \times 75 cm ² leaf area interaction | 8 | 1.3 \pm 0.152 | 8.5 | <0.0001 |

DF, degrees of freedom.

* Least square means.

to root 10 weeks after insertion in the propagators (Atangana et al. 2006), which is unusual for leafy stem cuttings of tropical tree species (8–10 weeks, Leakey et al. 1982). Interestingly, cuttings from Moabi were influenced by auxin application from week 8 to week 14, with IBA yielding the best rooting percentages, as found in most of tropical tree species studied (Leakey 2004), and no effects were identified from week 16 to the end of experiment. Also, combining IBA with sand and 75-cm² leaf area at week 14 yielded a higher rooting percentage than 75 cm²-treated cuttings in sand at week 22, indicating that rooting of Moabi leafy stem cuttings should be done within 14 weeks, when IBA is applied. Results from this study raised questions about a possibly period of action of root-promoting substances in rooting of leafy stem cuttings (Hartmann et al. 2002, p. 295), and more studies are needed to clarify this.

Although found to be influenced by rooting medium × leaf area interaction, the average number of roots per rooted cuttings did not attain the value of 2, whereas Tchoundjeu et al. (2004) reported an increase in the number of roots in *P. johimbe* cuttings treated with IBA. Auxins were also reported to increase the number of roots initiated per rooted cutting in *Shorea leprosula* Miq. (Dipterocarpaceae; Aminah et al. 1995). Auxins affect cell differentiation and promote starch hydrolysis and the mobilization of sugars and nutrients to the cutting base (Leakey 2004), which influence the number of roots per rooted cutting. Auxins are also interactive with rooting cofactors (Hartmann et al. 2002, p. 299–300), explaining between-species variation of their effects.

This study indicated that Moabi is amenable to vegetative propagation through rooting of cuttings using low-cost technology techniques within 14 weeks. This is an incentive for farmers developing rural nurseries for the domestication of high-value multipurpose agroforestry tree species indigenous to West and Central Africa (Tchoundjeu et al. 2006) and local institutes in charge of reforestation programs, as Moabi is an important agroforestry and forestry tree species in the region. Further work is required to increase rooting percentage and number of roots per rooted cutting in Moabi through identification of optimal environmental conditions to achieve good rooting, optimum pre- and postseverance treatments, and optimal morphological and physiological condition of the cutting material (as affected by stockplant environment) (Dick et al. 2004, Leakey 2004). From this study, recommendations for how best to propagate Moabi through rooting of leafy stem cuttings in nonmist propagators include using sand as the rooting medium, applying a 10- μ l droplet containing 50 μ g of IBA dissolved in ethanol at 95% concentration to the cutting base, and trimming the leaf of each cutting to 75 cm², with the propagation period lasting within 14 weeks.

Literature Cited

- AMINAH H., J.McP. DICK, R.R.B. LEAKEY, J. GRACE, AND R.I. SMITH. 1995. Effect of indole butyric acid (IBA) on stem cuttings of *Shorea leprosula*. *For. Ecol. Manag.* 72:199–206.
- ANDERSEN, A.S. 1986. Stockplant conditions. P. 223–255 in *New root formation in plant and cuttings*, Jackson, M.B. (ed.). Martinus Nijhoff, Dordrecht, Netherlands.
- ATANGANA, A.R., V. UKAFOR, P. ANEGBEH, E. ASAAH, Z. TCHOUNDJEU, J.-M. FONDOUN, M. NDOUMBE, AND R.R.B. LEAKEY. 2002. Domestication of *Irvingia gabonensis*: 2. The selection of multiple traits for potential cultivars from Cameroon and Nigeria. *Agrofor. Sys.* 55(3):221–229.
- ATANGANA, A.R., Z. TCHOUNDJEU, E.K. ASAAH, A.J. SIMONS, AND D.P. KHASA. 2006. Domestication of *Allanackia floribunda*: Amenability to vegetative propagation. *For. Ecol. Manag.* 237:246–251.
- DICK, J.McP., AND R. DEWAR. 1992. A mechanistic model of carbohydrate dynamics during adventitious root development in leafy cuttings. *Ann. Bot.* 70:371–377.
- DICK, J.McP., R.R.B. LEAKEY, C. McBEATH, F. HARVEY, R.I. SMITH, AND C. WOODS. 2004. Influence of nutrient application rate on the growth and rooting potential of the West African hardwood *Triplochiton scleroxylon*. *Tree Physiol.* 24:35–44.
- HARTMANN, H.T., D.E. KESTER, F.T. DAVIES JR., AND R.L. GEN-EVE. 2002. *Plant propagation: Principles and practices*, 7th ed. Prentice Hall, Upper Saddle River, NJ. 880 p (with indexes).
- HOWLAND P. 1975. Vegetative propagation methods for *Triplochiton scleroxylon* K. Schum. P. 99–109 in *Proc. symp. on variation and breeding systems of Triplochiton scleroxylon K. Schum.* Federal Department of Forest Research, Ibadan, Nigeria.
- IUCN. 2006. *IUCN red list of threatened species*. Available online at www.iucnredlist.org; last accessed Mar. 14, 2007.
- KHASA, P.D., G. VALLÉE, AND J. BOUSQUET. 1995. Provenance variation in rooting ability of juvenile stem cuttings from *Racosperma auriculiforme* and *R. mangium*. *For. Sci.* 41(2):305–320.
- KUBOTA, C., AND T. KOZAI. 2001. Mathematical models for planning vegetative propagation under controlled environments. *Hortscience* 36(1):15–19.
- LEAKEY, R.R.B. 2004. Physiology of vegetative reproduction. P. 1655–1668 in *Encyclopaedia of forest sciences*, Burley J., E. Evans, and J.A. Youngist (eds.). Academic Press, London, UK.
- LEAKEY, R.R.B., F.T. LAST, AND K.A. LONGMAN. 1982. Domestication of tropical trees: An approach securing future productivity and diversity in managed ecosystems. *Commonw. For. Rev.* 61:33–42.
- LEAKEY, R.R.B., J.F. MESÉN, Z. TCHOUNDJEU, K.A. LONGMAN, J.McP. DICK, A. NEWTON, A. MATIN, J. GRACE, R.C. MUNRO, AND P.N. MUTHOKA. 1990. Low-technology techniques for the vegetative propagation of tropical trees. *Commonw. For. Rev.* 69(3):247–257.
- LITTELL, R.C., G.A. MILLIKEN, W.W. STROUP, AND R.D. WOLFINGER. 1996. *SAS for mixed models*, 6th printing. SAS Institute Inc., Cary, NC. 633 p.
- LOACH, K. 1985. Rooting of cuttings in relation to the propagation medium. *Proc. Int. Plant Propagators Soc.* 35:472–485.
- MESÉN, F., A.C. NEWTON, AND R.R.B. LEAKEY. 1997a. Vegetative propagation of *Cordia alliodora* (Ruiz and Pavon) Oken: The effects of IBA concentration, propagation medium and cutting origin. *For. Eco. Manag.* 92:45–54.
- MESÉN, F., A.C. NEWTON, AND R.R.B. LEAKEY. 1997b. The effects of propagation environment and foliar areas on the rooting physiology of *Cordia alliodora* (Ruiz & Pavon) Oken cuttings. *Trees* 11:401–411.
- MIALOUNDAMA, F., M.-L. AVANA, E. YOUNBI, P.C. MAMPOUYA, Z. TCHOUNDJEU, M. MBEUYO, G.R. GALAMO, J.M. BELL, F. KOPGUEP, A.C. TSOBENG, AND J. ABEGA. 2002. Vegetative propagation of *Dacryodes edulis* (G. Don) H.J. Lam by marcots, cuttings and micropropagation. *For. Trees Livelihood.* 12:85–96.
- NYANSI, H.A.D. 2004. *Multiplication végétative de Garcinia kola*

- Heckel: Effet du substrat de propagation et de la surface foliaire sur la rhizogénèse des boutures de tige. Mémoire présenté en vue de l'obtention du diplôme d'Ingénieur des Eaux, Forêts et Chasse. FASA, Université de Dschang, Dschang, Cameroun. 52 p.
- SAS INSTITUTE INC. 2006. *SAS 9.1.3 service pack 4 for Windows*. Cary, NC.
- SCHNEEMANN, J. 1995. Exploitation of Moabi in the humid dense forest of Cameroon: Harmonization and improvement of two conflicting ways of exploitation of the same resource. *Bos. Newsl.* 31/14(2):20–32.
- SHIEMBO, P.N., A.C. NEWTON., AND R.R.B. LEAKEY. 1996. Vegetative propagation of *Irvingia gabonensis*, a West African fruit tree. *For. Ecol. Manag.* 87:185–196.
- SIMONS, A.J., AND R.R.B. LEAKEY. 2004. Tree domestication in tropical agroforestry. *Agrofor. Syst.* 61:167–181.
- TCHOUNDJEU, Z., E.K. ASAAH, P. ANEGBEH, A. DEGRANDE, P. MBILE, C. FACHEUX, A. TSOBENG, A.R. ATANGANA, M.L. NGO MPECK, AND A.J. SIMONS. 2006. Putting participatory domestication into practice in West and Central Africa. *For. Trees Livelihood.* 16:53–69.
- TCHOUNDJEU, Z., M.-L. AVANA, R.R.B. LEAKEY, A.J. SIMONS, E. ASAAH, B. DUGUMA, AND J.M. BELL. 2002. Vegetative propagation of *Prunus africana*: Effects of rooting medium, auxin concentrations and leaf area. *Agrofor. Syst.* 54:183–192.
- TCHOUNDJEU, Z., AND R.R.B. LEAKEY. 1996. Vegetative propagation of African mahogany: Effects of auxin, node position, leaf area and cutting length. *New For.* 11:125–136.
- TCHOUNDJEU, Z., AND R.R.B. LEAKEY. 2001. Vegetative propagation of *Lovoa trichilioides*: Effects of provenance, substrate, auxins and leaf area. *J. Trop. For. Sci.* 13(1):116–129.
- TCHOUNDJEU, Z., M.-L. NGO MPECK, E. ASAAH, AND A. AMOUGOU. 2004. The role of vegetative propagation in the domestication of *Pausinystalia johimbe* (K. Schum), a highly threatened medicinal species of West and central Africa. *For. Ecol. Manag.* 188:175–183.
- UNEP-WCMC. 2006. *Tree conservation information service*. Available online at www.unep-wcmc.org/trees/trade/bai_tox.htm; last accessed Jan. 30, 2007.
- VIVIEN, J., AND J.J. FAURE. 1996. *Fruitières sauvages d'Afrique (espèces du Cameroun)*. Nguila-Kerou, Clohars Carnoet, France. 416 p.
- WHITE, F. 1983. The vegetation of Africa, a descriptive memoir to accompany the UNESCO/AETFAT/UNSO vegetation map of Africa. *UNESCO Nat. Resour. Res.* 20:1–35.