Advances in mass clonal propagation of *Eucalyptus* urophylla x E. grandis in Congo

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The advantages of the new technique developed for efficiently propagating by rooted cuttings *E. urophylla x E. grandis* artificial clones in Pointe Noire, Congo, are obvious: higher rooting rates, better overall quality of the cutting-issued plants produced in much shorter delays with easier and cheaper maintenance. At the research level, the quality and reliability of the field clonal tests established from such rooted cuttings are greatly improved while the time needed for wise clonal selection and for effective genetic improvement can be considerably shortened.



Large scale propagation of *Eucalyptus urophylla x E. grandis* clones using intensively managed container-grown stock plants in Eucalyptus Fibre Congo (EFC) facilities, Pointe Noire, Congo.

Photo O. Monteuuis.

RÉSUMÉ

NOUVELLE TECHNIQUE DE PROPAGATION CLONALE INDUSTRIELLE POUR LES EUCALYPTUS UROPHYLLA X E. GRANDIS AU CONGO

La technique mise au point en 1978 à Pointe-Noire, au Congo, pour bouturer les clones d'eucalyptus hybrides naturels Eucalyptus PF1 et E. 12 ABL x E. saliqna s'est avérée inadaptée au bouturage des hybrides artificiels E. urophyla x E. grandis. En effet, seule une faible proportion des pousses récoltées sur les souches au champ après abattage et utilisées comme pieds mères extensifs s'enracinait. Les boutures difficilement produites montraient en outre une grande variabilité intraclonale qui s'avérait préjudiciable non seulement à la qualité mais aussi à la productivité des plantations résultantes. Le fait de remplacer les souches utilisées comme pieds mères par des pieds mères véritables cultivés de façon intensive en conteneurs permit d'améliorer considérablement la capacité au bouturage des nouveaux clones hybrides artificiels, ainsi que la qualité des boutures produites plus rapidement et à moindre coût. Les avantages de cette nouvelle technique sont manifestes. En matière de recherche, la qualité et la fiabilité des tests clonaux établis à partir de ces nouvelles boutures ont pu être grandement améliorées, permettant de sélectionner les nouveaux clones de façon plus rigoureuse dans des délais plus courts pour le plus grand profit de l'amélioration génétique. Les bénéfices sont encore plus flagrants sur le plan opérationnel pour la production efficiente à l'échelle industrielle de boutures destinées à alimenter des plantations clonales d'envergure, de qualité supérieure et homogène, tout en garantissant une forte productivité. Ces aspects sont discutés dans cet article, axé sur les particularités de cette nouvelle technique de bouturage industriel des clones d'E. urophylla x E. grandis.

Mots-clés: bouture, clonage, enracinement adventif, eucalyptus, gestion des pieds mères, pied mère hors-sol, propagation clonale industrielle.

ABSTRACT

ADVANCES IN MASS CLONAL PROPAGATION OF EUCALYPTUS UROPHYLLA X E. GRANDIS IN CONGO

The basic technique developed in Pointe Noire, Congo, since 1978 for propagating by cuttings the natural Eucalyptus PF1 and E. 12 ABL x E. saligna hybrid clones did not worked satisfactorily on the E. urophylla x E. grandis artificial hybrid clones. Only a small proportion of the cuttings collected from the field coppicing stumps used as extensive stock plants could be rooted. Moreover, the resulting plants demonstrated an unexpectedly high within clone variability detrimental to final crop quality and yield. Replacing such stumpderived stock plants by intensively managed container-grown ones resulted in a noticeable enhancement of cutting capacity for adventitious rooting as well as of the overall quality of the plants produced in much shorter delays with easier and cheaper maintenance. The relevant advantages of this new technique are obvious. At the research level, the quality and reliability of the field clonal tests established from such rooted cuttings are greatly improved while the time needed for wise clonal selection and for effective genetic improvement can be considerably shortened. The benefits are even greater for big scale operations, such as mass production of clonal offspring for large size clonal plantations of uniform superior quality. These aspects are argued in the current paper, focusing on the particularities of the new technique developed for efficiently mass producing by rooted cuttings E. urophylla x E. grandis clones.

Keywords: adventitious rooting, cloning, container-grown stock plant, eucalypt, mass clonal propagation, rooted cutting, stock plant management.

RESUMEN

NUEVA TÉCNICA DE PROPAGACIÓN CLONAL INDUSTRIAL PARA LOS EUCALYPTUS UROPHYLLA X E. GRANDIS EN EL CONGO

La técnica puesta a punto en 1978 en ' Pointe Noire, Congo, para la propagación por estacas de los clones de eucalipto híbridos naturales Eucalyptus PF1 y E. 12 ABL x E. saligna se reveló inadaptada para el estaquillado de los híbridos artificiales E. urophyla x E. grandis. En efecto, sólo una pequeña proporción de vástagos, cosechados en las cepas en el campo tras apeo y empleados como plantas madre extensivas, lograba arraigarse. Las estacas, de difícil producción, mostraban además una gran variabilidad intraclonal que perjudica la calidad y productividad de las plantaciones resultantes. El hecho de sustituir las cepas utilizadas como plantas madre por verdaderas plantas madre, cultivadas intensivamente en contenedores, permitió mejorar considerablemente la capacidad de estaquillado de los nuevos clones híbridos artificiales, así como la calidad de las estacas, de producción más rápida y barata. Las ventajas de esta nueva técnica son evidentes. En lo relativo a la investigación, la calidad y fiabilidad de las pruebas clonales desarrolladas a partir de estas nuevas estacas se pudieron mejorar bastante, esto permitió seleccionar los nuevos clones de forma más rigurosa y con plazos más cortos, favoreciendo así el mejoramiento genético. Los beneficios son aún más patentes a nivel operativo para la producción eficiente, a escala industrial, de estacas de calidad superior y homogénea para abastecer plantaciones clonales de importancia, garantizando al mismo tiempo una elevada productividad. Estos aspectos se analizan en este artículo, centrado en las peculiaridades de esta nueva técnica de estaquillado industrial de los clones de E. urophylla x E. grandis.

Palabras clave: estaca, clonación, enraizamiento adventicio, eucalipto, manejo de plantas madre, planta madre sin tierra, propagación clonal industrial.

Introduction

The striking growth superiority of the natural eucalypt hybrid 12 ABL x E. saligna and PF1 clones in comparison with pure eucalyptus species locally introduced was demonstrated for the first time during the 1960s in Pointe Noire, Congo (Chaperon, Quillet, 1977; Vigneron et al., 2000). This has accounted for the development of suitable nursery techniques for clonally propagating true-to-type by rooted cuttings the relevant natural eucalypt hybrids selected (Martin, Quillet, 1974; Chaperon, Quillet, 1977).

Since the late 1980s, artificial Eucalyptus urophylla x Eucalyptus grandis hybrid clones derived from a comprehensive genetic improvement program have been progressively replacing the former 12 ABL x E. saligna and PF1 clones used till then (VIGNERON, 1992; VIGNERON et al., 2000). These new hybrids, although superior in terms of yield and technological properties, did not respond however so satisfactorily to the mass clonal propagation methods developed for the 12 ABL x E. saligna and PF1 clones. With a view to improve this situation, special efforts were made in the early 1990s to intensify the cultivation and management of the field established stock plants (BASSET CANDEAU, 1993; LACLAU, BOUVET, 1994), but the results remained far below the expectations with too low rooting rates and a substantial variation in phenotypic conformity among the resulting clonal offspring. More consideration was therefore given to the possibility of using cuttings produced by container-grown stock plants instead of field established ones (MARIEN, SAYA, 2003), as successfully experimented with many different arborescent species in temperate and tropical countries (Monteuuis, 1993; MONTEUUIS et al., 1987 and 1995). This paper reports on the development of this technology for efficiently mass propagating E. urophylla x E. grandis selected clones at the Unité de Recherche sur la Productivité des Plantations Industrielles, "UR2PI" for short, in Pointe Noire.

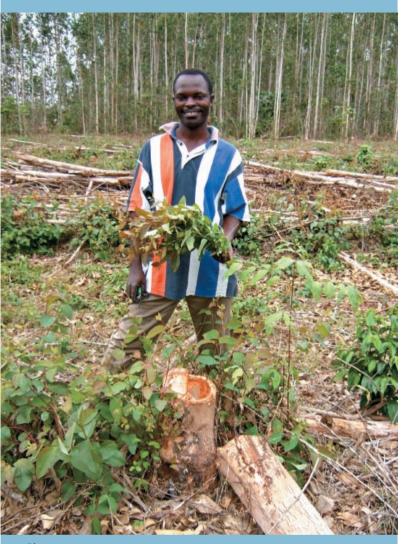


Photo 1.Collection of shoots from coppicing stumps in the field to produce BO rooted cuttings.
Photo O. Monteuuis.

Getting the first generation of rooted cuttings: the mobilization phase

The first and determining step of the technique used for clonally propagating by rooted cuttings field selected *E. urophylla x E. grandis* hybrids consists in getting the first generation of vegetative copies from the field growing selected ortets. This so-called "mobilization" phase, also referred to as "BO" generation (MONTEUUIS, 1993), results from the

rooting in suitable nursery conditions of nodal cuttings trimmed from 50 to 70 cm long sprouting coppices. These are produced by 25-35 cm high stumps of 3 year-old field growing trees, established at a density of 800 trees/ha (photo 1), 45 days on average after these latter had been felled, as described by MARTIN and QUILLET (1974).

These coppicing shoots, comprising 5 to 6 long internodes, are brought immediately after field collection to the nursery to be trimmed into cuttings. The reddish pigmentation of certain of these shoots reflects their strong vigour and has been observed, in eucalypts as well as in different

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Photo 2.
A BO nodal cutting trimmed from a stump coppice.
Photo O. Monteuuis.

other species, to be associated with a poor rooting ability (Monteuuis, 1993; MONTEUUIS et al., 1995). Only the semi-lignified middle portion of this coppice is used to make by cutting just above a node 1 or 2 cuttings of 8 to 10 cm as overall average length. These cuttings consist of a 5 to 8 cm long basal internode and two opposite half leaves separated by a second internode of varying length – it can be only 1cm long. Half of the surface of these 2 to 4 cm-long leaves is removed to reduce evapotranspiration and water stress risks, as illustrated in photo 2 and photo 6 b.

The so-prepared two-node cuttings are then soaked in an aqueous fungicide solution of 1.5ml/l of Banko Plus (550 g/l Chlorothalonil + 100 g/l Carbendazime) before dipping their base into a 0.8% powder concentration of Indole Butyric Acid "Chryzoplus" and insertion into 34 x 34 cm-wide by 9 cm-deep SAPPI trays consisting of $7 \times 7 = 49$ alveolar cells of 50 ml each (photo 3), filled with a rooting substrate made of 8 volumes of vermiculite and 2 volumes of perlite. As soon as completed with the 49 cuttings, the trays are transferred for 30 days to the rooting area, on benches at around 80cm above ground level, under 50% shade and



Photo 3.Rooting of BO generation nodal cuttings in Sappi trays. Photo O. Monteuuis.

mist water sprays maintained at 8 seconds every minute during the 6 hours following the transfer, to prevent hydrous stress. Spray regime is reduced to 15 seconds every 10 mn for the next 14 days, and finally to 15 seconds every hour for the 15 last days of the rooting phase, from 9 till 16 o'clock. During this rooting period, fertilization consists of weekly applications of 0.06g of NPK 1-1-5 in a granular formulation and 0.09 g of "Hakaphos Rouge" (NPK 8-12-24, + 4% magnesia and oligonutrients) in aqueous solution per cutting. Water solutions of fungicide -Banko Plus 1.5 ml/l - and insecticide - 10 ml/l of Cypercal 50 EC (50Cyperméthrine 50 g/l) - are also sprayed every week on the cuttings.

At the end of the 30 day-long rooting period, the misting regime is reduced to 15 seconds every two hours for 4 days, prior to transfer of the cuttings in the SAPPI trays for 45 days to acclimatizing conditions. These consist in exposing the cuttings to full natural light by totally removing the shading clothe installed over the rooting facilities, and to reduce the watering to 15 mn one to twice a day depending on the local climatic conditions to prevent water stress. During this acclimatization phase, feeding is

restricted to 5 g of NPK 13-13-21 provided in granular formulation per cutting, while fungicide and insecticide treatments remain the same as for the rooting phase.

The two-node cuttings trimmed from field coppices usually produce two shoots, one per axillary bud at the axil of the two lateral leaves. Usually, one of these two shoots outgrows the other one to form the main stem of the new plant, notwithstanding that the initial competition between the two shoots hinders its development. Moreover, such axillary shoots start to elongate at a certain angle of diversion from the main vertical axis of the cutting (photo 4). Manual shearing is therefore needed to keep only the more vigorous and upright axillary shoot from these field stump-issued rooted cuttings.

The rooted cuttings, maintained since the beginning in SAPPI trays, reach an overall size of 30-32 cm in height at the end of the 45 day-long acclimatization phase. They were previously used as planting material for field clonal tests or for industrial clonal plantations. With the new system, they are exclusively utilized for producing advanced generations of intensively managed stock plants, according to the following procedure.



Photo 4.The resulting rooted cuttings displaying varying degrees of stem straightness. Photo O. Monteuuis.

These B0 rooted cuttings are transplanted into 60 cm-long x 40 cm-wide and 16 cm-deep containers locally made by stacking two "stamp" plastic cases one over the other as illustrated in photo 5.

The so-obtained containers are filled with a cultivation substrate consisting of black fine sandy soil (8 volumes) mixed with wood charcoal (2 volumes) and enriched with 5 kg/m³ of NPK 13-13-21 basic fertilizer. This mixture is scalded in boiling water during 12 hours before utilization. Every "stamp" container contains 15 B0 generation-issued rooted cuttings of the



Photo 5.
Setting the newly rooted BO cuttings in « Stamp » containers to be used as stock plants.
Photo F. Mankessi.

same clone, planted at 12 x 12 cm each from another, and is placed on an 80cm-high elevated support under intermittent watering – 2 to 3 daily sprays of 5 minutes each – and full natural light. They are fertilized every week up to saturation with an aqueous solution of 5 g/l of NPK 13-13-21 plus 1 g/l of "Hakaphos Rouge" (NPK 8-12-24, + 4% magnesium and oligonutrients).

Soon after transplantation in such conditions, these B0 rooted cutting-issued plants are sheared back to reduce their main stem average height from 30-32 cm to about 5 cm. This operation is repeated a few times once or twice a week in order to stimulate the production of as many new axillary shoots as possible, which start sprouting from the main stem usually 7 days after shearing. From this step onwards, any elongating leader shoot must be removed as early as possible to reduce between-shoot differences that may induce further within-clone variation. After one month of such intensive pruning practices, the B0 containergrown stock plants produce numerous actively growing soft shoots with short internodes and juvenile-like opposite leaves. Such shoots are used to give rise to the second or B1 generation of container-grown stock plants.

Managing the subsequent generations of propagation by rooted cuttings

The actively growing juvenilelike shoots produced by the initial B0 generation of container-grown stock plants described above are collected as soon as they reach an overall length of 5 to 7cm, that corresponds to three nodes or pairs of opposite leaves in addition to the terminal shoot apex (photos 6). This must be considered as the standard for suitable cuttings. These terminal shoot cuttings will be rooted and then acclimatized following the same procedure and under the same conditions as for the BO generation of cuttings detailed previously.

Once they reach 30 to 32 cm in height, the container-grown plants issued from the B1 and subsequent generations of serial propagation by cuttings (Monteuuis et al., 1987; MONTEUUIS 1993; AIMERS-HALLIDAY et al., 2003) are shortened to 2 cm above the substrate level to be used as intensively managed stock plants, producing the first suitable cuttings 3 weeks later. By comparison, 5 cm was the shortest pruning height to keep B0 generation stock plants alive, from which suitable cuttings could be collected only after one month. Indeed, as observed for different species (FRANCLET, 1977; MONTEUUIS, 1993), the cloning ability of Eucalyptus urophylla x Eucalyptus grandis genotypes by rooted cuttings can be greatly promoted by maintaining the stock plants as miniaturized, compact and close to the root system as possible. This is achieved by frequent pruning and pinching operations which induce, after a few weeks, the formation of a 2 to 3 cm large burl in the upper part of the main stem, and from which most of suitable cuttings originate profusely (photo 7).

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Collecting regularly every 3 or 4 days from the stock plants all the newly formed shoots that can be used as suitable cuttings replaces the frequent hedging and pinching practices initially needed for stimulating the production of such shoots. All the young shoots produced from B1 or more advanced generations of such intensively managed stock plants look similar at this stage, displaying the same morphological features, i.e. opposite and decussate leaf phyllotaxy, short internodes and small leaves like juvenile seedlings. They also demonstrate a similar high capacity for adventitious rooting and true-to-type cloning from rooted cuttings. This contrasts with the BO generation cuttings collected from field coppicing stumps, which exhibit alternate leaves separated by much longer internodes, mature traits and which root in lesser proportions with only one or two long adventitious roots per rooted cutting (photos 8).

Under this new mass clonal production system, generations B0, B1 and B2 of rooted cuttings are used exclusively to produce the sufficient number of suitable stock plants needed to meet the annual requirement in plantable rooted cuttings. The current figures are amounting to 12 000 000 cutting-derived planting stock per year produced from 30 000 suitably managed container-grown stock plants. Only materials deriving from the fourth (B3) or more advanced generations of serial propagation are used for field planting. This is a fundamental difference with the previous extensive technique (MARTIN, QUILLET, 1974) utilizing exclusively Bo generation planting material obtained directly from stump coppicing shoots after felling of the 3 year-old ortets.



Photo 6 a.Morphological features of suitable cuttings produced by intensively managed container-grown stock plants.
Photo O. Monteuuis.



Photo 6 b.Morphological characteristics of BO nodal cuttings from stump (top) versus terminal shoot ones from intensively managed container-grown stock plants (bottom).
Photo A. Saya.



Photo 7.Most of the suitable cuttings originate from a burl developed in the upper part of the contained-grown stock plants induced by intensive pruning practices. Photo A. Saya.

Advantages of using cuttings produced from intensively managed container-grown stock plants

The inability of most of the E. urophylla x E. grandis genotypes to be mass-clonally propagated using the former extensive technique, which was successfully developed and experienced for many years with Eucalyptus PF1 and E. 12 ABL x E. saligna hybrid, demonstrates once again that eucalypts are liable to behave quite differently according to their genetic background. Significant differences in adventitious rooting and more generally in organogenic capacities have been also noticed both in nursery and tissue culture conditions between E. urophylla x E. grandis genotypes, even as closely related as half-sib (Monteuuis, unpublished data).

The new method developed has tremendously improved the mass clonal propagation by rooted cuttings of outstanding field clones like 18-147, which could not be efficiently mass produced with the former extensive technique.

The main advantages of this new container-grown and intensively managed stock plant technique are:

- It is more efficient than the previous field system, not adapted to the mass propagation by rooted cuttings of most of the *E. urophylla* x *E. grandis* field-selected ortets;
- Much higher quantity of rooted cuttings can be produced per stock plant, averaging 400 per annum versus 35 for field-established stumps. In other words, 1.12 m² of container-grown stock plants 15 units per 0.6 m long x 0.4 m wide "stamp" container produce annually the same quantity of rooted cuttings as 1 ha of stump-derived stock plants (800 stumps/ha) converted from industrial plantations.
- The quality of the resulting plants is improved: rooted cuttings with terminal bud produced from intensively managed container-grown stock plants give rise to straight, single-





Photos 8.Comparative characteristics of rooted cuttings produced from stumps in the field (to the left of each picture), and produced from intensively managed stock plants (to the right).
Photo A. Saya.



Photos 9. Ready for planting rooted cuttings derived from intensively managed container grown stock plants. Photo A. Saya.



stemmed and true-to-type clonal offspring. This contrasts with field system derived nodal cuttings which, once rooted, needed to be first sorted out, then hand pruned to keep only the most promising axillary shoot.

- Rooted cuttings can be produced sustainably all year around, whereas field cuttings can be collected to be rooted only twice a year, during the rainy seasons.
- The stock plant area is much smaller and located within the nursery for easier maintenance, monitoring and access than the field stump stock plants scattered unevenly over huge plantation areas.

The noticeable reduction of the within-clone variation or "C effects" (TIMMIS et al., 1987), by comparison with the previous field stump-derived system is likely to improve greatly the quality of the field clonal tests needed for the ongoing clonal selection programs, which can be analyzed on a more reliable and sound basis. Moreover, true-to-type clonal offspring can be produced much earlier in sufficient quantities for setting up once and for all at one time, only one set of rigorous clonal tests one year after the field selection of the 3year-old ortets. In this way, the time needed to achieve the clonal selection cycles can be reduced to 7 years versus 12 years with the previous extensive propagation system (table I), resulting in substantial space, time and money benefits.

Table I.
UR2PI former versus new clonal selection time frame.

Former cycle using field stump derived stock plants	Years	New cycle using container-grown stock plants
Field establishment of controlled pollination- derived progeny tests	1	Field establishment of controlled pollination- derived progeny tests
	2	
Field selection of candidate ortets for clonal tests (COC)	3	Field selection of candidate ortets for clonal tests (COC)
Production of clonal offspring from COC sprouting stumps	4	Intensive propagation from advanced generations of container grown stock plants and setting up of a complete (5x5) x 3 clonal test
Establishment of the first set (3 x 3) x 3 of clonal test plots	5	
	6	
	7	Clonal selection and Intensive propagation of the relevant clones selected for operational planting
Preliminary clonal selection from the year 5 clonal tests plots Production from field sprouting stumps of the relevant clones selected. Establishment of the second set or confirmation of (4x4) x 3 clonal test plots	8	
	9	
	10	
Final clonal selection from the year 8 confirmation clonal tests	11	
Mass production of the clones finally selected from field-established stumps for operational planting	12	

Discussion

The advantages of using intensively managed container-grown stock plants are becoming more and more obvious for many eucalyptus species or inter-specific hybrids (WENDLING, XAVIER et al., 2003; TITON, 2006). It can reasonably be assumed that the technique detailed in this paper for successfully mass propagating by rooted cuttings selected E. urophylla x E. grandis genotypes in Pointe Noire conditions can also benefit other eucalypt species in different environments. It is indeed much cheaper and easier to monitor and manage than the hydroponics or sub-irrigation systems which are currently more and more adopted for mass producing eucalypts and other forest tree species by rooted cuttings in many parts of the world (ALPOIN et al., 2004; JIMENEZ, 2005). The financial aspects, the overall efficiency, including the degree of sophistication and relevant skilfulness requirements, as well as the final destination of the planting material constitute indeed determining issues in choosing between nursery and in vitro clonal propagation system, and being aware that only tissue-cultured clones comply with international phytosanitary dispatching restrictions. All these crucial issues applied to the UR2PI E. urophylla x E. grandis selected clones mass produced for local utilization warrant the new nursery technique developed from intensively managed and container-grown stock plants.

The rationale for promoting mass clonal propagation by rooted cuttings of selected tree genotypes by intensive stock plant management has been argued and demonstrated for a long time on different arborescent species (FRANCLET, 1977). The advantages of preferring container-grown stock plants to field-established ones for achieving this goal can be summed up as follows:

Better control and therefore optimization of stock plant environment with regard to light, watering, soil fer-

tility, wind, etc.
• Proximity to the misting area resulting in shorter time and distance for collecting the cuttings that can be set sooner with less stress under proper rooting conditions;

- Easier maintenance weeding, feeding, pest and disease treatments – and control;
- More adapted to intensive management frequent pruning and pinching operations;
- Higher density of stock plants per area:
- Greater overall efficiency and cost effectiveness.

Less optimal field conditions may have accounted for the failure of the "semi-intensive" – 2500 stock plants/ha – and more particularly of the "intensive" – 20 000 stock plants/ha, which resulted in a premature and high stock plant mortality – field established stock plant systems tested on the same *E. urophylla* x *E. grandis* clones in Pointe Noire conditions during the early 1990s (LACLAU, BOUVET, 1994).

Maintaining by frequent pruning or pinching operations, the production of shoots to be used as cuttings close to the root system has been proven to promote their ability for adventitious rooting and true-to-typeness development once rooted (FRANCLET, 1977; MONTEUUIS et al., 1987; MONTEUUIS, 1993). Such practices shorten indeed the distance between the roots and the shoot apical meristems, whose role for true-to-type cloning is determining, in other words, between the "sources "and the "sinks" from a metabolic viewpoint. The architecture of the stock plant becomes simplified and the within-plant variability responsible for physiological gradients minimized for a more uneven physiological condition of the shoots, and assumedly a better morphologic uniformity of the resulting rooted cuttings (Monteuuis, 1989).

The beneficial influence of the serial propagation method for improving the ability for adventitious rooting and further true-to-type cloning of the cuttings has been particularly obvious from one generation of cuttings to the further one. The most striking difference observed in this respect was between the B0 and B1 generations, that is to say between cuttings collected from 3-year-old field established stumps and

the first generation of intensively managed container-grown and recently rooted stock plants. This is consistent with other findings on different species, especially when starting from mature original ortets (Monteuuis, 1993). Combining serial propagation to frequent hedging practices and cutting collections from the same containergrown stock plant is definitely easier to manage than a strict serial propagation system where cuttings are collected only once or twice from the same stock plant (Monteuuis et al., 1987; Aimers-HALLIDAY, 2003). This serial-hedged combined stock plant management has proven to work quite satisfactorily so far on the E. urophylla x E. grandis clones in Pointe Noire, similarly to what has been obtained with other tropical and temperate species in different conditions, including conifers (MONTEUUIS, 1993; AIMERS-HALLIDAY et al., 2003). The fact that the intensive propagation technique described has already been successfully applied to some stock plants for 5 years already without noticing any sign of decline demonstrates that the culture conditions have been quite appropriate. However, considering the great number of shoots produced continuously, risks of stock plant depletion must remain a crucial concern to care about. Thus, a systematic replacement at regular time period with new stock plants coming from the next generation of serial propagation seems highly recommendable.

This report emphasizes the importance of suitable nursery techniques for improving the ability for true-to-type cloning by rooted cuttings of *E. urophylla* x *E. grandis* clones. The physiological changes induced by such practices, which are responsible for this greater responsiveness of the plant material selected to be mass clonally propagated are under investigations at a more basic level. However, the practical benefits are from now on obvious, first for clonal selection, then for the large-scale utilization of the clones selected.

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