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EVOLUTION OF TECHNOLOGY FOR CLONING EUCALYPTUS IN LARGE SCALE

Teotônio Francisco de Assis¹

ABSTRACT

Mass vegetative propagation has become an important tool for increasing the competitiveness of forestry based industry. This method reaches its highest potential when is used to establish clonal forests of hybrids endowed with better wood quality and higher volumetric growth. However, in *Eucalyptus* species, the popular method of rooting stem-cuttings has limitation, like quick loss of rooting competence due to ontogenetic aging, intra-clonal variation resulting from topophysis, and poor quality root system, that negatively affect genetic expression of several clones.

This article reports two alternative super-intensive systems, micro- and mini-cutting, for cloning *Eucalyptus* at commercial scale. These systems have shown great potential of substituting, rooting stem-cuttings with technical and economical advantages. Micro-cutting uses the apices obtained from micro-propagated plantlets, while the mini-cutting is based on the rooting of axillary sprouts from rooted stem-cuttings. In both systems the plants are managed intensively to produce mini-cuttings. Field clonal hedges are replaced by indoor hydroponics mini-hedges, which provide high degree of juvenility of micro-propagated plantlets or rooted cutting. The success of the systems also is dependent upon the optimal nutrient concentration of the resulting mini-cuttings.

Compared to stem-cuttings, the rooting of micro- or mini-cuttings improves rooting potential, rooting speed, root system quality, and reduces costs. Additionally, these systems offer the opportunity of physiological homogenization of propagules and drastically reduce topophysis effects. The development of these super-intensive cloning systems has set the stage for a new phase of mass vegetative propagation of *Eucalyptus* and other woody species.

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INTRODUCTION

Since the first developmental stage until its recognition as an operational method of reproducing superior trees, cloning of *Eucalyptus* species is undergoing a continuous process of improvement through incorporation of new technical concepts and technologies at different phases of the process. Campinhos & Ikemori, 1983; Zobel & Ikemori, 1983; Ferreira & Santos, 1997; Denison & Keitzka, 1993.

In the last two decades, cloning *Eucalyptus* spp. has produced relevant progress for the forest companies, especially solving problems associated with diseases such as canker (*Cryphonectria cubensis*) and productivity improvement (Campinhos & Ikemori, 1983; Ferreira & Santos, 1997). Actually, the focus of

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cloning has shifted to industrial requirements, rather than remain limited to just disease resistance and volume increase. The wood properties that positively influence industrial processes and product quality are considered, especially where cloning has an important role to play.

In the era of global markets, the development of forestry plantations for industrial purposes must aim for, besides other objectives, increasing industrial competitiveness in the distinct market segments they interact with. In such scenario forestry based companies must consider the mode in which the forestry raw material can affect their competitive capacity. The modern concept of competitiveness includes producing products to meet the customer's requirements at low costs, in a sustainable manner and with minimum impact on the environment. Therefore, developing tree breeding programs to obtain quick gains and developing cloning systems to have a well established vegetative propagation method becomes important. The vegetative propagation methods should rapidly transform the genetic gains, obtained through breeding, into benefits for the industry. One of the most efficient tools to acquire these objectives is the combination of inter-specific hybridization and establishment of clonal forestry derived from superior hybrid individuals (Assis 2000).

In this context hybridization is an alternative of great impact in tree breeding programs, which can combine superior wood characteristics with tolerance to biotic and abiotic stress, thus representing a significant source of superior individuals, capable of introducing genetic gains in forest productivity and wood properties. Crossing species of different characteristics allows production of complementary wood properties in trees especially to meet industrial requirements.

Considering that *Eucalyptus* hybrids are heterogeneous, the effective and quick integration of genetic gains obtained with hybrids into the industrial process depends basically on existence of functional large scale cloning systems. Mass vegetative propagation perfectly complements hybridization for producing clonal forestry, and has some advantages over the sexual methods of mass reproduction of selected families, besides being the best way to commercially exploit the heterosis found in several *Eucalyptus* hybrid crosses. By capturing the total genetic variance (Zobel 1992), vegetative propagation allows for maximum benefits of wood properties and productivity, besides allowing for production of more uniform raw material, which from industrial point of view is highly beneficial to industrial process and product quality. Therefore, the tree breeding programs that focus on these aspects of forest industry will have great impact on the three important components of the competitive process: productivity, product quality and production costs.

This article basically describes the recent evolution of cloning *Eucalyptus* for industrial purposes in Brazil, with emphasis on the cloning techniques and on the systems used for mass production of plant propagules.

NEW CLONING TECHNIQUES

Considering the consolidation of cloning as a tool for the establishment of productive *Eucalyptus* clonal forestry and its positive effects on industrial process and product quality, the evolution of the systems used for cloning *Eucalyptus* certainly could be predicted. The precursor of this development started in the early 80's when cloning *Eucalyptus* by rooting stem-cuttings reached industrial dimension (Campinhos & Ikemori, 1983). Since that time, the major constraint for general adoption was its applicability only to

a small number of species and clones. Rooting stem-cutting was not suitable for a large number of economically important species, including those important for energy and charcoal, like *E. citriodora*, *E. maculata*, *E. paniculata* and *E. cloeziana*, and an important number of clones of rootable species had problems for commercial propagation. Most of the problems were associated with accelerated maturation process causing rapid loss of rooting-predisposition, and manifestation of topophytic effect. The phenomena of topophysis affects clones in different intensities, and is the main cause of intra-clone differences in growth and reduction of rooting ability. Franclet et al. (1987) emphatically pointed out that topophysis induced physiological differences and these differences can result in sufficient intra-clonal variability that can suppress the potential advantages of cloning. Another limitation of stem cutting was associated with alterations of root system architecture, leading to root deformation. In many clones such deformations prevented their full genetic expression, consequently reducing the ratio between selected trees and number of clones effectively used. Because such limitations of rooting stem-cutting, alternative methods were developed for commercial cloning of *Eucalyptus* species.

Micro-Cutting

Based on the work of Assis et al. (1992), this technique was developed in Brazil in the early 90's. The idea came up with the observations that rooting ability of stem-cuttings decreases with ontogenetic aging and the decline may be faster than reported in the literature. In *E. grandis* for example, the rooting competence decreased from the fourteenth node up (Patton & Willing, 1974), while it took longer in the *E. deglupta*. Assis et al. (1992) observed that clones of *E. saligna*, *E. grandis* and *E. urophylla* that had equally high proportion of stem-cutting rooting *in vitro*, showed differential levels of decline in the rooting percentage when managed in clonal hedges. This indicated that some factor related to clone growth, encompassing period between planting and cutting harvest (6 months), could be responsible for these differences. Preliminary tests done at Klabin Riocell (unpublished) showed, independent the species, almost 100% rooting of the very young mini-cuttings obtained from the cotyledonary leaf pair and the same tendency was maintained in the difficult to root species like *E. citriodora*, *E. cloeziana*, *E. paniculata*, *E. dunnii*, and *E. globulus*. However, with age, ranging from few days to some months, the cuttings harvested from such young plants showed a marked reduction in their rooting ability and in some cases such ability was totally lost. These observations suggested that the rooting potential reaches the maximum value at high juvenility level (mini-cuttings from cotyledons) and is similar in all species tested. But the decrease in the rooting ability with seedling age differed among species, which was similar to that found in the older materials in the field. This suggests that, at some stage, part of the juvenility obtained through rejuvenation process *in vitro* (Gonçalves et al. 1986) and/or on basal sprouts of cut adult tress (Hartney, 1980) is being gradually eroded during the growth of the clones in the clonal hedges.

Thus, hypothetically, the rooting ability of *Eucalyptus* clones *ex vitro* should increase if the "physiological distance" between the maximum juvenility stage, obtained *in vitro*, and the propagule collecting stage is reduced. To test this hypothesis Assis et al (1992) used shoot apex (micro-cuttings) of very juvenile micro-propagated plants of *E. saligna* clone as propagules. These micro-cuttings had 30% higher rooting than the stem-cuttings. In order to verify the results in a more representative sample, the rooting indices obtained by the traditional and the new micro-cutting method, in seven clones of *E. saligna* and five clones of *E. grandis* were compared. Considering all the

clones, the rooting of micro-cuttings, on an average, was 18% higher than the rooting of the stem-cuttings, which amounted to an increase in the range of 6.3 to 44.6 percent points over the average. In general, higher increases in rooting percentage were observed in clones with lower rooting ability with the stem-cuttings.

Other results were obtained from several other trials established to define substrate, growth substances, environmental conditions for rooting etc. One of the most significant findings of this new technology was complete elimination of the use of growth substances usually required for the rooting of stem-cutting (Assis et al. 1992). These substances did not increase rooting of micro-cuttings, instead in some cases reduced it, indicating that the endogenous auxin concentration in the juvenile tissues was sufficient to promote rooting. Based on these results, a super-intensive system of *Eucalyptus* propagation *ex vitro* was established.

The main feature of the technique is the use of juvenile plants or plants rejuvenated *in vitro*, as source of vegetative propagules. Shoot apices are used as micro-cuttings, which are placed to root in a glasshouse equipped with temperature and humidity control. The actual size of micro-cuttings is about 7 to 8 cm with two to three leaf-pairs. Presence of the shoot apex is important for quality of the root system, because its presence induces taproot-like system. The micro-stumps left after micro-cutting harvest, sprout rapidly producing new micro-propagules, which can be harvested for use within a period of 15 days in the summer and 30 days in the winter.

Since its first use, the micro-cutting technique is improving continuously by incorporating new research findings. The evidence of evolution of this technique are well documented in publications of Assis et al. (1992); Xavier & Comerio (1996); Iannelli et al. (1996); Assis (1997); Wendling & Xavier (1999); Higashi (2000), and Campinhos (2000). The participation of dedicated scientists such as Professor Acelino Couto Alfenas (U.F.V -Viçosa-MG) and Professor Antonio Natal Gonçalves (E.S.A.L.Q.- Piracicaba-SP) among others, was of fundamental importance for the establishment of an efficient super-intensive cloning system for *Eucalyptus* species. The technical contributions, reciprocally exchanged through a pre-competitive development model and intense information exchange, were the bases for the fast evolution of this new concept of cloning *Eucalyptus* in large scale.

Mini-Cutting

Probably, the first initiative to root mini-cuttings in *Eucalyptus* was taken in the early 80's, by using shoots obtained from the thinning operation of rooted stem-cuttings (unpublished). The early trials were discouraging due to inconsistent results that varied from highly positive to negative. Later studies showed that such inconsistencies were caused by nutritional deficiencies of the mother plants at the time of mini-cutting collection. A similar system called "cascade propagation", used in France, is described by Chaperon (1987). However, in the 90's, after the consolidation of the micro-cutting as a functional propagation system, that mini-cutting system became commercially viable for *Eucalyptus* cloning. Higashi et al., (2000) developed a functional and efficient mini-cutting cloning system and several other researchers added significant contributions to the development of this technique (Wendling et al.2000; Xavier & Wendling, 1988).

Mini- and micro-cutting techniques are very similar in concept and operational procedures, differing mainly in the origin of the initial

propagules. The micro-cuttings are obtained from shoot apices originating from micro-propagated plants, and the mini-cuttings come from axillary sprouts of plants cloned by stem-cuttings. After rooting of the first shoot, the two techniques are identical. In some clones using mini-cutting, some propagation cycles (serial propagation) are required to obtain the reactivation for acquiring full potential of rooting capacity. In micro-cuttings such propagation cycles comes naturally by monthly *in vitro* sub-culturing of explants. Micro-propagation is unnecessary for easy to root species because high levels of juvenility can be obtained easily by inducing basal shoots therefore in such cases mini-cutting is technically and economically feasible.

Despite the success of these techniques is believed to be related to maintenance of very juvenile stage (Assis et al. 1992, Xavier & Comerio 1996), new findings suggest that their high rooting potential are also related to the better nutritional status of the mini-cuttings. In general, mini-cuttings or micro-cuttings root better than juvenile stem-cuttings of the same clones produced in clonal hedges in field. Since both materials are juvenile, the mini-and micro-cuttings cultivated in well-balanced nutritive solutions root better as a result of better nutritional status. The rooting superiority of mini- or micro-cuttings may also be related to differential level of lignification in the two groups of propagules. Compared to stem cuttings, these micro- or mini-cuttings can be considered as "herbaceous", and using these techniques many complication associated with lignin formation and its concentration increase in tissues can be avoided.

Although, the two techniques are very similar, but received different names. The two terms mini-cutting and micro-cutting were designated, by convention, to systems of vegetative propagation that have their origin in rooted cuttings and micro-propagated plants, respectively. Thus a system originating from micro-propagated plants is termed as micro-cutting and that based on sprouts from rooted cuttings is called mini-cuttings. Nevertheless, there is a tendency to change this terminology to avoid confusion with the traditional term micro-cutting reserved for rooting of shoots produced *in vitro*. Therefore a system of vegetative propagation *ex vitro* based on mini-propagules could be termed as mini-cutting, independent of its origin, including those originating from a micro-propagation system. Therefore the term mini-cutting will be used to designate both techniques on this paper.

Advantages of Mini-Cutting

Compared to the traditional stem-cutting rooting, mini-cutting has many advantages leading to operational, technical, economical, environmental and quality benefits. Operationally, the labor demanded and cost is markedly reduced, due to elimination of labor intensive treatment with growth substances and many other operations required in an extensive outdoor clonal hedge management system. Many field operations like soil preparation, fertilization, irrigation, cultivation, weeding, pest and disease control, sprout transport etc are replaced by intensive activities in smaller indoor areas at much lower costs, where the amount of chemicals used is also drastically reduced.

The rooting ability of mini-cutting is much higher than the stem-cuttings. Although the benefits vary among the species and clones, in general in poor rooters species or clones with stem-cutting better compare with mini-cuttings, which may increase rooting by 40%. The main reasons for such increase are related to higher levels of juvenility and optimal nutritional content of the tissues, which improves the rooting predisposition and speed of

root initiation. The rooting speed of mini-cuttings has two other important consequences in a commercial cloning program: the time of the plants indoor is usually reduced to half compared to rooting stem-cutting, thus considerably improve the use of the facilities and reduce the exposures of basal tissue of mini-cuttings to pathogenic fungi. At the initial and more susceptible stage, the faster reaction of mini-cuttings inducing rapid protector callus formation at the basal end provides protection from pathogen resulting in reduced fungicide application.

Mini-cuttings produce better quality root system with a tendency for a taproot-like system in contrast to the predominant lateral root growth habit in the stem-cutting system. Apparently the connection between root and stem tissues in the mini-cuttings is more suitable due to lower lignification of the tissues involved. This kind of root system appears to be more responsive to fertilization and is more resistant to environmental factors and considerably reduces intra-clone variation. As pointed out earlier, the topophysis effects observed in several systems of vegetative propagation are highly harmful to clonal uniformity. In this respect mini-cutting offers the advantage of physiological homogenization of the propagules producing a much more uniform forest.

EVOLUTION OF MASS PRODUCTION OF VEGETATIVE PROPAGULES

When the rooting of stem-cutting was first introduced commercially in Brazil (Campinhos & Ikemori (1983), the vegetative propagules for establishing clonal forests were obtained from field plantations. This management strategy required annually reserving large areas, with the only objective of obtaining cuttings for commercial rooting programs. Although the commercial plantations could be used to produce propagules, the high time-demand to gather wood and the vegetative propagules made this alternative unfeasible. The clonal hedge based concept of intensive management model was introduced sometime later. This system demanded smaller area due to high shoot productivity per unit area (Carvalho, et al.1991; Higashi 2000). Despite being a tremendous advance for mass production of shoots, clonal hedges were still complicated to manage and poorly controlled. To meet the demand of clonal forestry, the system still required large areas, intensive labor, and large amount of nutrients and water. The propagators were mostly dependent on the weather since the system was completely at the mercy of environmental conditions.

Mini Clonal Hedges

At the beginning of the last decade, the development of micro-cutting technology for *Eucalyptus* (Assis et al. 1992) materialized the concept of super-intensive management of producing vegetative propagules at commercial scale. Like for cloning methods, the advent of the micro-cutting substantially contributed to the progress made in systems for large scale production of vegetative propagule *ex vitro*. Originally the system was based on mini-hedges established through rooted mini-cuttings, grown in small containers (dibble tubes). This system provided a series of technical and economical benefits as well as good root quality (Assis et al. 1992; Assis, 1997; Xavier & Comério, 1986). Despite great advance over the clonal hedges in field, mini-hedges faced some limitations. The outdoor mini-hedges were still hostages of climate, and problems related to adequate maintenance of nutritional status and leaf diseases continued, especially during winter. The main problems were, reduced photosynthesis rate, reduced nutrient uptake and high levels of nutrient loss by leaching during period of excessive rainfall or even during

irrigation. These limitation led to the development of indoor mini hedge system.

Indoor Hydroponics Clonal Hedges

The development of new technologies contributed to increase the efficiency of the existing systems. Hydroponics concepts for production of mini-cuttings was introduced first in operational indoor system based on drip fertigated sand beds (Higashi et al. 2000). The major contribution of this system was in terms of assuring an adequate nutritional status of the mini-cuttings, which is the key factor to obtain high rooting percentage in juvenile vegetative material. Using the same concept, Campinhos et al. (2000) developed a highly efficient method based on intermittent flooding system, where the containers of the mini-stumps are immersed in a nutritive solution for fertigation. These two hydroponics methods, sand-bed and intermittent flooding, are most widely used and the Brazilian companies use either of them.

Virtual Clonal Hedge

The observation that the shoots of first collection root better than those after third or fourth collection, suggested that vegetative propagules could be produced anywhere, without allocation of a definite physical area. Assis (1997) suggested a system where the mini-cuttings can be produced in virtual clonal hedges. Any rooted plant with normal growth when reaches a height of about 15cm, has its apex (7 to 8 cm) cut back to produce a new mini-cutting. As soon as the newly rooted mini-cuttings are about 15 cm high, their apices can be used as a new source of mini-cuttings, thus creating a virtual mini-hedge after some cycles of re-rooting, and requiring no specific area for production of vegetative propagules. The important attribute of this system is that the plants used for producing mini-cutting can be planted in field without losing quality of the intermittent flooding mini hedges.

The sprouting of harvested plants becomes an extra option of providing mini-cuttings. The blanks resulting from rooting failure can be filled with mini-cuttings produced on the mini-stumps of the harvested plants. Similarly, the sprouts produced on the pruned plants and the apices of the rooted mini-cuttings can be used continuously as self-sustained cyclic process. This system represents an excellent technical, economical, and operational alternative, but only works well if good nutritional conditions of the mini-cuttings are ensured. An extension of sub-fertigation to newly rooted mini-cuttings is the most suitable option to make virtual mini-hedging practical. The use of very soft mini-cuttings must be avoided to prevent fungal infection. The locations with wide climatic variations and without any environmental control are less suitable for establishing virtual mini-hedge system, therefore is not recommended in situations where environmental control is not feasible.

Advantages of Indoor Hydroponic Mini Hedging

The development of the concept of super-intensive hydroponic mini hedging, brought several benefits to commercial cloning of *Eucalyptus*. All indoor mini hedging systems have technical and economical advantages relative to normal outdoor clonal hedging. Higher productivity of mini-cuttings, lower labor demand, and low consumption of chemicals and water, represents major sources of economic gain. In addition, considering the important effect of the conditions under which the mother-plants grow, on the rooting success (Read,

1987; Hartman & Kester, 1983; Haissig, 1986; Andersen, 1986), the main technical advantages are related to such aspect. These systems allow the use of important theoretical concepts which are difficult to implement outdoor, such as CO₂ enrichment, control of temperature, light intensity and photoperiod. These factors in addition to the nutrition are of fundamental importance to enhance rooting predisposition. This system also enables application of pre-harvest treatments such as spraying mother plants with growth substance (phyto hormone) to increase rooting potential.

Propagules Productivity

Eucalyptus cloning in large scale started in Brazil using very extensive systems and the productivity of vegetative propagules was 114 cuttings/m² of hedges field (Campinhos & Ikemori, 1983). With the introduction of the mini-hedges the production increased progressively to 121 cuttings/m² (Carvalho et al. 1991), then to 1,752 cuttings/m² per year (Higashi et al. 2000) and presently is at about 24,000 cuttings/m²/year. Table 1 provides an overview of the evolution of vegetative propagules productivity in *Eucalyptus*. The super-intensive systems, especially hydroponics based, are much more efficient, having productivity 350 folds higher than the initial systems used in Brazil. Comparing the different systems, the capacity of producing vegetative propagules in the super-intensive systems is highly expressive, consequently, more cuttings can be produced per year per unit constructed area of mini hedge, besides they are ease of handle, manage and they have a lower cost per plant.

Variations in the productivity of mini-cutting observed in table 1 resulted from different hedging systems, from different genetic materials and also from different management practices. Clones of *E. grandis* x *E. urophylla* hybrids are generally much more productive in mini-cuttings than other species. The majority of the pure *E. saligna* clones are much more difficult to produce as mini-cuttings than the *E. grandis* x *E. urophylla* hybrid where production/mini-stump is almost double. Still, the rooting competence and propagule productivity is much higher than in the outdoor clonal hedges. The other source of variation in the productivity is related to the concept and kind of management adopted to utilize the sprouts.

Certain practices of management of mini-stumps in the sand beds produce vegetative propagules similar to the macro-cutting system. The sprouts are allowed to grow to bigger sizes, and after collection each sprout is divided into three mini-cuttings (basal, middle and apical). This system produces higher number of propagules/m² but it loses the best characteristic of the mini-cutting provided by the presence of shoot apex, responsible for producing high quality root system (taproot-like system, discussed earlier)

In both hydroponics systems (drip fertigation and intermittent flooding) losing mother plants is common due to salination, excessive harvesting, diseases etc. In this respect, the systems that use containerized mother plants, as in intermittent flooding, have advantage over the sand bed. In sand beds, the dead plant replacement and getting them to productive stage require more time compared to the intermittent flooding system.

The most attractive characteristic of virtual mini-hedges, that do not require allocated physical area for producing vegetative propagules, is that the mother plants are plantable. After supplying mini-cuttings, such plants are managed as normal rooted mini-cuttings. In the intermittent flooding system, the mother plants also can be planted provided the harvesting regime

has not degraded its quality. Under successive and heavy harvesting regime the quality of mother plants frequently declines.

Table 1: Propagules productivity in different clonal hedging systems used in Brazil*.

System	Species/ Spacing (m)	Productivity (Propagules/m ² /year)	References
Clonal bank	E. grandis x E. urophylla - 3,0 x 3,0	114	Campinhos & Ikemori (1983)
Clonal Hedging	E. grandis x E. urophylla - 1,0 x 1,5	121	Carvalho et al. (1991)
Clonal Hedging	E. grandis x E. urophylla - 0,5 x 0,5	1752	Higashi et al. (2000)
Mini hedging Out door	E. grandis x E. urophylla - 0,05 x 0,05	29200	Xavier & Comério (1996)
Hydro mini hedging (sand bed/drop irrigation)	E. grandis x E. urophylla - 0,1 x 0,1	41480**	Higashi et al. (2000)
Hydro mini hedging (interm. flooding)	E. grandis x E. urophylla - 0,05 x 0,05	25500	Campinhos et al. (2000)
Hydro mini hedging (interm. flooding)	E. grandis x E. urophylla - 0,05 x 0,05	24000	Klabin Riocell operational
Hydro mini hedging (intermittent. Flooding)	E. saligna and E. urophylla x E. globulus - 0,05 x 0,05	14400	Klabin Riocell operational
Virtual mini hedging	E. saligna and E. urophylla x E. globulus 0,05 x 0,05	-	Klabin Riocell operational

* Based on cuttings/mini-cuttings/stump considering 12 months of effective production, without discounting the replacement of dead plants.

** Subdividing sprouts in 3 mini-cuttings

One question that arises from the spacing in mini-hedges is about the influence of close spacing on the rooting of mini-cuttings and the mini-stump productivity. Trials at Klabin Riocell, showed that the spacing of 5 x 5 cm between plants does not interfere significantly in the rooting capacity of the mini-cuttings, but wider spacing permits higher survival rate of mini-stumps. But the final results are better with narrower spacing, since there is more than proportional compensation by existence of higher number of plants/m².

Temperature and Light

Temperature and light are good examples of the factors that can be controlled in mini hedges, to improve rooting predisposition of mini-cuttings. Light can be managed as to photoperiod and intensity. Light intensity can strongly influence cutting productivity and rooting by reducing or increasing endogenous phenolic substances that act as rooting inhibitors (Vieitez & Ballester, 1988) or promoter depending upon the concentration in the tissues and the species involved. The photoperiod, especially combined with

temperature can influence rooting predisposition of shoots. Lighting also can influence rooting by controlling internal levels of carbohydrates. Etiolation of mother plants can affect rooting and root number, which depending upon the specie may increase or decrease (Hansen, 1987). This variation appears to be linked to carbohydrate production (Nanda et al. 1971) and accumulation and transport of auxins (Hansen, 1987).

Temperature can influence rooting by interfering with nutrient uptake and metabolism, and its control, especially in sub-tropical climates, can be adjusted for optimum production of cuttings. Assis (1997) observed that rooting percentage of mini-cuttings decreases in cold winter months. Recent work at Klabin Riocell (Unpublished) showed that this problem could be solved by supplying additional light (14h/1000 lux) and increasing the temperature in environment of mother plants (>20 °C). Since the two factors were not separated, the effect of individual factor could not be determined. However, considering that nutrient concentration is of prime importance for rooting process, and that their uptake depends on the metabolic activity, it can be assumed that both the factors contributed to reestablishment of normal rooting competence. In general, in areas of cold climate, rooting is limited to only warmer months, therefore as discussed above, if light and temperature are controlled the use of greenhouse space can be optimized by extending the rooting period for the entire year.

Nutrition

The most important advantage of hydroponics mini-hedges is the possibility of providing well-balanced nutrition to mini-cuttings. The effect of macro- and micro-nutrients on root initiation of cuttings on several plant species, has been well documented in the literature (Andersen, 1986; Read, 1980; Hansen, 1987; Haissig, 1986; Hartman & Kester, 1983). As discussed earlier, nutrition is the key factor affecting rooting predisposition, because of its involvement in determining the morphogenetic response of the plants. In hydroponics mini-hedging system the nutrients are supplied in ideal concentrations to promote rooting. Hydroponics, without the influence of rainfall and or overhead irrigation, allows better nutrition control of mother plants. The use of indoor hydroponics mini-hedges allows nutrient supply in a correct concentration, avoiding the nutritional imbalance commonly observed in outdoors clonal hedges; besides there is no leaching by rainfall and the response to nutrient application is rapid. According to Haissig (1996), a feasible nutritional balance of mother plants can be linked to production of tryptophane, the precursor of IAA and to storage substances.

Calcium plays an important role in the rooting process, mainly because it acts as peroxidase activator (Haissig, 1986). According to practical experience of Klabin Riocell, the percentage rooting of mini-cuttings decreased considerably if the leaf calcium concentration dropped below 0.7%. This situation was observed especially in winter when, in the absence of heating, leaf calcium concentration was substantially lower than in warmer months. Calcium deficiency induces shoot apex necrosis in mini-cuttings, which can cause problems of rooting and mini-cutting development (McComb & Sellmer, 1987). This phenomena was commonly observed on the initial, operational scale, outdoor mini hedges.

In general, moderate deficiency of nitrogen can improve rooting (Haissig, 1986), but at high levels more energy is required for vegetative growth and especially for leaf expansion. Consequently, carbohydrates are not stored at suitable levels reducing the C:N. On the other hand, extreme

nitrogen deficiency can reduce rooting since it is necessary for nucleic acid and protein synthesis. Based on the considerations of Hartman & Kester (1983), and Haissig (1973b, 1986) a general model for leaf nutrient concentration can be established. The mother plant must be well nourished with macro nutrients like phosphorus, potassium, calcium and magnesium and moderately deficient in nitrogen.

The effects of micro-nutrients on rooting are variable. Manganese, due to its inhibitory effect should be used at minimal concentrations, but boron and zinc are essential for rooting process (Andersen, 1986). Zinc increases the endogenous content of auxins by increasing tryptophane proportions while boron is involved on rooting and root growth. Table 2 shows nutrient concentrations in leaves of well nourished plants (Roberto Ferreira Novais, pers. Comm.) for rooting mini-cuttings. Monitoring leaf nutritional concentration of the mother plants, as a guide to assure these levels of macro and micro-nutrients has been an important factor in improving the rooting competence of mini-cuttings.

Table2 Concentrations of macro- and micro-nutrients in leaves of well nourished *Eucalyptus* plants.

MACRO NUTRIENTS (%)						MICRO NUTRIENTS (mg/kg)				
N	P	K	Ca	Mg	B	Mn	Zn	Fe	cu	S
2,5	0,2	1,5	1,0	0,25	40	100	50	100	10	0,15
to	to	to	to	to	to	to	to	to	to	to
3.0	0,4%	2,0	1,5	40	70	500	60	200	15	0,25

Phytosanitary Advantages

Shoot contamination by splashing of soil during rainfall or over-head irrigation (Ferreira, 1996) is a very well known sanitary problem associated with outdoor clonal hedges. As many pathogenic fungi are soilborne, the cuttings produced either in clonal banks or in outdoor clonal hedges, are frequently contaminated by these fungi. Thus one of the advantage of using indoor hydroponics is the preventive phytosanitary control. In the absence of leaf wetness occurrence of leaf diseases is greatly reduced, thus producing pathogen free cuttings that increases the results of rooting. However, all these benefits of indoor system can be lost or become disadvantageous in the absence of efficient controls to minimize effects of external factors such as extreme temperature and humidity.

MANAGEMENT OF MINI-STUMPS IN HYDROPONICS SYSTEMS

The management of mini-stumps is rather simple, but some aspects must be considered for the technique to be successful. Mini-cutting collection should be done selectively to avoid mini-stump degradation. Desalination should be done at least once a week by generously irrigating the substrate to leach the surface-accumulated salt. In non-automated nutritive solution preparations, the entire solution should be changed every 15 days. In intermittent flooding better results are obtained when the electric conductivity is adjusted to 1,8 - 2,2 in the winter, and to 0,8 - 1,0 in the summer. Avoiding cut and abscised leaves to fall into the nutritive solution (Alfenas, 2001) prevents biotic and abiotic problems on mother plants.

USE OF MINI-CUTTING FOR CLONING OTHER WOODY SPECIES

The applicability of mini-cutting technique has been tested also on other, broad leafed and conifers woody species. In general, the methods used are similar to those of *Eucalyptus*, with minor adaptations. The results have shown that this technique can be fully applied to species like *Pinus taeda*, *P. elliottii*, *Acacia mearnsii* and *Ilex paraguayensis* and many other woody and non-woody species. The best results were obtained with juvenile plants as is the case with difficult to root *E. citriodora*, *E. maculata* and *E. paniculata*. Thus, mini-cutting is feasible to be used consistently for family multiplication of these species. Use of mini-cuttings can be a possible alternative for production of family forests by multiplication of full-sib or tested half-sib families.

Klabin Riocell started a small program of establishing *E. globulus* clonal plantation, based on adult and early selected trees resistant to rust caused by *Puccinia psidii* and other diseases. The preliminary results suggest that mini-cutting is a very promising technique to commercially propagate this species. Compared to stem-cutting, the mini-cutting gave better results in rooting proportion and root system quality. It appears that the propagation of *E. globulus* is profitable, since it is considered to have different and higher nutritional requirements. The development of special nutritive solutions for *E. globulus* tends to improve the results and it is clearly possible to establish efficient clonal program for this species.

In *Acacia mearnsii*, mini-cutting has shown good results, despite being considered as recalcitrant species with regard to vegetative propagation (Matheson 1990). Despite reasonable success obtained by Assis et al. (1993) in adult trees, application of mini-cuttings gave better results, indicating possibility of developing clonal forestry of this genus. The key point in this case is to achieve juvenile or rejuvenated shoots to start the process. Through induction of basal shoots, without tannin oxidation normally present in the chopped trees of this specie, it is necessary to establish the initial healthy rejuvenated plants, which should done using standing trees to avoid problems with tannin.

In *Pinus*, only juvenile seedlings were tested as mother plants. *Pinus elliottii* seedlings can be easily rooted by mini-cuttings with more than 85% success. *Pinus taeda* also roots well though somewhat less (76%) than *P. elliottii*. The results suggest the possibility of developing forestry families programs with both species based on the systems used for *Eucalyptus*. Managing *Pinus* mini-hedges in hydroponics is efficient for providing a good nutritional status to the mini-cuttings. The productivity of mini-propagules, without any change in the nutritive solution, was 9600/m²/year. The results of mini-cutting productivity and rooting proportion are highly encouraging.

REFERENCES

ALFENAS, A.C. Síntese das observações fitopatológicas e sugestões de controle em minijardim clonal e casa de enraizamento da Klabin Riocell. Relatório Técnico de Viagem. U.F.V., Departamento de Fitopatologia. Viçosa-MG. 10p.

ANDERSEN, A.S. Environmental influences on cuttings of non-woody species. In: JACKSON, M. B., ed. **New root formation in plants and cuttings**. Dordrecht: Martinus Nijhoff Publishers, 1986.p.223-253.

ASSIS, T.F. Production and use of *Eucalyptus* Hybrids for Industrial Purposes. In: HYBRID BREEDING AND GENETICS OF FOREST TREES. Dungey, H. S., Dieters, M. j. and Nikles, D. J. (Compilers). Pp. 63-75. **Proceedings** of QFRI/CRC-SPF Symposium, 9-14 April 2000, Noosa, Queensland, Australia. (Department of Primary industries, Brisbane).

ASSIS, T.F. & ROSA, O. P. & GONÇALVES, S. I. Propagação clonal de *Eucalyptus* por microestaquia. In: CONGRESSO FLORESTAL ESTADUAL, 7. Nova Prata, 1992. **Anais**. Santa Maria: UFSM. 1992. P.824.

ASSIS, T.F. Propagação vegetativa de *Eucalyptus* por microestaquia. In: IUFRO CONFERENCE ON SILVICULTURE AND IMPROVEMENT OF EUCALYPTS, Salvador, 1997. **Proceedings**. Colombo: CNPF/EMBRAPA, 1997. V.1, P.300-304.

ASSIS, T.F. & HIGA, A.R. & ROSA, O.P. & BAUER, J.F.S. Propagação vegetativa da acácia negra (*Acacia mearnsii*). In: CONGRESSO FLORESTAL PANAMERICANO, 1/CONGRESSO FLORESTAL BRASILEIRO, 7. Curitiba, 1993. **Anais**. São Paulo: SBS-SBEF, 1993. V.1, P.150-152.

CAMPINHOS, E. & IKEMORI, Y.K. Introdução de novas técnicas na produção de mudas de essências florestais. **Silvicultura**, v.8,n.28, p.226-228, 1983.

CAMPINHOS, E.N. & IANNELLI-SERVIN, C.M. & CARDOSO, N.Z. & ALMEIDA, M.A. & ROSA, A.C. Hidrojardim clonal Champion: Uma otimização na produção de mudas de eucalipto. **Silvicultura**, 80: 42-6, 2000.

CARVALHO, P.L.T. & MOREIRA, A.M. & SOUSA, A.J. & BERTOL, R & MAGNAGO, J.M. & BUFFON, J.B. & AZEVEDO, J.A. Jardim clonal como área de multiplicação de estacas na Bahia Sul Celulose S/a. In: SIMPOSIO IPEF, 2, São Pedro, 1991. **Anais**. Piracicaba: IPEF, 1991. P.71-75.

CHAPERON, H. Vegetative propagation of *Eucalyptus*. In: SYMPOSIO SOBRE SILVICULTURA Y MEJORAMIENTO GENÉTICO DE ESPECIES FORESTALES, Buenos Aires, 1987. **Anais**. Buenos Aires: C.I.E.F, 1987. V.1 P. 215-223.

DENISON, N.P. & KIETZKA, J.E. The development and utilization of vegetative propagation in Mondi for commercial afforestation programmes. **South African Journal of Forestry**. 166: 53-60. 1993.

FERREIRA, F.A. Enfermidades do eucalipto no Brasil. **Informe Agropecuário**, v.18, n.186, 5-19, 1997.

FERREIRA, M & SANTOS, P.E.T. Melhoramento genético florestal dos Eucalyptus no Brasil: breve histórico e perspectivas. In: IUFRO CONFERENCE ON SILVICULTURE AND IMPROVEMENT OF EUCALYPTS, Salvador, 1997. **Proceedings**. Colombo: CNPF-EMBRAPA, 1997. V.1P.14-34.

FRANCIET, A. & BOULAY, M. & BEKKAOUI, F. & FOURET, Y. & VERSCHOORE-MARTOUZET, B. & WALKER, N. Rejuvenation. In: Bonga, J.M. and Durzan, D.J. ed. **Cell and Tissue Culture in Forestry**. Vol. 1. Dordrecht: Martinus Nijhof, 1987. P.232-248

GONÇALVES, A.N. & CROCOMO, O.J. & ALMEIDA, C.V. & UNTERPERTINGER, J.P. & CHAVES, R.A.B. Reversion to juvenility in micropropagation of *Eucalyptus*. In: INTERNATIONAL ASSOCIATION OF PLANT TISSUE CULTURE, 6.,1986, Minneapolis. **Abstracts...** Minneapolis, 1986.

HAISSIG, B.E. Metabolic processes in adventitious rooting of cuttings. In: Jackson, M.B. **New root formation in plants and cuttings**. Dordrecht: Martinus Nijhof, 1986. p.141-189.

HAISSIG, B.E. Metabolism during adventitious root primordium initiation and development. **New Zeland Journal of Forestry Science**, v.4,n.2,p.324-337,1973.

HANSEN, J. Stock plant lighting and adventitious root formation. **HortScience**, v.22,n.5,p.746-749,1987.

HARTMAN, H.T. & KESTER, D. E. **Plant propagation principles and practices**. New Jersey: Prentice-Hall, 1983. 727p.

HARTNEY, V.J. Vegetative propagation of the *Eucalyptus*. **Australian Forest Research**, v.10,p.191-211,1980.

HIGASHI, E.N. & SILVEIRA, R.L.V.A. & GONÇALVES, A.N. Propagação vegetativa de *Eucalyptus*: Princípios básicos e a sua evolução no Brasil. 2000. 10p. (IPEF-ESALQ-USP. Circular Técnica, 192).

IANNELLI, C. & XAVIER, A. & COMÉRIO, J. Micropropagação de *Eucalyptus spp.* na Champion. **Silvicultura**, **66**: 33-5, 1996.

MATHESON, A. C. Breeding strategies for MPTs. In Tree improvement of multipurpose species. Multipurpose tree species network technical series. Vol 2, 1990. P. 67-99.

McCOMB, B.H. & SELLMER, J. C. General media and vessels suitable for woody plant culture. In: BONGA, J. M. DURZAN, D. J. **Cell and tissue culture in forestry**, 1. Dordrecht: Martinus Nijhoff, 1987.p.4-16.

NANDA, K.K. & JAIN, M. K. & MALHOTRA, S. Effects of glucose and auxins in rooting etiolated stem segments of *Populus nigra*. **Physiologia Plantarum**, v.24,p.387-391, 1971.

PATON, D.M. & WILLING, R. R. Inhibitor transport and ontogenetic age in *Eucalyptus grandis*. In: **Plant growth substances**. Tokyo: Hirokawa, 1974,p.126-132.

READ, P.E. Introduction to the symposium. **HortScience**, v.22, n.2, p.736-737, 1987.

VIEITEZ, F.J. & BALLESTER, A. Effect of etiolation and shading on the formation of rooting inhibitors in chestnut trees. **Phyton**, v.48,n.1/2,p.13-19, 1988.

XAVIER, A. & COMÉRIO, J. Microestaquia: uma maximização da micropropagação de *Eucalyptus*. **Revista Árvore**, Viçosa, v.20, n.1,p.9-16, 1996.

XAVIER, A. & WENDLING, I. Miniestaquia na clonagem de *Eucalyptus*. 1998, 8 P. (SIF/UFV. INFORMATIVO TÉCNICO SIF, 11).

WENDLING, I. & XAVIER, A. & GOMES, J.M. & PIRES, I.E. & ANDRADE, H.B.
Propagação clonal de híbridos de *Eucalyptus spp* por miniestaquia. **Revista
Árvore**, Viçosa, v.24, n.2, p.181-186, 2000.

ZOBEL, B.J. & IKEMORI, I. Vegetative propagation in *Eucalyptus*. In: Clonal
Forestry: its impact on tree improvement and our future forests. Zsuffa, L. &
Rauter, R.M.; % Yetman, C.W. eds. Pp.136-144. **Proceedings** of 19th MEETING OF
THE CANADIAN TREE IMPROVEMENT ASSOCIATION part 2. 1983. August, Toronto.

Zobel, B.J. Clonal forestry in the Eucaypts. In: Ahuja, M.R. & Libby eds.
Clonal Forestry II: Conservation and application, Springer-Verlag, Berlin,
1992. P.139-148.