

CLONAL PROPAGATION OF EUCALYPTUS IN BRAZILIAN NURSERIES

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Abstract

Brazil has established extensive *Eucalyptus* plantations to support a growing forest products industry. During the past 25 years, the country has been a pioneer in developing clonal propagation systems to regenerate these highly productive plantations. Original clonal selections optimized disease resistance, coppicing ability, and volume growth, while recent priorities have moved to improve volume growth and wood quality. The techniques used for the establishment and management of clone banks has seen fundamental changes during the development of clonal systems. From early clone banks, established at wide spacings over large areas, there has been a gradual shift to intensively managed smaller units. The evolution in clonal techniques has increased planting stock quality and lowered prices. Brazilian industrial programs commonly produce millions of cuttings from tissue culture techniques and intensively managed hedges in green houses. Specific technologies have been developed regarding container medium, fertilization, and environmental management. These technologies are typically produced through cooperative industry and academic programs.

Key Words

Vegetative propagation, clonal orchards, rooted cuttings

The word 'Brazil' often conjures up images of Rio de Janeiro, Carnival, sparkling white sand beaches, and the Amazon River. But Brazil has much more to offer, namely a significant and highly productive forest products industry. Most of the forest industry concentrates in the southern one-third of the country between 20° to 30° south latitude, particularly the states of Sao Paulo, Minas Gerais, and Bahia. Brazil is a large country, and forestry plantations can be found in the northern part of the country near the equator as well.

Although Brazil grows loblolly, slash, and Caribbean pine, the *Eucalyptus* planting program is over 80% of the total annual planting program, according to the Brazilian Pulp and Paper Association (BRACELPA 1999). *Eucalyptus* is used for pulp and paper, charcoal, and some solid wood products. The more commonly planted *Eucalyptus* species are *grandis*, *urophylla*, *saligna*, and several hybrids, particularly *grandis x urophylla*, or

urograndis. Rotation lengths are normally five to eight years while average growth rates are reported at 20 to 40 m³/ha/yr. *Eucalyptus* is cultivated using intensive practices that include mechanical and/or chemical site preparation, leaf cutting ant control, fertilization, hand planting containergrown stock, watering, and chemical, mechanical, and manual weed control (McNabb 1994). A typical planting program is 5,000 to 15,000 ha/year, requiring large scale nursery activities. The majority of this *Eucalyptus* planting program is based on the vegetative propagation of individually tested and selected clones.

Brazilian forestry has been a pioneer in the development of nursery techniques for the clonal propagation of *Eucalyptus*. Originally, clonal propagation was viewed as a method to improve plantation productivity and uniformity, disease resistance, and coppicing ability (Campinhos and Ikemori 1983). The objectives of the program

have gradually changed over the years to emphasize wood volume plus wood quality. Coppicing is no longer an objective, as the rate of clonal selection and improvement results in new clones being planted in successive rotations. Some of the first work on clonal propagation was done in the early 1970s at the Piracicaba campus of the University of Sao Paulo (Poggiani and Suiter Filho 1974). The process was moved to large scale production forestry by the private sector at Aracruz Florestal in the late 70s and early 80s (Campinhos and Ikemori 1983). The pioneering work of Aracruz has been widely recognized and has resulted in their forestry personnel winning the Wahlenburg prize for advances in forestry technology. Although the current clonal process is basically the same today as it was 15 years ago, there have been significant changes in efficiencies and techniques, particularly in the last five years.

THE CLONAL SELECTION AND PROPAGATION PROCESS

Individual trees are first selected in existing plantations for the morphological characteristics of growth, health, and branching patterns. Trees can be selected as early as three years after planting. Selected trees are then cut down and the coppice sprouts are used to vegetatively propagate a number of cuttings sufficient for a field testing program. Simultaneously, wood samples are analyzed for wood properties such as lignin content, specific gravity, and fiber length. Clonal tests are conducted on a variety of sites, with each of these sites carefully characterized by thorough in-house soil testing and analysis. Final selection for inclusion in the planting program is based on field performance, nursery performance (including rootability), and wood properties. Once selected, clones are then placed in a clone bank to be used for mass propagation.

The first clone banks used for cutting production were established at a 3- by 3-meter spacing (1,111 plants per hectare) and cultured similar to a plantation (Higashi and others 2000). Each individual plant was managed as a "hedge" with multiple sprouts removed from each stool. One or more stems were usually left to serve as a carbohydrate source for the production of future cuttings. Over time this system developed into a closer spacing at 0.5- by 0.5-meters with 40,000 plants per hectare. Now called a "clone garden,"

they were moved adjacent to the nursery, irrigated, and fertilized. Each stool was still continually hedged, but cutting production began earlier, and the frequency of harvesting increased. As a result, the ratio of clone garden to area planted increased from 1:44 to 1:525 (in other words, 1 ha of clone garden could produce enough cuttings to reforest 525 ha with planting stock) (Higashi and others 2000). The Trouts removed from hedges were transported to the nursery and processed into cuttings of 10- to 15-cm lengths in the case of clone banks and 6- to 8-cm lengths in the case of clone gardens. A cutting consisted of a semihardwood stem section with a single pair of leaves that were usually cut in half. The base of the stem was dipped in an IBA treated talc (usually 5,000 ppm) and transplanted to the growing container. For the past ten years Brazilian nursery managers have mostly used the "Hawaiian dibble tube" with about a 3-cm top circumference, 10- to 15- cm in length, and around a 50-cc volume. Container medium varies by company but is normally a mixture of composted pine bark, vermiculite, and carbonized rice hulls. It is typically a light, well aerated mixture that is well drained yet having good water retention properties.

After cuttings are planted into individual containers, they are placed under misting in a greenhouse or shade house. Rooting is commonly above 75% and robust enough to grow out of the bottom of the containers during this phase. After approximately 45 days, the rooted cuttings are removed from misting to an area covered with 50% shade cloth. At this point, empty containers (dead cuttings) are removed and the live plants are separated by size. Cuttings remain under shade for an additional 10 to 20 days and then are moved to full sunlight. After an additional 30 days they are ready for planting in the field. During this process, the cuttings are routinely fertilized, usually through the irrigation system. Smaller plants may receive increased fertilization. One of the most perplexing problems encountered in this system was an increase in the variability of cutting rootability and growth in the nursery. The continual coppicing of *Eucalyptus* hedges resulted in harvesting cuttings of a variety of physiological ages and sizes. The Brazilian nursery community referred to this as the "C effect" and considerable effort was applied to

solve the problem (Xavier and Comerio 1996). While the clonal garden was able to maintain the juvenility of cutting material necessary for successful rooting, over a period of time the degree of juvenility began to vary, resulting in reduced rooting success, variable rooting timing, and increased sorting. All of this translated into higher production costs and lower planting stock quality. The current clonal garden techniques have practically eliminated this effect and greatly increased stock quality.

CURRENT PROPAGATION TECHNIQUES

The majority of current *Eucalyptus* clonal propagation in Brazil is based on indoor clone gardens. These gardens are planted in raised beds of fiberglass or concrete 20 to 30 cm deep, 40 to 60 cm wide, and 8 to 12 m long. They are usually filled with washed sand and may be irrigated by internal flow from one end to the other or by drip irrigation from above with drainage at one or both ends. Nutrition management in these indoor clonal gardens is an integral part of their success (Higashi and others 2000), and considerable research has been devoted to finding the most satisfactory combinations of macro- and micro-nutrient fertilization to maximize production. The clonal material for establishing indoor gardens is initiated and maintained by using tissue culture techniques. Clones that have been identified through the clonal testing program are first established in test tube media under controlled light and temperature conditions. As the explants callous and sprout (they are not allowed to root), shoot tips are removed and transplanted to "elongation" tubes where they grow to between 2 to 3 cm in length. Next these elongated shoot tips are transplanted to container media for rooting and then used for planting in the indoor clone garden. The utilization of tissue cutting techniques and "micro-propagation" is now an integral part of most industrial *Eucalyptus* cloning programs in Brazil.

Harvesting of the "mini-hedges" for nursery propagation begins 20 to 30 days after planting the indoor clone bed. It is important that the harvesting of plant material occurs when sprouts reach a constant height from the bed in order to maintain a consistent level of juvenility, giving the clone garden a "mowed" appearance. Shoot tips of 2- to 3-cm lengths are harvested for use in

cutting propagation. Each cutting has a single pair of leaves with 50% of the leaf blade removed. The cutting is planted into the standard container and follows the standard cutting propagation series of misting, shading, full sun, and well-monitored liquid fertilization. These shoot tip cuttings do not require basal hormone treatment to ensure rooting. In fact, even without IBA application, rooting generally increases by about 10% over stem section cuttings. There are also significant changes in planting stock quality. When using semi-hardwood cuttings, shoots and roots tended to initiate from lateral buds along the stem. When using the 2- to 3-cm shoot tip softwood cutting, the shoot tip forms the new apical meristem and the lead growing tip does not originate from the side of the plant. In addition, roots tend to grow straight down from the base of the cutting as opposed to growing from the side of the stem. The result is a faster-growing, more vigorous nursery stock.

The combination of tissue culture techniques and indoor hedging gardens results in a number of significant improvements in the production of planting stock: the time required for nursery production of planting stock is shortened by about 20%, rooting is increased by about 15%, planted stock is more uniform, and the plantations themselves are more vigorous and uniform, resulting in faster crown closure. Furthermore, not only is the indoor clonal mini-garden more economical to manage than larger outdoor gardens, more land is available for plantations because the land area previously used for the outdoor clonal garden is freed up (lanelli and others 1996). The result is a highly proficient system that produces healthy, vigorous, genetically advanced planting stock for as little as \$0.03 to 0.05 in USA currency.

FUTURE DIRECTIONS

Much of the recent progress in *Eucalyptus* clonal regeneration in Brazil has come about through cooperative efforts of universities and the forest industry. Several Brazilian universities have forestry research cooperatives similar to those found in the United States that routinely develop collaborative projects of mutual benefit. Clone based tree improvement and propagation has been one of the success stories of that collaboration. The Brazilian research community continues to place a high priority on plantation regeneration,

and there are several areas of on-going research to improve clonal *Eucalyptus* propagation. Some companies are interested in "closing the system" in their greenhouses and production areas. Because of environmental impact concerns, companies are searching for ways to circulate and reuse irrigation water. Aside from occasional pesticide application, there is heavy fertilization used during the propagation process, and a "closed system" would eliminate or greatly reduce the release of nitrogen, phosphorus, and other fertilizers into the environment. Other improvements may be made in the selection of soil medium used in the indoor mini-garden beds, looking for a well-aerated medium with good moisture availability that can be reused. Improved rootability of the unsterile cuttings used in the first stage of tissue culture can also be studied. The resolution of these and other problems will ensure that the Brazilian system will remain on the forefront of efficient *Eucalyptus* culture.

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