Attack of the Clones: Somatic Embryogenesis in Forestry

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Wood is one of our most important natural products. It is impossible to imagine modern life without wood and its applications, yet only recently have we begun to divert wood harvesting from now dwindling natural forests. If consumption continues at the current rate, we will soon be faced with a shortage of wood and natural forests. One solution is to grow our trees in a managed, organized and intensive manner. This is the solution that plantation forestry offers. By implementing biotechnology it is conceivable that tree plantations could meet most human demand. Native forests would thus be left untouched for future generations to enjoy.

Wood consumption

Global wood usage is estimated to be between 3.5 and 4 billion m3 per year. (Sutton,W. 1999). When this value is averaged across the 5.6 billion people on earth we get the more meaningful number of just under 2 L of wood/person/day. Certain countries, such as the USA and Canada, consume 3.5 times the global average (Bowler 1992) while other less developed countries use much less.

The FAO estimates that at their peak, forests covered 30% of the earth surface, or about 3.9 billion ha. Of this area, approximately 80% has been affected in some way by human activities such as logging, agriculture, and settlement. With current projections of wood usage, it appears that there may soon not be enough forestland to support human expansion. Since drastic conservation efforts appear slow and ineffective, there is an obvious need for a more efficient approach to producing wood and meeting consumption demands (Strauss 1999). The one new approach to forestry that is revolutionising wood production is plantation forestry.

Plantations

Tree plantations are not a new idea. There are examples of species such as Cunninghamia lanceolata (Chinese fir), which have been grown in high-density tree farms for at least 800 years. (Minghe 1999). Other examples include Teak and Mahogany trees that have traditionally been grown for their wood's characteristics. Maple and Rubber trees have also been farmed, not exclusively for wood, but also for their economically important sap. These types of tree farms have historically been relatively small, family run operations. In contrast, contemporary tree plantations are generally industrial scale operations, expanding over thousands of hectares. Because of this difference, it was only in the late 1970s that forestry companies realized the benefits of plantations and began investing huge amounts of resources in such enterprises (Sedjo 1999). In this short period of time, over 100 million ha of plantation tree species have been planted throughout the world. (Bazett, 1993).

There are several significant environmental advantages to growing trees in a plantation, rather than relying on natural forests. Firstly, plantations provide an opportunity to conserve remaining natural forests for other purposes. Robert Sedjo (1999) summarised it best by writing that plantations should "*be used to deflect logging away from natural forests*": It should be possible to grow all the wood the we need, on managed plantations, and eliminate the need to log wild forests. The use of trees by countries in North America to acquire Carbon Credits and help to buffer climate change is another consideration. By planting plantation forest for the specific purpose of being a "Carbon Sink", nations could be compliant with the Kyoto Protocol. Plantation forests should offer a viable means of both conserving natural forests and reducing the amount of CO2 in the atmosphere

The other assets of plantation forests are related to their productivity. Like with most other domesticated crops, by managing tree farms in an intensive and organised manner, forestland productivity can be increased several fold. Using an example from Sedjo (1999), we can calculate northern Boreal forest (such as is found to grow across Canada and northern Europe) to have a productivity of 1-10 m3/ha/yr. Similarly we can calculate the productivity of tropical plantations to be between 30-70 m3/ha/yr. This significant increase is due to the correlation between forest productivity, and solar input and temperature (Robinson 1999). Not only do tropical plantation species grow extremely fast, but by having trees that grow to full size in 7-30 yrs (tropical plantation) rather than 60-100 yrs (northern native forest), the crop rotation time is reduced substantially. With a shorter rotation time landowners do not have to wait as long to profit from their initial investment. It is not surprising that most commercial forestry companies are moving their operations South to benefit from the longer growing season, more favourable growing conditions, and shorter rotation times.

Major players in plantation forestry include the South Eastern United States, Brazil, Chile, Australia, and New Zealand. In the case of Brazil, it is estimated that 100% of the wood harvested for pulp and paper comes from intensive tree plantations (Sedjo 1999). It is also estimated that a plantation the size of Sweden, grown in Brazil, could fulfill current global wood demands (Robinson 1999). These regions all have one thing in common. They are all found at relatively low latitudes, where growing seasons are long, and neither temperatures nor precipitation are limiting.

Quality improvements are another motive for a transition to plantation forestry. The primary quality trait for most forest product companies is uniformity. The fact that plantations are managed systems makes it easy to control the tree in ways that are not practical in a native forest. Pest management and fertilising help optimise growth, while pruning and weed control can



Figure 1. A Loblolly Pine.

ensure that a tree grows into the desired form. As well, plantations are generally stands of roughly the same age and dimensions. This degree of uniformity also allows for automated processing and a more homogeneous end product. The synergistic effect of improved quality and increased productivity is a huge economic benefit for the forest owner. Shorter rotation times, cheaper harvesting, and lower processing costs are the main economic benefits of plantation forestry.

The Trees

To date, it is estimated that humans have used over 1000 tree species as a source of wood. Dozens of these tree species are grown in plantation format, but there are five taxa that are particularly prominent (Sutton W. 1999). These five tree species are: Douglas-fir (*Pseudotsuga menziesii*), Loblolly pine (*Pinus taeda*), Radiata pine (*Pinus radiata*), Poplars (*Poplus spp.*), and Eucalyptus (*Eucalyptus spp.*) (Sutton W. 1999). The basic characters they share are:

- 1. Fast growth on a variety of geographies
- 2. Relatively disease and pest resistant
- 3. Can thrive in high density planting
- 4. Responsive to genetic improvement, be it breeding or genetic modification
- 5. Responsive to management techniques, such as pruning or cultivation
- 6. Provide wood of desirable quality and versatility.

Each of these five trees has unique advantages. Ranging from the ability of Poplars to withstand substantial ranges of stresses (Mann and Plummer 2002) to the fact that Brazilian grown eucalyptus can grow to the height of 35m and be ready for harvest in 7 years (Sedjo, 1999), there is a consensus throughout the industry that these trees are fixtures in the plantation landscape.

Plantation sites are usually chosen because of proximity to roads and processing infrastructure, as well as a moderate landscape (rather than steep mountains) that lends itself well to mechanized management and harvesting (Sutton W. 1999). Quite often plantations utilize former or abandoned agricultural land. The biggest hurdle that limits the so-called domestication of trees is the fact that generation times are so long. Whereas crops such as corn have been bred for thousands of years, gone thought thousands of generations, and looks very little like their original ancestors, Pines have generation times in the tens of years and have undergone very few breeding cycles (Merkle and Dean, 2000). As a result, plantation pines have barely diverged in phenotype from their wild cousins. This is where biotechnology can potentially speed the domestication of trees for the purposes of agro-forestry plantations.

Clonal forestry

Vegetative (clonal) propagation of forest trees is the logical extension of simply farming trees. By propa-

gating plants in a clonal manner it is possible to massproduce the elite performers in a population of any given trait, without having to wait for hundreds of generations of breeding (Williams 2001). For this reason, horticulturalists have been regenerating plants from grafts or rooted cuttings for centuries. Examples of plants that are propagated through clonal methods include most fruit trees, grape vines, ornamental roses, tulip bulbs, bamboo, and bananas. An instance of trees being clonally propagated for the purposes of wood production is Cumminghamia lanceolata (Chinese fir). This species has been propagated from cuttings, and grown on large-scale plantations for at least 800 years (Minge and Ritchie 1999). It also appears that certain genotypes were selected for particular sites and performance traits.

Preserving and optimizing performance traits, such as growth rate, stress tolerance, or wood quality, are the essence of clonal forestry. Any trait (growth rate, for instance) in a population such as a natural forest or seed orchard will have a normal distribution. By selecting individuals from the population that have exceptionally high growth rates, mass producing these individuals as identical clones, and planting them in a plantation, the mean growth rate of the plantation should be significantly higher than that of the original source population. According to Ben Sutton (2002), the theoretical increase in mean growth rate between a source population and the clonal population could be 25-40%.

Attree and Fowke (1993) describe several other applications of clonally propagating forest trees, other than increasing a plantation's average growth rate. Examples of these include:

- Cloning species such as Norway spruce or some Larches that are difficult to commercialize in plantations because they do not produce large amounts of seed
- Customizing plantations to tolerate environ mental stresses, or to extend a species' habitat range
- Selecting for pest resistant genotypes
- · Conserving/ rescuing endangered trees
- Propagating ornamental species of interest; such as extremely colourful phenotypes of Colorado blue spruce.

 Production of well formed Christmas trees, without the need for costly pruning or training.

The major technical limitation when propagating trees vegetatively from shoot cuttings is that rooting the cuttings is not always feasible. Also for many species of trees, there is no practical method of rooting cuttings, or when there is, the source plants can have production limitations. The best alternative propagation system is called somatic embryogenesis (SE). The propagules produced by this process are often dubbed "Artificial seeds", because of the fact that plant embryos are multiplied *in vitro* in the absence of a seed coat.

A summary of the process is as follows (Attree and Fowler1993), (Pullman 2003):

- 1. Seed is collected for controlled breeding crosses between two elite quality parent trees.
- 2. The embryo is dissected form the seed and placed on specially formulated Initiation Media. The embryo reverts back into a mass of non-differentiated tissue called embryogenic callus.
- 3. This resulting mass can be cryogenically frozen in the form of a library, bulked up in bioreactors, or maintained on Maintenance Media.
- 4. The embryogenic callus can then be placed on Maturation Media, rich in Abscisic Acid



Figure 2. An embryogenic callus.

(a plant growth regulator that is integral to inducing differentiation).

- 5. After several weeks most of the embryogenic callus will have differentiated into viable embryos.
- 6. These embryos can then be germinated and grown into tree as a normal seed.

By using such techniques it is theoretically possible to generate an infinite number of genetically identical embryo propagules from a single seed. Somatic embryo technology is also important from a transgenics standpoint: Whereas most transgenic corn lines are the result of a single transformation event that was then bred into numerous commercially important cultivars, this strategy is not practical for long generation time species such as pine. Since breeding a trait (trans-gene) into a pine cultivar would take decades, it is much faster to transform a pine genotype of interest and clonally propagate it *ad infinitum*. For this reason, a reliable clonal propagation platform is essential for the commercialization of genetically engineered trees.

The Critics

The main criticism of plantation forestry, in particular clonal forestry, is that it lowers biodiversity. This is true if only the biodiversity of the plantation itself is considered. While it is the case that plantations are not going have the same species diversity as a native forest, they are not intended to. Plantations are designed to produce a maximum amount of wood with a minimum footprint, not to simulate a natural forest. If plantations are viewed in the context of a tree farm that reduces logging of natural forests at a global level (thus maintaining ecosystem diversity), then lower biodiversity in at a local scale is acceptable. There is also the criticism that the monoculture of clonal forestry is risky, because a single pathogen could impact all trees equally. In the instance of crops such as grapes or apples, which are clonally propagated, landowners plant a range of genotypes in order to ensure that they are not entirely dependent on a single genotype that could be susceptible to certain pests. The same is true for clonal forests. By maintaining high genotypic diversity within a plantation by using a mosaic like distribution and small planting blocks, it is possible to buffer the effects of any pest of the entire plantation (www.cellfor.com).

The reality of plantation forestry is that plantations are not going to be forests, but rather an entity that produces wood in the most efficient way possible. By applying a combination of conventional breeding, clonal propagation, genetic modification, and marker related technologies; it should be possible to maximize the yield of plantations, and to eliminate the need to log native forests. By doing so, natural forests would be relieved of the pressures imposed by logging and could be set aside for other valued functions such as wildlife habitat, recreation, scenery, and biodiversity (Sutton 1999). As for meeting wood consumption demands, plantations will certainly help meet increasing demand for inexpensive wood and fiber, but consumption rates cannot continue to increase indefinitely. Eventually conservation and reduction will have to be considered if a sustainable level is to be achieved. Finally, there can be no illusion that by growing trees in plantations, deforestation will cease. Globally, agriculture and the expansion of cities are a larger source of deforestation that logging. In conclusion, biotechnology will be essential to ensure that wood needs are met in a long term and sustainable manner, through high yielding plantation forests.

References

- 1. Sutton, W.R.J. The need for planted forests and the example of Radiata pine. *New Forest.* **17**, 95-109 (1999).
- Bowler, J.L. Responsible Environmentalism-Forests, People, Raw materials. Hamilton Rodis Manorial Lecture series No. 1 Department of forestry, College of Agriculture, University of Wisconsin, Madison. (1992).
- Strauss, S., Boerjan, W., Cairney, J., Campbell, M., Dean, J., Ellis, D., Jouanin, L., Sundberg, B. Forest biotechnology makes its position known. *Nature* 17, 1145 (1999).
- 4. Minghe, L. & Ritchie, G.A.Eight hundred years of clonal forestry in China: traditional afforestation with Chinese Fir (Cunninghamia lanceolata) *New Forest* **18**, 131-142 (1999).

5. Sedjo, R.A. The potential of high-yield plantation forestry for meeting timber needs. *New Forest* **17**, 339-359 (1999).

- Bazett, M. Industrial wood: Shell/WWF tree plantation reviews Study #3 (Panda house, Weyside Park, Godalming Surrey, 1993).
- 7. Robinson, C. Making forest biotechnology a commercial reality. *Nature*. **17**, 27-30 (1999).

8. Mann, C.C. and Plummer, M.L.Forest Biotech edges out of the lab. *Science* **295**, 1626-1629 (2002).

- Merkle S.A. and Dean J.F.D Forest tree biotechnology. *Current Opinion in Biotechnology* 11, 298- 302 (2000).
- Williams, C.G. Forestry's third revolution: integrating biotechnology into Pinus taede L. breeding programs. *SJAF* 25, 116-121 (2001).
- 11. Sutton, B. Commercial delivery of genetic improvement to conifer plantations using somatic embryogenesis. *Ann. For. Sci.* **59**, 657-661 (2002).
- Attree, S.M. & Fowler L.C. Embryogeny of gymnosperms: advances in synthetic seed technology of conifers. *Plant Cell, Tissue and Organ Culture* 35 1-35 (1993).
- 13. Cellfor Inc. (2003) *When can we grow a better tree?* Retrieved August 17, 2004 from: www.cellfor.com