RESEARCH NEEDS AND THE VALUE OF FOREST BIOTECHNOLOGY AT UNION CAMP CORPORATION

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Abstract: -- Union Camp has had an active research program in forest biotechnology for twelve years. The first objective of this program is to develop genetically improved planting stock and deploy these as clones on an operational scale. Once superior clones are identified, the next objective is to make further gains in productivity through genetic engineering. Toward this goal, in 1995, Union Camp became the first US Forest Products Company to field test genetically engineered clones of sweetgum.

Our experience allows us to assign a relative value to forest biotechnology (i.e., genetic engineering and genetic mapping). In addition, it has brought sharply into focus the research needs for operational clonal forestry and the non-traditional genetic improvement of pine and hardwood. The research needs and the value of biotechnology from Union Camp's point of view will be presented.

Keywords: Biotechnology, clonal forestry, genetic engineering, genetic mapping

INTRODUCTION

The goal of Union Camp's forest biotechnology program is to improve forest productivity, (i.e., growth rate) by assisting in the development of clonal forestry in loblolly pine and hardwoods. Once superior clones have been identified, further gains in productivity are expected to be made through genetic engineering of those clones. Union Camp also has a program in genetic mapping. The objective of the mapping program is to characterize the individual genetic factors that contribute to' growth rate. Loblolly pine is the focus of the mapping effort, not only because relevant mapping population are available in that species, but also because the development of molecular markers for growth rate would enable the use of cloning techniques developed for immature, but unproved genotypes.

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To successfully develop plantations consisting of genetically engineered clones of pine and hardwood, three components are necessary - germplasm, large-scale vegetative propagation and transformation. This paper will outline Union Camp Corporation's efforts in each of these three areas for both loblolly pine and a representative hardwood, sweetgum. Areas in which further research is needed to make operational plantations of genetically engineered trees a reality are highlighted.

HARDWOOD BIOTECHNOLOGY (SWEETGUM)

Species. Sweetgum has been the hardwood species that has received the most attention. Sweetgum is the "russet potato" of Southeastern hardwood species. That is, sweetgum is not outstanding in any one characteristic, but is good in many different traits. For example, sweetgum will grow on a wide variety of sites, has few disease and insect pests, and is an acceptable furnish for bleached Kraft pulps. From a biotechnological standpoint, it is relatively easy to vegetatively propagate (from tissue culture and rooted cuttings) and genetic transformation methods are well established.

Germplasm. Unlike loblolly pine, Union Camp does not have a long tradition of selection and breeding with sweetgum. Neither can we afford to wait 40 years until we have achieved the same level of success with hardwoods as we have with pine. Therefore our short-term to sweetgum improvement program is to select trees from genetic tests and test them as clones. The success of this approach is based on our ability to vegetatively propagate selection age trees. Cloning selection age trees has been a viable approach.

Shoot cultures from selection age trees are established by the method of Sutter and Barker (1985). Plantlets micropropagated from mature trees are then used as stock plants for rooted cutting production. Selected trees are tested as clones by propagating them as rooted cuttings from the micropropagated stock plants. Clonal testing has been underway for nine years and during this period, several hundred selections have been evaluated. At present, a small number of these clones have been chosen for scale-up. The magnitude of genetic gain in growth rate projected with these clones is similar to that seen in eucalyptus (Wright 1995).

Vegetative Propagation. Methodology for the large-scale production of sweetgum from rooted cuttings has been developed. Stock plant management, rooting conditions (both in containers and in the nursery), and regimes to grow rooted cuttings into plantable seedlings are in place. Micropropagation was used initially for clone capture (as noted above) but is now under investigation as a means of large-scale vegetative propagation.

Genetic gain in volume growth from utilizing clones (in the form of lower wood costs) will not be realized if the cost for producing clones planting stock is too high. Figure 1 shows the break-even cost of planting stock (relative to seedlings) as a function of genetic gain. A considerable increase in planting stock cost can be tolerated to vegetatively propagate elite material.

Micropropagation has traditionally been one of the most expensive ways to propagate woody plants. The labor associated with the intensive handling is the most significant component of the cost associated with micropropagation. However, the advent of automation technology may be able to go a long way toward reducing labor cost by eliminating the multiple handling of cultures and microshoots. Figure 2 gives an indication of how the propagation rate (i.e., the number of microshoots harvested from culture and set as cuttings) influences the cost of planting stock. Propagation rates on the order of four shoots per minute are needed to match the cost of seedlings. These propagation rates require that conventional, agar-based tissue culture systems become about 100 times more productive, and that each microshoot be touched only once by human hands.

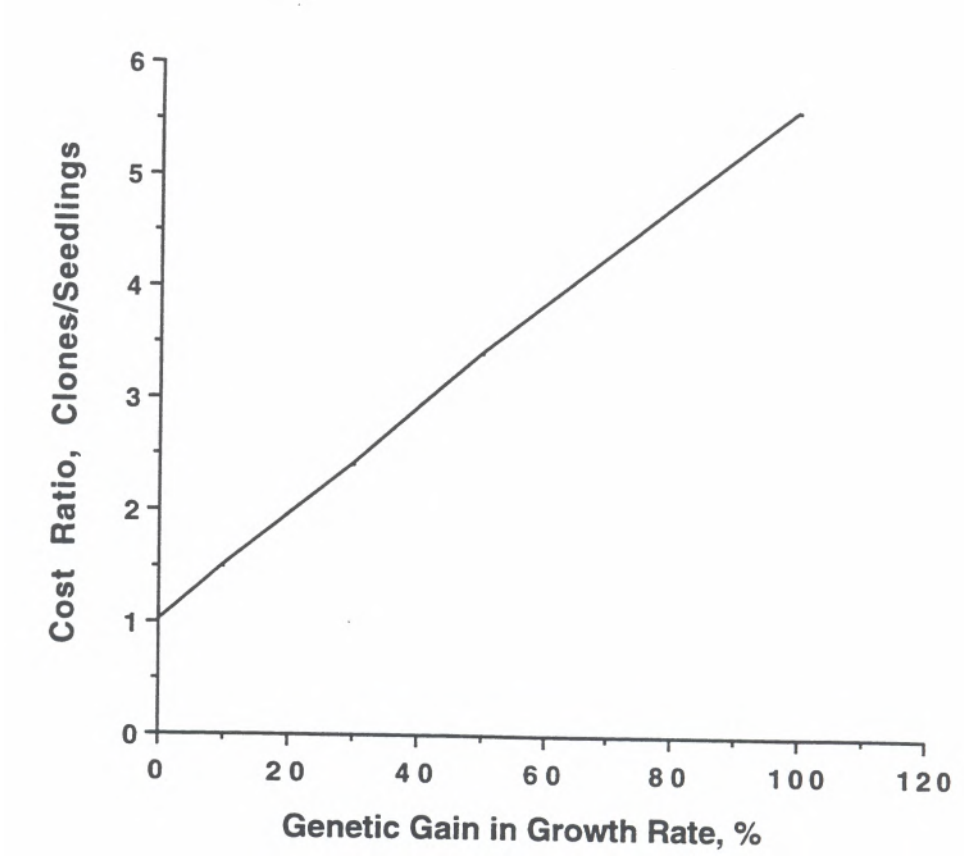

Figure 1. Break-even cost of sweetgum clones (relative to seedlings) as a function of genetic gain.

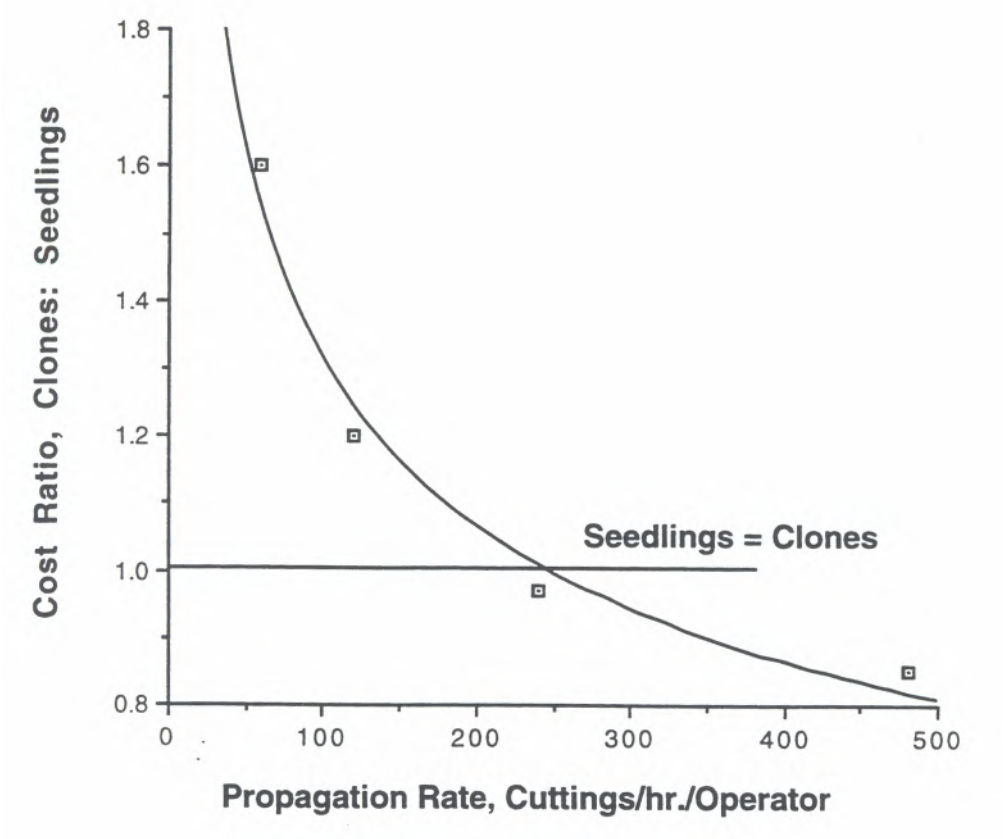

Figure 2. Effect of propagation rate on the cost of vegetatively propagated of planting stock.

Developing low cost vegetative propagation methods (for both pine and hardwood) is an area where there is a considerable need for innovation if we are to bring about clonal forestry in both pine and hardwood. Government and university researchers have avoided investigation of propagation systems as it is difficult to get funding for what is considered technology, not science. As a result, we find ourselves in the frustrating position of having genetically improved material that we cannot deploy as clones on a significant scale. To many tissue culture remains an art, and many laboratories continue to rely on procedures developed in the 1950s.

Genetic Transformation. *Agrobacterium-based* transformation of sweetgum has been previously reported (Feirer and Wann, 1989). This transformation method coupled with regeneration of shoots from leaf pieces (Brand and Lineberger, 1988) is routinely used at Union Camp to introduce foreign genes into elite clones of sweetgum. Our program has proceeded to the point that in 1995, Union Camp became the first US Forest Products Company to test genetically engineered trees in the field.

Union Camp's field test consists of sweetgum transformed with the tfdA gene, a gene that encodes for a enzymes that detoxifies the common herbicide 2,4-D (Stermer and Wilmitzer, 1989). In some cases, trees resistant to 2,4-D under greenhouse conditions did not express a

level of resistance in the field that would be suitable for operational use. This observation underscores the need to test each genetic transformation event individually.

Herbicide resistance would be of considerable value to hardwood plantation forestry. For example, if site preparation and vegetative competition control costs could be reduced to that of pine (as pine is basically herbicide resistant) the reduction in hardwood production cost could be as much as 25%. While herbicide resistance is likely to be one of the first traits commercialized in transgenic trees, the real impact of genetic engineering in forestry will come when genes that exert major effects on growth are identified. For example, if a gene was discovered that increased growth rate by a factor of two, this would cut wood production costs in half. Identification of genes that can exert a major influence on growth is the second area of research needed in forest biotechnology.

Our USDA-APHIS Permit to field test genetically engineered sweetgum stipulates that we must monitor the trees for flowering and prevent the release of transgenes (in the form of pollen or seed) should they flower. Containment is required because our field test, located near Belleville, GA, is in close proximity to native populations of sweetgum. The need to prohibit flowering in our field test implies that the USDA will require sterility in the operational deployment of transgenic trees. Therefore, it could said that before we pursue any other traits, sterility has to be the number one trait to target for genetic engineering.

The regulations surrounding release of genetically engineered organisms are still changing. It may be that for those engineered traits that would not constitute a plant pest risk (i.e., the trait would not increase the "weediness" of the native population) the requirement for sterility might be waived. Even if sterility were waived, corporations may not wish to risk the potential negative public perception of lack of containment of transgenes even if they were for traits deemed benign by governmental agencies. Therefore, the need for sterile trees may hinge on non-technical issues more than anything else.

Approaches to genetically engineering sterility have centered on the disruption of the developmental genes that orchestrate flowering (Strauss et al., 1994). There is a need to extend this technology to our Southeastern species. It should also be appreciated that even if genetic constructs that conferred sterility were available today, it would take years to evaluate their effectiveness in the absence of techniques for inducing precocious flowering. Early flowering (or perhaps more appropriately, accelerate maturation) is needed to compress the juvenile phase to a time period that would allow rapid assessment of sterility. These experiments are inherently difficult to perform because they require the evaluation of a negative finding (i.e., no flowers) under otherwise permissive conditions (i.e., physiological phase change).

Early flowering can therefore be considered to be enabling technology for transgenic trees. Floral stimulation techniques in juvenile loblolly pine are well established (Burris et al., 1991), such that seedlings can be induced to flower in 2-3 years. Less is known about floral stimulation in hardwoods. Techniques that will reliably induce flowering in hardwoods in as short a time frame as possible are therefore an urgent research need.

LOBLOLLY PINE BIOTECHNOLOGY

Germplasm. Union Camp has been selecting and breeding loblolly pine for more than forty years. This program has progressed from a time when planting stock was comprised of seed orchard mixes, then single, open pollinated family plantings and soon, full-sib families. Largely due to the foresight of Marvin Zoerb, large blocks of trees from full-sib crosses were planted as long ago as thirteen years in anticipation of a time when we would be planting full-sib families operationally. These planting have proved to be ideal mapping populations for the development of molecular markers (see below).

Vegetative propagation. Union Camp is actively investigating vegetative propagation of pine through rooted cuttings, micropropagation and somatic embryogenesis. Vegetative propagation from tissues older than seedlings has not been particularly fruitful, and attention has been focused on cloning options based on juvenile starting material - most notably somatic embryogenesis. Embryogenesis has good potential for scale-up as evidenced by the activity of several commercial concerns for pines and spruces. Embryogenesis is also appealing as a means of genetic transformation of conifers. This year Union Camp will establish its first clone test using somatic seedlings.

The use of juvenile starting material to develop clones would benefit greatly from early selection. Union Camp has been involved with physiological selection based on microcalorimetry since 1991 (Warn et al, 1991). Recently, we have extended our early selection program to genetic mapping. Molecular markers for growth would be of tremendous benefit since they have the potential to be independent of both age and environment.

Our genetic mapping program initially begun as a research contract utilizing our selection age full-sib plantings. Figure 3 shows a frequency distribution of volume index in one of these populations. Table 1 depicts the effect that marker-aided selection would have on wood cost if markers could be found that afforded the selection of trees with the percentage of volume above the mean shown. Marker selected trees would have to produce greater than 15% more wood above the mean to recover the costs of screening and vegetative propagation. However, if markers could be found that accounted for even 25% of the variation, their value to a mid-sized forest products company such as Union Camp would be well into millions of dollars per year. Markers have been found that account for a 7% increase in biomass (in the form of increased wood specific gravity) in radiata pine (Gleed et al., 1995) and 161% increase in volume in a hybrid eucalyptus cross (Chaparro et al, 1995). Given this spread in variation accounted for, it seems entirely reasonable that genetic markers that account for a significant portion of the variation in growth rate will be found in our mapping populations.

Table 1. Postulated effect of genetic markers for volume growth on pine wood costs (Base case = full-sib seed)

Wood Volume, % Above Mean	Wood Cost Ratio, Marker Selected/Full-sib mean
10	1.04
25	0.92
50	0.77
75	0.66

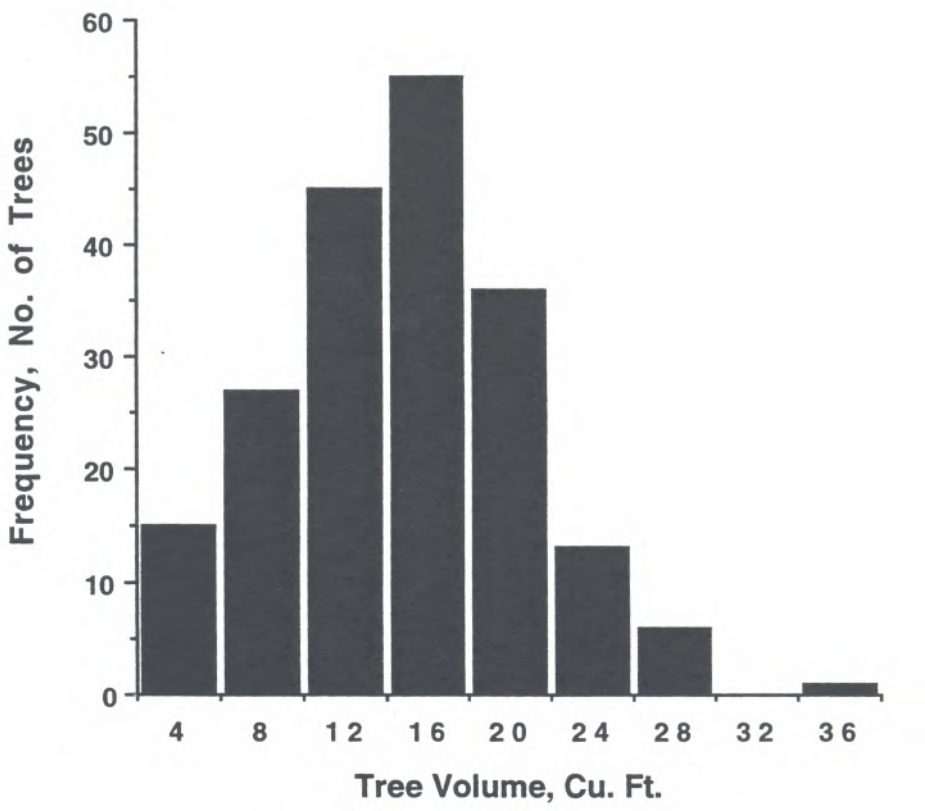

Figure 3. Volume distribution of a ten year old full-sib loblolly pine mapping population (location 1; n=200)

Large-scale plantings of full-sib crosses of selection age trees are ideal mapping populations and facilitate the search for molecular markers for growth rate. However, it would be extremely difficult to develop mapping populations for every parental combination we would want to use. For that reason, it would be highly desirable to have markers that are independent of genetic background. Markers that fall into this category are the genes

themselves, or simple sequence repeats (SSRs). Developing genetic markers that were independent of genetic background is a research priority.

Genetic Transformation. Unlike hardwoods, genetic transformation of loblolly pine is not yet routine in our laboratory. *Agrobacterium* has been used to transform embryogenic tissue of loblolly pine (Fierer and Warm, 1989). Further, transgenic conifers have been regenerated from embryogenic tissue (Ellis et al., 1993).

As with hardwoods, we have asked what traits should be targeted for genetic engineering. As noted, herbicide resistance is of limited value in pine. However, the Nantucket Pine Tip Moth causes considerable damage to young trees is a potential target for genetically engineered resistance. Noteworthy in this regard is the observation that several bt toxins are effective against tip moth. We postulate that a 10% reduction in wood costs could be made if engineered resistance to tip moth replaced spraying with pyrethrins. While insect resistance offers some reduction in wood costs, this reduction would pale in comparison to what might be accomplished if genes were found that had a major impact on growth rate. As with hardwoods, discovering single genes with profound effects on the growth of pine is an area where research is needed.

CONCLUSIONS

Throughout this paper we have highlighted where progress needs to be made before forest biotechnology can be utilized on an operational scale. The research topics identified are broad in scope, and as such are ideally suited for academic or governmental research. Value to forest products companies can only be derived from research in the above areas when an organization applies the findings to its own particular set of circumstances or genotypes. The areas where forest biotechnology research is needed are summarized below:

- Large-scale, cost-effective vegetative propagation technology for both pine and hardwood.
- Genetic constructs that, when transformed into trees: (1) confer sterility and (2) increase vegetative growth rate.
- Tissue-and age-specific promoters for transgenes.
- Early flowering in hardwoods.
- Molecular markers for growth, especially in loblolly pine, that are independent of genetic background.

Developing clonal forestry with genetically engineered trees is a daunting task and technical barriers still remain. While Union Camp has enjoyed a measure of technical success, the progress we have made has also been dependent on findings from academic and governmental

laboratories. By working together with the research community, we hope to help focus efforts on what should be done, rather than what can be done.

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