ADDENDUM

At the request of the Forest Biotechnology Committee the following condensed report on a questionnaire and Workshop organized by this committee is published as an addendum to these proceedings. The full length report is available from A. M. Stomp, North Carolina Biotechnology Center, Box 13547, Research Triangle Park, N.C. 27709-3547.

The committee has met once, on May 1, since the Workshop and adopted "Forest Biotechnology Committee" as its official name and agreed on a mission statement. Its activities will initially be focused on information exchange and the fostering of cooperation among various groups of scientists and users, including forest biotechnologists, forest tree breeders and forest physiologists.

VIEWS ON FOREST BIOTECHNOLOGY: REPORT FROM A CONFERENCE ON TREE IMPROVEMENT BY GENETIC ENGINEERING

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(Condensed by J. P. van Buijtenen)

INTRODUCTION

A three-day Workshop was held in North Carolina in January of 1987 to focus the thoughts and ideas of both scientists and industrial managers on the current technical state and future potential for tree improvement using genetic engineering approaches. Participants, from three principal interest groups, included government (USDA Forest Service and North Carolina Biotechnology Center), industry (timber, pulp and paper, and genetic engineering companies), and academic professionals from both the research and business management areas. Four goals were defined for the Workshop:

1) Assemble leading scientists, R&D directors and project managers for the purpose of establishing communication and initiating a framework for further cooperation in forest biotechnology R&D.

2) Define the present informational and policy constraints impeding immediate progress and efficient utilization of resources in the application of existing biotechnologies to current problems of forest tree use and biomass production.

3) Define existing opportunities for advancement in forest biotechnology.

4) Pinpoint areas where further basic research is required to maximize forest tree production and utilization.

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To accomplish these goals, the Workshop was structured with two parts. The first consisted of a questionnaire sent to research and management professionals to poll perceptions on the current state and potential value of genetic engineering to tree improvement. The second phase was the Workshop which assembled 30 participants from the principal interest groups to address the goals outlined above. This meeting was held at the Aqueduct in Chapel Hill, North Carolina under the joint sponsorship of the North Carolina Biotechnology Center, the United States Forest Service and the School of Forest Resources of North Carolina State University. What follows is the results of the questionnaire and the Workshop.

THE QUESTIONNAIRE

A questionnaire designed to poll current perspectives on the status and future direction of forest biotechnology was prepared and mailed to 138 professionals in the three interest groups. Following are the questions and a summary of the responses:

1) What is your perception of the current state of forest biotechnology? For example, what areas do you feel are developed or developing, e.g. clonal forestry, tissue culture, identification of early markers for superior trees, gene identification, gene transfer, etc.? What areas do you feel are not developed?

More than half of those responding felt that clonal forestry, i.e. multiplying selected material for operational planting through any vegetative method, is the most advanced technology. Methods cited for clonal forestry included rooted cuttings, micropropagation and tissue culture. A definite difference is perceived between propagation/plant regeneration methods for angiosperm vs gymnosperm species, with more difficulty encountered with gymnosperms. Both somatic embryogenesis and plant regeneration from small numbers of cells, e.g. shoot regeneration from callus, was cited as a critical need.

The general consensus was that DNA (gene) transfer was in its infancy but that method development was starting, e.g. the recent successful transfer of glyphosate resistance to hybrid poplar. About one third of those responding felt that much more work should be done to develop DNA transfer methods for important forest tree species, especially conifers.

Concern about a critical lack of information focused around four points: 1) Virtually no information exists on the physiological and molecular mechanisms of interesting phenotypes, e.g. drought resistance, disease resistance, wood characteristics. 2) Very little information exists about genomic structure, gene identification and mapping, and gene regulation for any forest tree species. 3) Mechanisms are not currently in place to integrate new biotechnology efforts with existing tree breeding work. This aspect was pinpointed as critical for long-term testing or genetically manipulating plants and for the development of biochemical markers which could be used for early screening of progeny. The development of "early" markers was considered a top priority problem. 4) There is controversy over the characteristics which make a 'superior" tree. (Editor's note: Desirable traits will depend much on how the trees will be used).

2) What do you think are the most important processes and properties contributing to efficient productivity of forest trees? What do you think are the biological bottlenecks to increasing forest productivity and/or tree use?

The answers fell into two categories, abiotic and biotic stresses and limitations inherent in the trees themselves. Abiotic and biotic stresses were considered more important and were ranked as: disease resistance, nutrient utilization (especially nitrogen), and cold and water stress.

Overall, aspects of tree biology which may limit forest productivity were not considered as important as environmental limitations. Areas of interest included increasing photosynthetic efficiency, growth rate, wood quality, fiber length, other fiber characteristics, cellulose and lignin amounts and characteristics, and all aspects of canopy development including light interception, and root-shoot ratio. Other characteristics of interest included respiration, juvenile-mature transition and floral induction, pollination and fertilization as it pertains to both increased production of seed, identification of male steriles and total loss of reproductive capacity.

One frequently discussed item was genotype x environment interaction. Tailoring trees to site was thought as having significant potential for increasing stand productivity. Another idea frequently mentioned was developing the exploitative tree, one that was more environmentally opportunistic and thus could capitalize, through rapid growth, on favorable environmental conditions.

3) What are the most economically important forest tree species? Are there species which could serve as model systems for the development of technologies?

Selection of species was a regional issue, therefore no one species could be singled out as the most important. Coniferous species were considered more important than angiosperm species in the U.S., Canada, northern Europe and Central America. Loblolly pine (Pinus taeda) and Douglas fir (Pseudotsuga menziesii) are considered far more important than other coniferous species.

4) With respect to each of the important properties and processes you listed in question 1 above, what do you think are the best biotechnological approaches for increasing the efficiency of productivity? Why? For example, consider recombinant DNA techniques, the use of somaclonal variants, organelle transfer, clonal forestry, sexual tree breeding and

generation of molecular or cellular characteristics for early selection of superior individuals.

A unanimous view in favor of strong programs in conventional breeding was articulated. More work should be done on precocious flowering to accelerate breeding programs. The short-term gains from biotechnology are expected to come from some sort of clonal propagation of desirable phenotypes. Long-term gain is expected to come from recombinant DNA/gene transfer studies, but not through genetic engineering but through the molecular markers identified by this technology and the increase in understanding of the molecular mechanisms controlling tree phenotypes.

5) What processes and properties listed in question 1 do you think are most tractable for improving tree use or productivity? for example, consider herbicide resistance, insect and herbivore resistance, wood quality, tree growth rate, tree morphology, soil nutrient utilization, photosynthetic efficiency and novel tree use or secondary product production.

6) What do you think are the most rewarding/least risk/most sensible targets for increasing productivity in the short-term? mid-term? long-term?

Questions 5 & 6 dealt with what are the most important areas for research vs what are perceived as the most scientifically tractable projects for the short-, mid- and long-term. Virtually all responders agreed that the most important research effort should be in studies of tree physiology, biochemistry, cell and molecular biology and genetic structure and regulation with wood quality, phosynthetic efficiency, pest (disease, insect and herbivore) resistance and tree morphology receiving major emphasis. Although many were quick to acknowledge that genetically engineering herbicide resistance is feasible now and has been done in hybrid poplar, skepticism was voiced as to its value and economic justification. A large percentage of responders were concerned about the lack of good tissue culture methods for routine propagation of plants from single cells or cell clumps and for mass propagation of selected individuals of economically valuable species, especially conifers.

7) What sort of organization and strategy would you prefer to follow-up this Workshop, continue your involvement and maximize future cooperation of the principle interest groups in forest biotechnology?

Two suggestions received much attention, the creation of a newsletter and the creation of a new, broadly based organization. The organization would be a small one with representatives of government, wood and pulp & paper industries, genetic engineering concerns and academic and institute researchers to periodically discuss and review progress in the field and means to promote continued research. Other suggestions were to affiliate with existing groups such as TAPPI, the Society of American Foresters and IUFRO working parties.

THE WORKSHOP

The Workshop assembled 30 participants from four major interest groups. They were asked to develop a strategy for the application of biotechnology to tree improvement which incorporated both scientific tractability and economic reality. The approach included the following tasks:

1) Prepare a prioritized list of economically important phenotypes for potential improvement by genetic engineering.

2) Determine the current extent of basic understanding of the molecular and genetic basis of economically important phenotypes.

3) Determine the current application of biotechnology to tree species.

4) Identify the critical technologies and information which are presently unavailable and necessary to apply genetic engineering to tree improvement.

Potential Traits for Genetic Engineering

Workshop discussion quickly focused on the fact that much of the future application of biotechnology to forestry will be determined by the perceived return on investment. In prioritizing a list of phenotypes for genetic improvement, difficulty arose in that it was impossible to determine cost or economic return on any phenotype. However, general guidelines on the cost side were given by Dr. Ron Sederoff, USDA-Forest Service, and on the return side by Dr. Pat Trotter, Weyerhaeuser Company.

Two factors determining the cost of manipulating a single gene were identified by Dr. Sederoff: 1) the amount of information that is known about the gene's identity at the molecular level and 2) the availability of methods for engineering in the selected species. A convenient way to express this is in scientist years, with the average cost of a scientist year averaging $100,000. If the gene product's identity, i.e. the enzyme or protein, is known and no new technology needs to be invented, the time required to isolate (clone) the gene will be about 2-3 years; to put that gene into a plant will be an additional 2-3 years. If molecular information is not available or if technologies, such as tissue culture methods or DNA transfer methods, need to be developed, the time required to move a gene into a plant becomes much longer. Extrapolating from the few examples that exist, such as herbicide resistance, a reasonable time period required before application would be 8-10 years.

Obtaining estimates on the return side of the investment equation is equally difficult. Several determining factors were identified by Dr. Trotter. The largest of these is the cost of getting the raw material to the mill gate. Genetic manipulation which lowers this cost could be valuable. Most of the value-added gain is accrued in processing the raw material, therefore, genetic manipulation for quality or new products could be valuable. However, other factors play a role as well. When the raw material is plentiful, cost is low and market share is more important in determining profit. Under these conditions quality and product innovation are paramount. When wood is in short supply, bigger harvests are more important and faster growth rate giving higher yield increases profits. In either case, very large changes in returns, e.g. for the largest companies amounts on the order of $100 million, are required to attract investment. Clearly, there is no general formula for investment strategy.

The group's consensus of economically important phenotypes is given in Table 1. These characteristics have always been important factors in determining profitability and fall into two categories, "insurance" traits and "investment" traits. Insurance traits are those which protect trees from biotic and abiotic stresses, thereby preventing crop loss, i.e. increasing yield. Investment traits are those which alter the tree for specific processing or end-use, thereby increasing yield, value or creating a new product. Economic returns of all traits listed is dependent on environment.

Table 1.--Economically Important Traits.

Insurance Traits	Investment Traits
Biotic Stress	Wood Quality
Disease resistance	Chemistry
Insect resistance	Fiber length
Abiotic Stress	Growth Characteristics
Drought	Juvenile to mature transition
Heat & cold	Carbon allocation, harvest index
Air pollution, ozone	Morphology, apical dominance
Herbicide resistance	Growth Rate
	Nutrient Utilization

Requirements to Engineer These Traits

As outlined by Sederoff, the ability to manipulate any of the phenotypes listed in Table 1 will be proportional to our understanding of the genetic basis of the particular trait and the availability of methodologies to manipulate the trait at the molecular level. The group agreed that the largest need exists for information on the molecular basis and genetic regulation of economically important phenotypes. This need exists in agronomic crop species as well, however often less is known about tree species. Method development also lags. Two areas require immediate attention: 1) tissue culture regeneration for mass production of plants and 2) DNA transfer methods, with special focus on coniferous species.

Method Development

Method development in tree species generally lags far behind agronomic crops. Where Japanese breeding programs with Cryptomeria japonica are more than 200 years old, conventional sexual breeding of forest tree species are less than 50 years old. In the oldest of these conventional U.S. programs, breeding is now approaching the third generation. Long breeding cycles and progeny testing coupled with the lack of breeding tools such as inbred lines, greatly limits the usefulness of conventional breeding programs to produce improved planting stock in a reasonable time scale. Therefore, tree improvement via genetic engineering has great potential if critical methods and information are forthcoming.

Key biotechnological methods critical for molecular investigations of genetic regulation are only beginning to be developed for use with tree species. Tissue culture methods are generally better developed for angiosperm tree species than gymnosperm species. A complete regeneration system from protoplasts to shoots exists for hybrid poplar. Somatic embryo systems are available for sweetgum, American elm, and Norway spruce. Shoot regeneration from callus exists in several angiosperm species including Betula sp., Populus sp. and Eucalyptus sp.

Critical to the application of biotechnology to trees is the transfer and expression of DNA. DNA transfer and expression has been demonstrated in hybrid poplar by the transfer of a gene giving tolerance to the herbicide glyphosate. Although actively being pursued in several laboratories, these key techniques have yet to be developed for gymnosperms.

In summary, methods for molecular investigations and genetic engineering are not generally available in forest tree species. The two most critical needs are tissue culture regeneration and DNA transfer, the latter being completely unavailable in coniferous species for the production of plants with new genes. Without these vital technologies, gene identification becomes more difficult and genetic engineering impossible.

Lack of Basic Information

Even with full method availability, a genetic engineering approach to tree improvement would be impossible due to the almost complete lack of molecular information about the genetic basis of economically important phenotypes. This point is reiterated both in the questionnaire responses and in Workshop discussions. It is generally felt that the pace of method development outstrips the increase in understanding. A positive correlation exists between methods and information; as methods become more universally available, the rate at which information is gathered becomes greater.

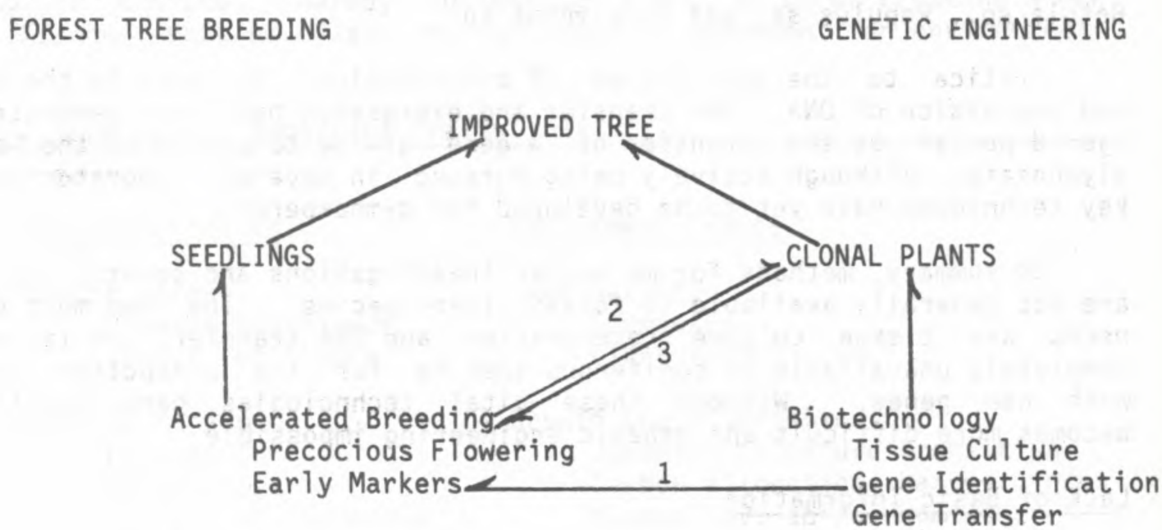
Currently, the molecular basis of two of the traits listed in Table 1 are known. Genes are available which confer resistance to certain herbicides. One such gene, Aro A, has been transferred to, and expressed in hybrid poplar. The group generally agreed that herbicide resistance was not a phenotype of major economic importance, and would therefore only merit limited investment. The other trait about which a considerable amount of molecular information is known 'is lignin biosynthesis. The economic importance of this trait could possibly justify the amount of research investment necessary to reach the level of understanding required for genetic engineering. Very little molecular information is available for the other traits listed in Table 1. Therefore, given the complete lack of molecular information on many economically important phenotypes, it is imperative that research immediately utilize existing tools to identify valuable genes.

STRATEGIC PLAN FOR THE APPLICATION OF BIOTECHNOLOGY TO TREE IMPROVEMENT

The group agreed that work to advance our abilities to genetically engineer trees needs to take place on two fronts: 1) continue to develop new and better methods to study the genetics of trees at the cellular and molecular level and 2) greatly increase the research effort on the genetic regulation of economically important traits.

The relationship between forest tree breeding and biotechnology is pictured in Figure 1.

Figure 1.--Relation between Forest Tree Breeding and Biotechnology.



Strategy

Workshop participants unanimously agreed that both conventional breeding and genetic engineering should be used for tree improvement. Presently, seedlings are used for reforestation. Parents are selected for general combining ability, therefore limiting the gain to the additive genetic component. It takes about 15 years for a seed orchard to reach full production and it takes at least 16 years to complete a breeding and testing cycle in loblolly pine. This cycle could be materially shortened if early markers related to future performance were available.

Biotechnology may hold promise for development of early markers (arrow 1). A key step in genetic engineering is the identification of genes regulating the phenotype of interest. Methods exist for screening individual organisms once the genes have been identified. Once a valuable gene has been identified and isolated (cloned), the next step in engineering an improved tree is to transfer that gene into a tissue culture regeneration system to see if the gene is functioning as desired in the whole plant. Once this has been determined and any corrections made, tissue culture methods can again be used to regenerate large numbers of plants for operational plantings. Mass propagation methods could also be used to propagate the best individuals in the breeding population (clonal forestry), thus capturing both the additive and non-additive genetic components (arrow 2). Plants produced through genetic engineering could also be placed into the breeding population to move select genes into reforestation stock while maintaining broad genetic diversity of other traits (arrow 3).

Economic Impact of Biotechnology

The commitment to this research effort must be large and long-term. Is the potential economic gain from the application of biotechnology able to justify the long-term investment?

Table 2 gives the group's estimate of the potential increase in forest value and the time scale for application of the strategy for integrated tree improvement.

Table 2.--Gain Potential and Time Scale for Different Tree Improvement Techniques.

	Accelerated Breeding	Clonal Forestry	Gene Transfer
Potential Increase of forest Value above present	50%	40%	100%
Period of Application	present-long-term/	present-long-term/	mid-long-term/
Time before Application	currently available	available in short-term	available in long-term

* short-term: 3-5 years; mid-term: 6-10 years; long-term: 11-20 years

The potential impact of individual molecular and cellular techniques on the future success of tree improvement using conventional breeding, clonal forestry and gene transfer techniques can be examined as well. Table 3 estimates the magnitude of impact and the estimated time before specific technologies could be applied to the genetic manipulation of trees.

Another way to conceptualize the impact of biotechnology on tree improvement is through specific examples. For example, consider lignin biosynthesis. The lignin content of loblolly pine, a major pulp wood species, averages 28%. Lowering the lignin content to 23%, could result in a 10% increase in fiber yield. This increased yield would return $100 million annually to North Carolina pulp mills alone (H. M. Chang, personal communication). A way to look at the economic value of genetic gain would be to reduce it to the added value per acre (SAF Forestry Handbook and J. P. van

Buijtenen, personal communication). One percent genetic gain has been valued at $4.00/acre in 1987. If one assumes a minimum profit margin of 50%, then only half of the value of the genetic gain can be used to pay for its cost, or $2.00 per acre for 1% gain, dividing this by the average number of trees (loblolly pine) planted per acre, and adding this amount to $.03, a typical current price for bare-root loblolly pine seedlings, generates Table 4.

Table 3.--Impact and Time Scale of Specific Technologies.

Technique	Accelerated Breeding M/T*	Clonal Forestry M/T	Gene Transfer M/T
Markers/RFLP[1/]	high/short		medium/mid
Mapping	medium/mid		low/mid
Haploids	low/long		
Rejuvenation		high/mid	
Mass propagation		high/short	
Gene identification	medium/mid		high/mid-long
Vector Development			low/short
Somaclonal variants			low-medium/short
Organelle transfer			low/mid-long
Regeneration of transformed plants			high/short

* M:magnitude of the impact; T:time before techniques will be developed.
1/ RFLP: restriction fragment length polymorphisms.

Table 4.--Target Price of Propagules based on Estimated Genetic Gains.

Gain	Added value/acre	Target price/propagule @ 700 trees/acre
5%	$ 20	$ .045
10%	40	.06
25%	100	.09
50%	200	.17
100%	400	.31

CONCLUSIONS

The potential for using biotechnology to modify economically important genetic traits in trees is large and long-term. Both the questionnaire and the Workshop participants identified two categories of traits for modification: 1) "insurance" traits which protect against crop loss from biotic and abiotic stress, and 2) "investment" traits which alter the tree to lower production costs, and create better or new products. Modification of trees by genetic engineering requires information identifying the specific molecular and cellular processes which determine the trait of interest and a sophisticated set of tools, called biotechnology, to manipulate the tree's biology. Both the basic information and the tools are critical; lack of either greatly inhibits or often prevents progress. Key tools requiring immediate development are plant regeneration from cells (tissue culture) and gene transfer methods, especially in coniferous species. In addition, we have virtually no knowledge of the molecular processes underlying the most important economic traits of trees. Research in basic molecular genetics of interesting traits is essential for future application of genetic engineering to tree improvement.