NORTH CENTRAL FOREST EXPERIMENT STATION BIOTECHNOLOGY PROGRAM-APPLICATION TO TREE IMPROVEMENT

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Abstract.---In 1983 the USDA Forest Service initiated a new research program on the genetic engineering of forest trees. One-half of this initiative is the Biotechnology Multiproject Research Program of the North Central Forest Experiment Station (NC). The NC Biotechnology Program is centered at Rhinelander, Wisconsin, and also has scientists at St. Paul, Minnesota, and Madison, Wisconsin. The Program has nationwide responsibilities and cooperators at another Forest Experiment Station, five universities, and three biotechnology companies. The overall Program purpose is genetic tree improvement, complementing conventional breeding technologies. Program structure, studies, and early results are described.

Additional keywords: Genetic engineering, somaclonal, protoplast, recombinant DNA, herbicide resistance, disease resistance, Populus.

In 1983 the USDA Forest Service initiated a research program on the genetic engineering of forest trees, probably the first such major program in the world. One-half of this initiative is the Biotechnology Multiproject Research Program of the North Central Forest Experiment Station (NC). The NC Biotechnology Program, formally organized in 1984, has a nationwide responsibility. Centered at the Forestry Sciences Laboratory in Rhinelander, Wisconsin, the Program has scientists at Rhinelander, and Madison, Wisconsin, and St. Paul, Minnesota, and research cooperators at another Forest Service Experiment Station, five universities, and three firms in the biotechnology industry.

Two previous papers have described the NC Biotechnology Program (Nelson and Haissig 1984, Nelson et al. 1984). My purposes in this paper are to describe and update Program:

. structure

• studies

. early results

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STRUCTURE

Strategic Plan

We recognized early that strong strategic planning was necessary for success in biotechnology. In the formative stages, we collaborated with an internationally known biotechnology consulting firm, L. William Teweles & Co., on a strategic plan for forest biotechnology research (Kidd 1984). A unique research program evolved from that collaboration as well as from extensive further analysis by Program managers and scientists.

The Program's strategic plan is based on the following postulates about conventional tree breeding:

. The new biotechnologies and conventional tree breeding are complementary, rather than competing, technologies. Both are essential components of successful tree improvement. For example, resistance to a specific stress imparted through biotechnological techniques is of little value when the trait resides in an otherwise maladapted genotype. Improved and elite genotypes and populations resulting from conventional breeding programs provide the most desirable starting material for further specific biotechnological improvement.

. One of the most important benefits of the new biotechnologies is the potential ability to reduce the long time periods required for tree improvement using conventional genetic technology alone. Three recent major studies of biotechnology (Burg et al. 1983, National Academy of Sciences 1983, Skelsey 1984) have identified woody perennial crops as prime targets for genetic engineering A major conclusion was that combining the new biotechnologies with conventional breeding may produce relatively greater payoffs in forestry than in any other agricultural area (Skelsey 1984), largely because of the time-saving potential of biotechnology.

. The new bioiotechnologies provide potential means for introducing rare or foreign critical traits into otherwise desirable forest tree germplasm. Introducing such traits into tree genomes may be impractical or impossible through conventional breeding alone.

. The new biotechnologies may aid in the development of practical means for capturing non-additive, as well as additive, genetic variation in improved tree populations. The established biotechnology of micropropagation provides the main vehicle for capturing such improvement. The new biotechnologies of somaclonal selection, somatic hybridization, and perhaps, microinjection and recombinant DNA may improve the frequency and reliability of whole plant regeneration in micropropagation systems.

The NC Biotechnology Program is modeled after a typical startup biotechnology company, a unique organizational plan for a public research effort. The Program is based on four essential synergistic factors (fig. 1), also present in new private biotechnology firms:

. entrepreneurship

. focus

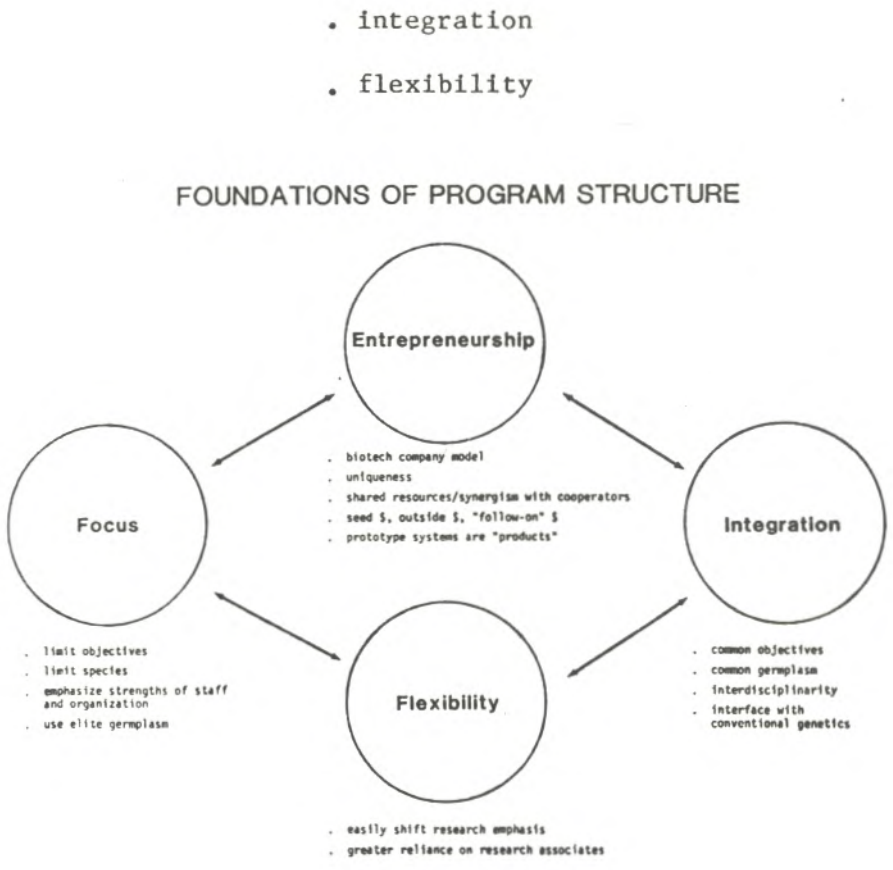

Figure 1. Principles of strategic planning and organization for the North Central Forest Experiment Station Biotechnology Program.

Entrepreneurship for the NC Program involves the forementioned emulation of biotechnology industry strategies. These strategic characteristics include ensuring uniqueness for the research program, sharing resources and developing a synergistic relationship with cooperators, and using "seed money" to help attract outside research contracts and grants and further "follow—on investment." In contrast to a private biotechnology firm, the NC Program produces prototype genetic transformation systems for forest trees, rather than commercial products.

Focus in the research program includes limiting the number of objectives and target species. It involves carefully selecting species based on both biological flexibility and commercial importance. The NC Biotechnology Program has optimized the use of our limited resources by emphasizing research on biologically amenable model species, while still maintaining research on some commercially important species at a lower but meaningful level. Focus in the NC Program also encompasses an emphasis on the strengths of the staff and parent organization in choosing research objectives. This analysis of endogenous strengths includes scientific, logistic, and financial considerations. An important part of research focus in the NC Program is the use of elite foundation stock from conventional genetics programs as starting material for biotechnological improvement, as mentioned above.

Integration within the NC Program means ensuring commonality across the Program, including all Forest Service and cooperating scientists. The commonality includes common objectives and common germplasm. Integration in the NC Program also includes interdisciplinary research planning and execution and a strong interface with conventional genetics and breeding efforts. The latter involves not only the selection of elite and well-defined germplasm as experimental material for the biotechnology research, but also the joint planning of how the biotechnologically improved genotypes will be delivered to users. In some cases the latter consideration may involve incorporating the improved trait in seed. Classical genetic analysis is an important part of analyzing and verifying the genetic effects of the new biotechnologies and constitutes another conventional genetics-biotechnology interface within the NC Program.

Flexibility in the NC Program includes building in the willingness and ability to shift research emphasis to follow up promising results. Research in biotechnology is "high risk" in that positive results often cannot be predicted. The history of research in this area also includes the common occurrence of unexpected results and important spinoff applications. A biotechnology research program must be flexible to capitalize on this situation. Another component of flexibility is a greater reliance on research associates and other employees on temporary appointments than has been common in Forest Service research.

Objectives

The NC Biotechnology Program is using established biotechnologies (conventional genetics and breeding, tissue culture) and new biotechnologies (somaclonal/gametoclonal selection, somatic hybridization, recombinant DNA) to accomplish three objectives:

- impart herbicide resistance to selected forest trees
- impart disease resistance to selected forest trees
- develop genetic guidelines for the regeneration of selected forest trees in tissue culture (regeneration genes)

The rationale for the choice of these objectives is fully explained in two previous papers (Nelson and Haissig 1984, Nelson et al. 1984). Current research is on the technologies of somaclonal selection, tissue culture, and breeding. We expect to gradually increase our commitment to somatic hybridization and recombinant DNA.

Species

The NC Program is working with some species selected primarily for biological reasons (models); some chosen primarily because they are commercially important, and one species selected for both biological (model) characteristics and potential commercial importance:

Model	Model/Commercial	Commercial
Populus spp.	*Pinus banksiana*	*Pinus taeda*
Larix spp.		*Pinus resinosa*

About 75 percent of current Program research is on Populus.

Poplars were selected as our primary experimental species because they are highly amenable to regeneration in a variety of tissue culture systems, can be easily vegetatively propagated, are the subject of ongoing breeding programs, are diploid, have a small genome size (Dhillon et al. 1984), are genetically variable, and have ample background information in genetics, physiology, and plantation silviculture. Because of these characteristics, the USDA Forest Service Genetic Engineering Workshop (F. T. Ledig, personal communication) recommended poplars as model species to include in forest tree biotechnology programs.

Staff

The NC Program currently includes three plant physiologists, a plant geneticist, a plant anatomist, a tissue culturist, and two plant pathologists (fig. 2). The Program Leader and Research Work Unit (RWU) NC-1403 are in

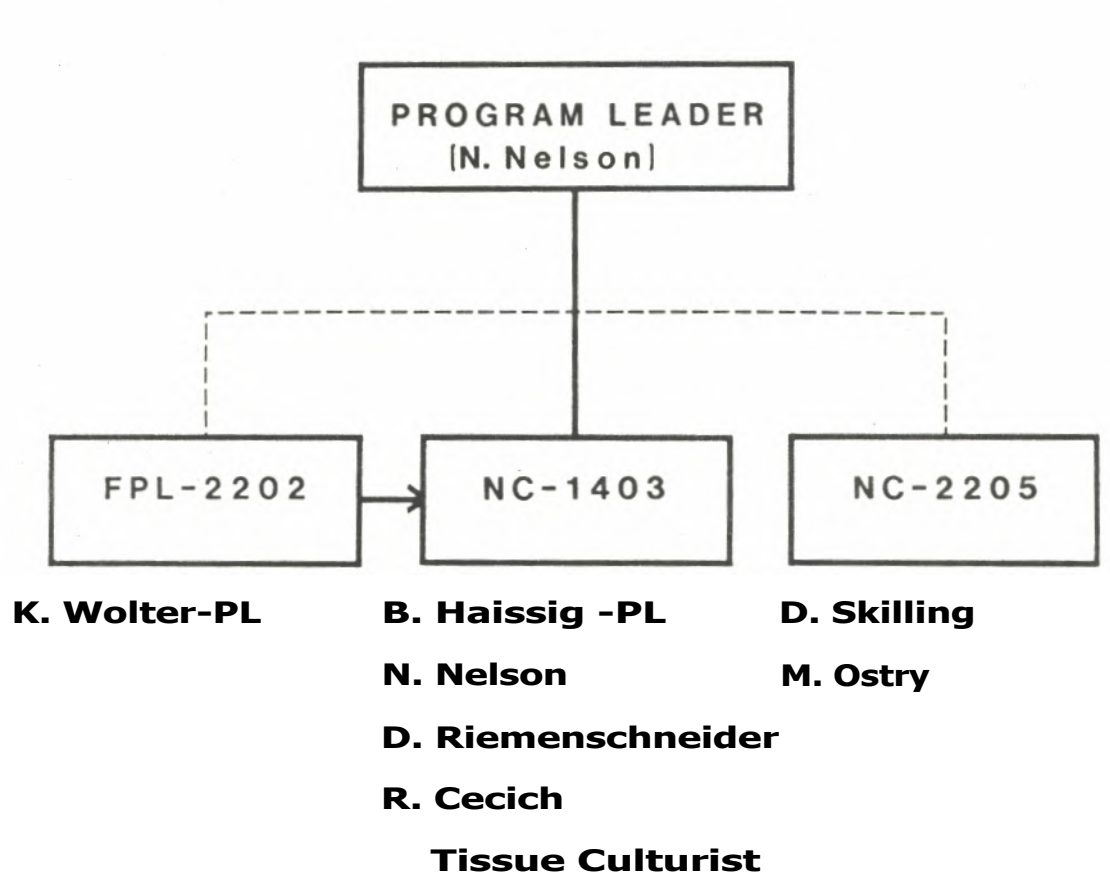

Figure 2. Organizational structure and in-house scientific staffing of North North Central Forest Experiment Station Biotechnology Program. Numbers are Research Work Units (RWU). NC-1403 is the Program core unit. PL is Project Leader of RWU. Arrow indicates research contribution to the core unit.

Rhinelander, Wisconsin; RWU FPL-2202 is in Madison, Wisconsin; and RWU NC-2205 is in St. Paul, Minnesota. NC--1403 is responsible for Program research on herbicide resistance and regeneration genes. FPL-2202 contributes tissue culture research to the work of NC-1403. NC-2205 is responsible for Program research on disease resistance.

Cooperators

Program cooperators, listed in table 1, work closely with the Forest Service scientists in the Program, using germplasm that is common across the Program. This planned research focus and coordination have created a synergistic organization.

STUDIES

The current studies of the NC Biotechnology Program are listed in table 2. Studies planned to begin soon are listed in table 3.

Table 1.--Research cooperators of the North Central Forest Experiment Station Biotechnology Program.

Government		University		Biotech. Industry	
Institution	Investigator	Institution	Investigator	Institution	Investigator
Southern Forest Exp. Station	O.Wells	Wisconsin	B.McCown	L.Williams Teweles & Co.	G.Kidd
		Minnesota	P.Read W.Hackett	DNAP	M.Sondahl
		N.C. State	S.Dhillon (J.Miksche)	Calgene	M.Moloney J.Fillatti
		Michigan Tech.	D.Karnosky A.Diner		
		New Hampshire	S.Minocha		

EARLY RESULTS

Most studies of the NC Biotechnology Program were initiated less than 1 year ago. Nevertheless, Program scientists have obtained a number of significant results. Some of the most noteworthy findings are listed below:

A strategic plan for forest biotechnology was developed (Kidd 1984).

A dozen Populus clones have been established in sterile shoot culture. Most have been established in a proliferative state (McCown 1984). Shoots of one clone were rooted and planted in a field test plot of 200 trees in northern Wisconsin (B. McCown and D. Riemenschneider, personal communication).

Callus and leaf disc cultures have been established for several Populus clones. Nine clones have exhibited shoot and root formation from callus through organogenesis. High frequency regeneration of shoots from leaf discs can be readily obtained with two of these clones (T. Ettinger, B. McCown, N. Nelson, M. Ostry, P. Read, unpublished data).

Table 2.--Current studies of the North Central Forest Experiment Station Biotechnology Program.

Study	Principal investigators	Species	Program objective[a]	Biotechnology emphasis
Stratigic plan in biotechnology for forest trees[b]	G.Kidd	all	all	general
Genetics of whole plant regeneration *in vitro*	B.Haissig,B.McCown, D.Riemenschneider, R.Cecich	*Populus*	rg	genetics/breeding, tissue culture
Anther culture for haploidy induction in *Populus*	K.Wolter	*Populus*	all	tissue culture
Tissue culture systems for red pine	M.Sondahl	*Pinus resinosa*	all	tissue culture
Genetics of hexazinone resistance in jack pine	D.Riemenschneider	*Pinus banksiana*	hr	genetics/breeding
Imparting glyphosate and sulfonylurea resistance to elite poplar germplasm	N.Nelson, B.Haissig	*Populus*	hr	somaclonal selection
Imparting *Septoria* resistance to elite poplar germplasm	M.Ostry,P.Read, W.Hackett	*Populus*	dr	somaclonal selection
Imparting scleroderris and needlecast resistance to selected *Larix*	D.Skilling, A.Diner, D.Karnosky	*Larix*	dr	somaclonal selection
Cotyledon and embryo culture of red pine to generate somaclonal variation	S.Minocha, N.Nelson, D.Riemenschneider	*Pinus resinosa*	all	somaclonal selection
Protoplast technology for *Populus*	B.McCown	*Populus*	all	somatic hybridization
Nuclear DNA changes in leaves of *Populus* and *Larix* during the growing season	S.Dhillon, J.Miksche, R.Cecich	*Populus*, *Larix*	all	recombinant DNA
Conferring glyphosate resistance on selected *Populus* through genetic transformation with *aro* A gene	M.Moloney,J.Fillatti, B.Haissig, B.McCown	*Populus*	hr	recombinant DNA

a/ All = all objectives, rg = regeneration genes, hr = herbicide resistance, dr = disease resistance.

b/ Completed.

Table 3.--Future studies of the North Central Forest Experiment Station Biotechnology Program, planned to begin in 1985.

Study	Principal investigators	Species	Program objectives[a]	Biotechnology emphasis
Genetic modulation of somaclonal variation in poplars	B.Haissig, N.Nelson D.Riemenschneider	*Populus*	hr	genetics/breeding, tissue culture, somaclonal selection
Genetics of regeneration *in vitro* for loblolly and jack pines	D.Riemenschneider, O.Wells, B.Haissig	*Pinus taeda*, *Pinus banksiana*	rg	genetics/breeding, tissue culture

a/ hr = herbicide resistance, rg = regeneration genes.

. An in *vitro* system has been developed for one *Populus* clone that allows continued viability of individual protoplasts and division and growth of these protoplasts to the large calli stage (> 1000 cells per callus) (B. McCown, unpublished data).

. An embryogenic cell suspension culture system has been developed for one *Populus* clone, apparently the first report of embryogenesis from cell suspension for the genus (B. McCown, unpublished data). This system is potentially useful for somaclonal selection for chemical and disease resistance as well as for genetic transformation through recombinant DNA.

. *Populus* shoot cultures were found to exhibit the highest sensitivity to cytokinin of any dicot deciduous tree species so far examined (Sellmer et al. 1985). This cytokinin sensitivity differs markedly by clone (Sellmer et al. 1985).

. Callus was obtained from the anthers of three male *Populus* clones in our research on haploidy induction and gametoclonal variation. Viable callus formed in 4 to 6 weeks, with spontaneous root formation after one or two subcultures. Sporadic shoot formation was also observed. No evidence of haploidy in any of these calli, roots, or shoots has yet been found (K. Wolter, unpublished data). Several experimental parameters are being modified to increase the probability of achieving haploidy.

. *Populus* cells were found to have very small chromosomes and only 1.5 pg of DNA per nucleus (Dhillon et al. 1984). At least 95 percent of the higher plant species so far examined have more nuclear DNA than this (J. Miksche, unpublished data). This surprisingly small genome size should facilitate genomic analysis through recombinant DNA approaches. Cytogenetic analysis, however, is made more difficult by the small chromosome size (R. Cecich, unpublished data).

. A leaf disc bioassay for *Septoria* susceptibility in *Populus* developed at NC Station has been refined so that there is a high correlation between bioassay results and *Septoria* resistance in field plantations (M. Ostry, unpublished data). This leaf disc bioassay is being used in our somaclonal selection system for *Septoria* resistance.

. Successful infection of *Populus* shoot and leaf disc cultures with *Agrobacterium tumefaciens* has been achieved, with gall formation in shoots in *vitro* (J. Fillatti and M. Moloney, unpublished data). *Agrobacterium* is the transformation vector in our North Central Station—Calgene—University of Wisconsin recombinant DNA herbicide resistance work (table 2).

. Large and highly significant differences were found between open pollinated families of *Pinus banksiana* in tolerance to the triazine herbicide, hexazinone (D. Riemenschneider, unpublished data). Work will soon begin on the mode of inheritance of this tolerance.

. Cotyledon culture systems of high regenerative capacity have been developed for *Larix decidua* (A. Diner, D. Karnosky, D. Skilling, unpublished data).

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