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Genetic Improvement and
Evaluation of
Black Cottonwood for
Short-Rotation Biomass
Production

Final Report

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Genetic Improvement and Evaluation of Black Cottonwood for Short-Rotation Biomass Production

**Final Report
1987 to 1992**

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GENETIC IMPROVEMENT AND EVALUATION OF BLACK COTTONWOOD FOR SHORT-ROTATION BIOMASS PRODUCTION

A FIVE YEAR PROGRESS REPORT (1987 - 1992)

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30 APRIL 1993

ABSTRACT

This project was initiated in 1978 to serve three objectives: 1) develop genetically improved poplar cultivars offering increased productivity under short-rotation culture; 2) identify the major components of productivity in poplar and determine ways in which they can be manipulated, genetically and culturally; and 3) engage in technology transfer to regional industry and agencies so as to make poplar culture in the Pacific Northwest economically feasible. The project is aimed at capturing natural variation in the native black cottonwood, *Populus trichocarpa* T. & G., and enhancing it through selective breeding. Major emphasis has been placed on hybridization of black cottonwood with *P. deltoides* and *P. maximowiczii*, more recently with *P. nigra*. First-generation (F_1) hybrids have consistently outperformed black cottonwood by a factor of 1.5-2. The high yields of woody biomass obtained from these clonally propagated hybrids, in rotations of 4-7 years, have fostered the establishment of large-scale plantations by the pulp and paper industry in the region. Physiological studies have helped to elucidate hybrid superiority and several of the underlying mechanisms.

This five-year progress report summarizes key results from the period 1987-92, as published by the authors and their co-workers in 72 abstracts, theses, book chapters and journal articles. Significant developments described include 1) new insights into patterns of genetic differentiation in natural populations of black cottonwood east and west of the Cascades, 2) continued breeding of new genetic material, with increased emphasis on advanced generation hybrids, 3) incorporation of molecular techniques in the genetic analysis of hybrid pedigrees and the establishment of a genetic linkage map of *Populus*, 4) comprehensive analysis of clonal evaluation trials comprising 1,300 clones at 14 field sites, 5) elucidation of how productivity is affected by factors of water relations and canopy architecture, spanning from the cell to the stand levels, and 6) studies showing how several of these tree variables can be influenced by nitrogen fertilization, with resulting effects on stand productivity. An active program of technology transfer has provided for a regular exchange of information among researchers and industrial collaborators. The rapid expansion of poplar culture in the region will continue to present new research challenges and is likely to benefit from the aggregate expertise in this research program.

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GENETIC IMPROVEMENT AND EVALUATION OF BLACK COTTONWOOD FOR SHORT-ROTATION BIOMASS PRODUCTION

INTRODUCTION

Goal

Increase the production of woody biomass in *Populus* through the development of genetically improved cultivars that are suitable for an operational short-rotation culture (SRC) in the Pacific Northwest.

Objectives

1. Develop genetically improved poplar cultivars offering increased productivity under SRC.
2. Identify the major components of productivity in poplar and determine ways in which they can be manipulated, genetically and culturally.
3. Engage in technology transfer to regional industry and agencies so as to make poplar culture in the Pacific Northwest economically feasible.

The goal and objectives of this project are well aligned with those of the Biofuels Feedstock Development Program, which are: to establish a technology that is capable of producing predictable quantities of terrestrial biomass in a cost-effective and environmentally acceptable manner. In fact, over its 15-year history this project has helped to prove that the Department of Energy's goals are indeed attainable in the Pacific Northwest.

Background

This project is a **tree domestication program for biofuels and fiber feedstock**. It has used a multi-disciplinary approach to develop a new crop material and a matching cultural system by which to produce feedstock predictably and repeatably. Key elements in its success have been (1) the choice of species and the approach to its genetic improvement, (2) the careful assessment of how improved cultivars differ from unimproved stock, and how this improvement may be further enhanced, and (3) the successful transfer of the concept from the research scale to the operational scale. *Populus* was an attractive choice: its rapid growth, easy clonability and capacity to resprout met important requirements of the SRC concept. Its species diversity and the ease with which it can be bred and hybridized offered excellent opportunities for genetic improvement. Most importantly, a Northwest species, *P. trichocarpa* T. & G., the native

black cottonwood, still occurred in extensive natural populations with large amounts of genetic variation to be tapped.

Rapid gains in productivity were achieved by hybridizing *P. trichocarpa* with *P. deltoides*, resulting in a doubling of yield in four-year rotations (Heilman and Stettler, 1985). Hybrid superiority over black cottonwood was further documented by an expanded hybridization program (Stettler, *et al.*, 1988). The production levels obtained, 20-25 Mg/ha/yr of dry woody biomass, were sufficient to encourage the pulp and paper industry to establish larger-scale field trials with the new hybrids. Today, the area of hybrid poplar plantations on both sides of the Cascades exceeds 7,000 ha and is planned to be tripled within the next three years. More than 2000 clones are being tested in numerous field trials in British Columbia, Washington and Oregon. Two of the companies, James River Corporation and Boise-Cascade Corporation, have embarked on breeding programs of their own, patterned after the UW/WSU model.

To better understand the nature of hybrid superiority, or heterosis, a major research effort in production physiology was launched in the early 1980s. It demanded, on the one hand, a more careful study of black cottonwood and its natural variation; and on the other, the comparative study of hybrids and their two parental species in replicated trials. This is where the favorable attributes of *Populus* came fully into play: its crossability, clonability, resprouting and rapid display of phenotypic variation. In an initial series of experiments, nine groups of morphological, anatomical and physiological factors were identified as likely causes of heterosis (Hinckley, *et al.*, 1987). These studies have continued and are now being pursued using pedigree material of the F₂ and B₁ generation to shed light on the genetic control of individual productivity traits. Parallel molecular studies have established a linkage map of the *Populus* genome, based on restriction fragment length polymorphisms (RFLPs) and RAPD markers (Bradshaw and Stettler, 1993a and b). Efforts are underway to associate molecular markers with specific quantitative and qualitative trait loci. Eventually, this should facilitate the screening of parents and progenies and should help in the development of long-term breeding strategies.

In summary, this project has grown into a major research program on *Populus*. In its entirety, it now involves a total of 12 senior investigators including the four principal investigators of this project. These scientists are housed in eight different departments of two universities (University of Washington, Washington State University). The group's annual research budget is in the range of \$1.2-1.6 million, most of it from federal grants, a modest but increasing amount from state and private sources. Apart from serving a regional need and meeting DOE's objectives, the group has firmly established *Populus* as a model species for basic studies in tree growth and differentiation.

TABLE 1: List of Project Researchers and Collaborating Scientists**PROJECT RESEARCHERS*****Principal Investigators***

Dr. Harvey D. Bradshaw, Jr., Research Assistant Professor, Biochemistry Department, University of Washington
 Dr. Paul E. Heilman, Soil Scientist, Washington State University Research and Extension Center, Puyallup
 Dr. Thomas M. Hinckley, Professor, College of Forest Resources, University of Washington
 Dr. Reinhard F. Stettler, Professor, College of Forest Resources, University of Washington

Postdoctoral Fellows, Graduate Students and Technicians

Dr. Jeffrey H. Braatne, Postdoctoral Fellow, College of Forest Resources, University of Washington
 John Reed, Graduate Student, College of Forest Resources, University of Washington
 Hong Tian, Graduate Student, College of Forest Resources, University of Washington
 Rongling Wu, Graduate Student, College of Forest Resources, University of Washington
 Gordon Ekuon, Technician, Washington State University Research and Extension Center, Puyallup
 Diane M. Fogel, Technician, Washington State University Research and Extension Center, Puyallup
 Indulis A. Muiznieks, Technician, Washington State University Research and Extension Center, Puyallup
 Kevin Otto, Technician, Department of Biochemistry, University of Washington
 Carl Riches, Database Manager, College of Forest Resources, University of Washington
 Brian Watson, Technician, Department of Biochemistry, University of Washington
 Sarah Wolpow, Technician, College of Forest Resources, University of Washington

COLLABORATING SCIENTISTS

Dr. John H. Bassman, Associate Professor, Washington State University, Pullman
 Dr. Reinhart Ceulemans, Associate Professor, University of Antwerp, Belgium
 Dr. Gary A. Chastagner, Pathologist, Washington State University Research and Extension Center, Puyallup
 Dr. David W. Evans, Agronomist, Washington State University Research and Extension Center, Prosser
 Dr. Milton P. Gordon, Professor, Department of Biochemistry, University of Washington
 Dr. Richard R. Gustafson, Associate Professor, College of Forest Resources, University of Washington
 Dr. Judson G. Isebrands, Physiologist, Forestry Sciences Laboratory, US Forest Service, Rhinelander, Wisconsin
 Dr. Patricia Layton, Scott Paper Company, Scott Plaza 3, Philadelphia, Pennsylvania
 Peter McAuliffe, Scott Paper, Ltd., New Westminster, British Columbia
 Dr. George Newcombe, Postdoctoral Research Associate, Washington State University Research and Extension Center, Puyallup
 Dr. Giuseppe Scarascia-Mugnozza, Associate Professor, University of Tuscia, Viterbo, Italy
 Dr. Brian J. Stanton, Northwest Fiber Supply Division, James River Corporation, Camas, Washington
 Cees Van Oosten, Woodlands Services Division, MacMillan Bloedel, Ltd., Nanaimo, British Columbia
 Dr. Elizabeth Van Volkenburgh, Associate Professor, Botany Department, University of Washington
 Dr. Ken Wearstler, Jr., Boise Cascade Corporation, Wallula, Washington

VISITING SCIENTIST

Dr. Marc Villar, Center for Forest Tree Improvement, INRA, Orléans, France

Format of Report

This report is intended as a summary of the past five years' work and will focus on the DOE-sponsored research conducted by the four principal investigators and their co-workers during the period 1987-1992. It will summarize the main findings from the many articles published by the group in technical journals. A cumulative list of all publications derived from this project is given at the end of the report.

SIGNIFICANT DEVELOPMENTS 1987-92

Molecular Tools: One of several noteworthy developments during the past five years has been an increased emphasis on genetic analysis by molecular means. It evolved out of our past collaboration with Dr. Milton Gordon from the Department of Biochemistry and assumed a dimension of its own under the direction of Dr. Harvey (Toby) Bradshaw, Jr. A key construct resulting from this work, a low-resolution linkage map of the *Populus* genome, will provide a new organizing principle for much basic information in poplar. With currently 241 RFLP loci covering approximately 90 percent of the genome, the map is already dense enough to be related to quantitative trait loci (QTLs) governing commercially important traits. For certain traits, *e.g.*, *Melampsora* susceptibility, correlations may be sufficiently high to allow early marker-aided selection of seedlings. Molecular information will also help to elucidate the genetic basis of hybrid superiority and, thereby, provide guidance for the development of poplar breeding programs. Tangible by-products of this work have been the identification of triploids among F_2 hybrids, the discovery of lethal recessives, and the adoption of DNA fingerprints as a means of clonal identification. As a consequence of this molecular approach, emphasis in our breeding has shifted from the production of F_1 material to that of informative advanced hybrid generations (F_2 and B_1). Altogether, the increased reliance on molecular tools has added greater precision to many facets of this project and has developed a firmer genetic basis for all future work.

Physiology: Production physiology is now the major focus of physiological research in short rotation hybrid *Populus* culture. Since productivity is the function of tree growth rate at the stand level, the functional aspects of tree morphology, growth and physiology must ultimately be addressed in the context of the stand and its field environment. These aspects include water relations, nutrient requirements, photosynthetic efficiency and canopy leaf area and its horizontal and vertical distribution. We find not only rapid development of high levels of leaf area by these hybrids, but also much higher efficiencies of these high leaf areas in producing biomass than is the case with most tree species. Furthermore, measurement of water use by stands of hybrids indicates very high water use efficiency (water use per unit biomass production).

Commercial Production: A most remarkable development is the establishment in the arid regions of the Pacific Northwest of commercial-scale plantations for fiber production

where season-long irrigation is required. Furthermore, because of the indicated high productivity and the areas of land available and suitable for this purpose, potential acreage under these circumstances is quite large. Already, the acreage scheduled by the two pulp and paper companies involved is approximately 20,000 hectares. Although the clones developed in the program are highly productive under these conditions, efforts are needed to develop new clones and investigate special cultural requirements including irrigation, fertilization and pest control practices.

Melampsora Rust: The appearance in 1991 of two exotic species of *Melampsora* rust, *M. medusae* and *M. larici-populina* in commercial plantations of TxD hybrids in the Pacific Northwest send reverberations throughout the hybrid poplar community. Fortunately, the initial level of alarm was substantially tempered as more was learned about the disease, the range of susceptibility of hybrid cultivars and the minimal impacts on native species of poplar and conifers. Nevertheless, this development has brought pathology to the forefront in the process of clonal breeding and selection. It has also dramatically conveyed the need for better disease control strategies in plantation management.

GENETIC VARIATION IN BLACK COTTONWOOD

Black cottonwood has a large distribution range and occurs widely in extensive natural populations. This was early recognized as a major asset to this research program as it promised an abundance of genetic variation to be available for the development of a new crop. Measuring and interpreting this variation has thus been a continuous effort, albeit with changing emphasis over time. Early attention focused on the gains to be made from clonal selection of native material. As hybridization gained prominence, attention shifted to the parental qualities of black cottonwood and the underlying genetics. Later, as hybrid plantation culture became accepted in the region, new concerns emerged as to the future role of natural populations, the importance of biodiversity in riparian ecosystems and their protection against degradation. Throughout these studies, a variety of methods have been explored of how to measure genetic variation, ranging from common-garden studies to growth-chamber experiments, and from DNA probes to phenotypic expression of traits that are pertinent to productivity.

Early study of 50 clones had revealed a noticeable, if moderate, trend in West Cascade black cottonwood: northern populations had larger leaves on fewer, more horizontal branches than southern populations, when grown at Puyallup (Weber, *et al.*, 1985). This trend was confirmed in a second, larger study, completed during this reporting period (Rogers, *et al.*, 1989). It was based on a sample of 308 clones, the majority of which were derived from open pollination and were grown in a more northern location at Alger, WA. The study offered further insight in the structure of genetic variance: three-year growth showed the largest amount of variation at the clonal level, followed by

lesser amounts at the family and population levels. Volume growth was significantly correlated with single-leaf area and several branch traits. Comparison between the two studies also revealed strong clone-site interactions. Whereas several southern populations had outperformed northern populations at Puyallup, the reverse was true at Alger.

Genetic differentiation in an east-west direction, across the two mountain ranges that most strongly affect the Washington climate, became the object of a major study carried out by Joan Dunlap and culminated in her PhD thesis (Dunlap, 1991). This investigation represents the most carefully designed and executed study on natural variation in black cottonwood to date. It has given us new insights in the genecology of the species, important information on sampling and testing methods, and a wealth of data that will serve as baseline information for many years to come. Results of this work have been published (Dunlap, *et al.*, 1992) or submitted as manuscripts; two are companion articles (Dunlap, *et al.*, 1993a,b) and are in review by the *Canadian Journal of Forest Resources*; a third (Dunlap, *et al.*, 1993c) is under review by the *Canadian Journal of Botany*.

Noteworthy design features of Dunlap's research were (1) the two pairs of valleys chosen for sampling, each pair offering an E/W comparison, the Dungeness/Hoh across the Olympic Mountains and the Yakima/Nisqually across the Cascades; (2) the systematic transect sampling scheme for each valley; (3) the choice of the two common-garden sites, capturing contrasting environments, and (4) the robustness of the statistical design: each plantation contained 12 copies of each of 128 clones in a randomized complete block design with three blocks. Although more reliable data came out of the Puyallup plantation (more homogeneous site, less droughty, better maintenance), the Wenatchee plantation was valuable in providing a sharp contrast.

Dunlap's common-garden studies showed stronger genetic differentiation of black cottonwood across the mountain ranges than had been found previously parallel to them. Material from mesic sources grew better than that from xeric sources at both locations. Further genetic differentiation was manifest *within* valleys: material from the upper elevations behaved differently from that of the lower elevations. This was especially pronounced with clones from the Yakima River. This eastside river descends the cooler upper slopes of the Cascades and then traverses the arid shrub steppe in its lower reaches before joining the Columbia. Black cottonwood along that river reflects the selective pressures of the local environment. Material grown from the lower, xeric portion was branchier and more densely covered with smaller and thinner leaves than material from the upper portion. Lower-Yakima clones were also much more susceptible to the *Melampsora occidentalis* leaf rust, especially when grown on the West side.

Growth-chamber experiments complemented the common-garden studies (Dunlap, *et al.*, 1993c). Cuttings from the lower Nisqually and Yakima were potted and grown, a complete set each in a maritime (Puyallup) and continental (Wenatchee) climate. After two months, both sets were transferred to growth chambers, where their photosynthetic rates were determined at four light levels (200, 500, 800, and 1500 $\mu\text{Mol m}^{-2}\text{s}^{-1}$) and two temperatures 24° and 32°C). Yakima plants had significantly higher rates at high light and also at moderate light in high temperature than Nisqually plants. But a certain acclimation was also manifest: at the two moderate light levels in high temperature, the Wenatchee-grown plants, regardless of source, had higher rates than those grown at Puyallup. Later field measurements showed eight lower-Yakima clones to photosynthesize at a higher rate than eight upper-Yakima clones.

All in all, Dunlap's research points to a closer attunement of black cottonwood to the local environment than previously appreciated, involving many morphological, anatomical, physiological, phenological and biochemical traits. This finding has at least two practical implications. First, it points to a greater genetic diversity being available in black cottonwood both for selection and breeding. Whether some of these traits, *e.g.*, drought and heat tolerance, are worth exploiting in a hybridization scheme or whether they are more profitably borrowed from another species, *e.g.*, *P. deltoides*, remains to be clarified. Second, conservation and restoration of riparian ecosystems have to be pursued on a sufficiently small scale to safeguard local genetic diversity. Dunlap's finding also raises the question of how genetic differentiation can arise and be maintained in populations along the same river in the face of a steady down-river gene flow via sexual and asexual propagules. This question will be addressed by a new study, initiated by John Reed. It will use molecular tools in descriptive and experimental approaches and will focus on the distinctive populations along the Yakima River.

One final and important piece of methodological work by Dunlap, a C-effect study, showed that it matters in certain materials from which position along the stem a cutting is taken on stool-bed ortets (Dunlap, *et al.*, 1992). The study examined the growth of hardwood cuttings taken from five stem positions, evenly distributed along the length of an annual sprout, in ten clones each from three river valleys. What was especially significant about this C-effect was that it faded during the second year of growth but reappeared again in the third. The lesson learned from this carefully designed study is that the cuttings' positional origin may bias genetic evaluation, especially in tightly-spaced multiclonal plantations. This bias can be minimized by the use of cuttings with comparable, preferably large, diameters and functional lateral buds.

BREEDING

Breeding was a major activity in 1987, 1988 and 1990. During these years, more than 20,000 seedlings were raised from 120 different crosses, resulting in a total of 4700 clone selections. A first set of materials included new F_1 hybrids, such as *P. trichocarpa*

x *P. maximowiczii* (TxM) and *P. trichocarpa* x *P. nigra* (TxN) crosses. The former were intended for the cool, moist environment west of the Cascades. Pollen from select materials was exchanged and resultant seed shared with geneticists at Oji Paper Company in Kuriyama, Japan. Several hundred TxM hybrid selections are in Phase I trials, and many have shown superior performance to TxD hybrids in the coastal climate of the lower Columbia Valley and on Vancouver Island. Hybrids with *P. nigra* were targeted for the rapidly expanding poplar culture east of the Cascades. That region has a more continental climate with greater temperature amplitudes and more wind. Early trials in Boardman, OR, had shown promising results with several Euramerican hybrids (DxN), in contrast to many large-leaved TxD materials. The *P. nigra* pollen for the crosses was provided by V. Steenackers, Belgium, and came from performance-tested clones in Belgium and France. Mean two-year height growth of the TxN material in the seedling beds at Puyallup (1.45 m) was less than commonly measured in TxD or TxM hybrids (2-2.5 m). However, the real test will be their performance in eastern Washington. Data from such trials, conducted by Boise-Cascade Corporation in Wallula, WA, will be available by the end of 1993.

A second type of materials resulted from advanced-generation hybridization. By 1988, many F_1 hybrids from our first round of breeding (TxD) had become sexually mature. They were then used for F_2 (TDxTD) and B_1 breeding (TDxD), both among unrelated and related individuals (half and full sibs). A total of 22 crosses generated 1500 seedlings. Both generations showed ample variation in all traits observed, as found earlier in B_1 and F_2 hybrids (Stettler, *et al.*, 1988). But B_1 families had higher average survival (76%) than F_2 families (58%) and a higher representation among the top 50 clones (24) than the latter (16) after two years of growth. Mean height-growth differences between crosses of related vs. unrelated individuals were not significant but "unrelated" crosses contributed three times more clones to the top 50 than "related" crosses. Field testing of selected clones from this material is underway. Early comparison of F_1 breeding with that of F_2/B_1 suggests that considerably larger numbers of seedlings have to be screened in advanced generations, especially in the F_2 , to identify well-performing hybrids. However, certain trait combinations may only be obtainable via advanced-generation breeding and may make the added effort worthwhile. This is especially true for an easily clonable material such as poplar. New emphasis on disease tolerance may give further impetus to this breeding pathway.

Advanced-generation breeding also served an additional purpose, namely to help to elucidate the genetic control of productivity traits in poplar. The development of a long-term breeding strategy for poplar that maximizes gains in productivity, while incrementally improving the breeding stock, demands a better understanding of heterosis and its underlying genetics. Molecular tools now offer new means of gaining insight in this important phenomenon, which has been so central to past efforts in poplar improvement. To exploit these molecular opportunities fully, attention was directed in 1990 to provide for complete three-generation pedigrees with adequate seedling

numbers. More details are given on this material in the next section on molecular genetics.

Finally, the breeding program shed light on geographic and genetic patterns of susceptibility in *P. trichocarpa* to the native leaf rust, *Melampsora occidentalis*. This study involved the collaboration of two pathologists, Drs. G. Chastagner and T. Hsiang, and was based on 29 crosses performed among *P. trichocarpa* from east and west of the Cascades. Field rust susceptibility of several thousand seedlings growing at Farm 5 in Puyallup was scored in three consecutive years. Both the degree of infection and mortality were highest in east by east (ExE) crosses, followed in descending order by ExW, WxE and WxW crosses (Hsiang, *et al.*, 1993). Narrow-sense family heritability for rust susceptibility was estimated from parent-progeny correlations in the third, most severe, year to be $h^2 = 0.79$ (Hsiang, *et al.*, 1993). Seedlings from parallel crosses with *P. maximowiczii* showed low susceptibility (TxM crosses) or virtually no infection by *M. occidentalis* in the case of MxM, TDxM and MxTD crosses. Results from this study show that black cottonwood from the east slope of the Cascades tends to have higher rust susceptibility than westside material, although with much variation at the clonal level. Presumably, the hotter, drier summer conditions in that area reduce pathogen pressure and result in less stringent selection for tolerance in the host. Once challenged in the coastal climate, however, susceptible clones will become quickly apparent. The high degree of genetic control in this trait parallels findings by Wilcox and Farmer (1967) on the susceptibility of *P. deltoides* to *M. medusae*. Thus, rapid genetic gains can be predicted in breeding for rust tolerance from a careful selection of parents, both at the species and individual tree level. Specific screening tools for this purpose may soon be available in the form of molecular markers.

MOLECULAR GENETICS

Introduction. The concept of interspecific hybridization in breeding *Populus* for increased biomass production is now well-established. Yet, the genetic mechanism(s) responsible for the observed heterosis remain obscure. Accordingly, a large portion of our breeding program has shifted from interspecific hybridization to the production of experimental advanced-generation pedigrees designed to shed light on the genetic basis of hybrid vigor. Three-generation pedigrees founded by interspecific hybridization between *P. trichocarpa* and *P. deltoides* have been developed over the past 12 years, with large segregating backcross (B_1) and F_2 progenies produced in 1988 and 1990. The availability of these pedigrees has enabled us to extend the analysis of patterns of inheritance to the molecular level, using detailed genetic linkage maps to study the effect of small regions of a chromosome on productivity-related phenotypes such as growth, form, and disease resistance. More than 350 RFLP and RAPD markers have been placed on the linkage map, making the *Populus* map one of the most detailed for any woody plant. Unexpected insights into the reproductive biology of *Populus* have been gained as a result of this work.

The mapping pedigree. The primary mapping pedigree is descended from *P. trichocarpa* 93-968 (female) and *P. deltoides* ILL-129 (male). F_1 hybrids (family 53) from these parents were produced in 1981; 11 of the hybrids were selected for testing in clonal growth trials. In 1988, sib-mating between 53-246 and 53-242 produced F_2 family 331, and a backcross between 53-246 and ILL-129 was made (family 342). Preliminary linkage mapping data indicated the desirability of increased numbers of segregating progeny, so the F_1 , F_2 , and backcross breedings were repeated in 1990. An additional backcross between 93-968 and 53-242 was added to make the breeding design symmetrical (family 354). Over 500 advanced-generation offspring from this pedigree are maintained in the seedling beds and clone banks at Farm 5.

Construction of a linkage map of the *Populus* genome. Linkage mapping experiments were begun in 1989, with the goal of producing a low-resolution map of the 19 *Populus* chromosomes useful for mapping quantitative trait loci (QTLs) important for biomass productivity. Random *Pst*I fragments of *Populus* DNA were cloned and used as hybridization probes to identify RFLPs between the parents of the mapping pedigree. Probes recognizing single- and low-copy number sequences in the *Populus* genome were used to follow segregation of RFLPs in the F_1 , F_2 , and backcross generations of the mapping pedigree. Cosegregation of RFLP markers and approximate map distances between markers were calculated using maximum likelihood methods. Currently, 241 RFLP loci have been mapped. We have estimated the genome length of *Populus* as 2900cM, giving an average spacing between markers of about 12cM. The map is sufficiently dense to begin mapping QTLs using phenotypic data collected from a replicated clonal trial (see below).

Triploidy in TxD hybrids. One surprising result from the early genome mapping experiments was the discovery of triploids among the F_1 hybrid family 53 (Bradshaw and Stettler, 1993a). When the inheritance of RFLPs heterozygous in the female *P. trichocarpa* 93-968 was followed, it was found that 3 of the 11 F_1 hybrids often received both alleles instead of the single allele expected. Subsequent determination of nuclear DNA content by fluorescence flow cytometry verified that 2 of the hybrids were triploid (3 complete chromosome sets instead of the normal 2 sets) and the third was aneuploid (more than 2 chromosome sets). Phenotypic characteristics, such as leaf color, epidermal cell size, fertility, and fiber length in the wood, were markedly different in the triploid hybrids. The whiter abaxial leaf surface in the triploids was used as a visible marker to screen all hybrids in the clone banks at Farm 5. All ten female *P. trichocarpa* that are represented in the stoolbed by at least four offspring have produced one or more triploid hybrids when crossed with *P. deltoides*; one female produced triploids exclusively. Evidently, unreduced ($2n$) gametes are found at moderate to high frequency in *P. trichocarpa* females. Interspecific hybrids often arise from the fertilization of these $2n$ gametes, but intraspecific crosses have yielded nothing but diploid offspring, implying differential selection operating on ploidy level during embryo

development. We have also found triploids in the F_2 of the mapping pedigree, and marker inheritance has shown that these are derived from unreduced F_1 female gametes. The potential growth advantage of triploids in *Populus* (as seen in aspen) is being tested in a replicated trial at Farm 5. Each of 51 triploids is paired with a diploid full-sib (where possible) in two-tree plots. After two years of growth, it is clear that triploids as a class do not exceed their diploid sibs in volume.

Segregation distortion due to genetic load. Distortion of marker segregation from expected Mendelian ratios is commonly observed in many plant species and hybrids. The mechanism of distortion is seldom known, but can be due to differential chromosome loss, pollen-lethal alleles, interaction among genes involved in genetic isolation between species, or to expression of recessive lethal alleles (genetic load). In our mapping studies, we identified a region of the *Populus* genome where the *P. trichocarpa* allele was almost never found in homozygous form in the F_2 offspring (Bradshaw & Stettler, 1993b). Detailed mapping and breeding experiments, as well as development of PCR-based marker systems allowing us to determine the genotype of very small embryos, showed that in this case segregation distortion was due to a lethal recessive allele found in the *P. trichocarpa* 93-968 and transmitted to her F_1 offspring. When F_2 offspring homozygous for the lethal allele were observed from the zygote stage, we found that embryo development was slowed; the few that survived to the seed stage and germinated failed to grow. The lethal allele appears completely recessive, having no measurable effect on tree growth when heterozygous. Genetic load will be an important consideration if inbreeding is to be used in future mating designs for *Populus*.

DNA fingerprinting for clonal identification. As *Populus* breeding and testing are scaled up for commercial applications, verification of clonal identity will become simultaneously more important and more difficult. The RFLP and RAPD markers developed in conjunction with the genome mapping effort have proven useful for genetic fingerprinting. Even within a full-sib family, sufficient polymorphism can be found to distinguish among individual offspring. More comprehensive efforts to catalogue fingerprint data, while beyond the scope of our previous and proposed work, would help standardize genetic material used for poplar research worldwide.

Replicated field trial of the three-generation mapping pedigree. In 1991, a replicated clonal trial (FT 7.23) was installed at Farm 5 to follow the segregation of morphological, physiological, and genetic variation relevant to biomass productivity. *P. trichocarpa* 93-968, *P. deltoides* ILL-129, the two F_1 s (53-246 and 53-242), and 107 F_2 and B_1 clones were included in the trial. The plantation is laid out in a modified complete randomized block design, with 2-tree plots in each of three blocks. Within each block the clones are grouped into three size classes to minimize competition. The rank of each clone was determined by growth performance in the seedling bed. The

trial is to run for six or seven years, with a 50% thinning after the third year to allow for destructive sampling.

After nearly two years of growth, this trial has provided a rare opportunity to visualize the remarkably high level of phenotypic and genetic variation in advanced generations of interspecific hybrids. Many visitors have toured the plantation and most have become convinced that this kind of material is uniquely suited for teasing apart the components of hybrid superiority in *Populus*. Segregating variation has been documented for several traits known to be important for biomass productivity, including crown architecture and geometry, leaf size, shape, and display, phenology and resistance to three species of *Melampsora* leaf rusts. As the linkage map nears completion, each of these aspects of growth and development can be dissected into its genetic components (QTLs). The morphological and physiological trait correlations we have observed in our previous studies in the relatively homogeneous F_1 generation can be tested and validated in the context of F_2 and B_1 populations with much higher levels of variation. The results of this work will be an improved understanding of the underlying genetics of tree growth, with immediate application to the design and implementation of breeding programs for hybrid *Populus*.

TESTING/EVALUATION

Selection and evaluation of high yielding hybrid *Populus* clones are major tasks in the effort to develop a short rotation technology for the Pacific Northwest (PNW). They have been an important component of this project during the past five years. Extensive testing is needed because the climate of the PNW varies greatly, ranging from mild and wet coastal areas with a cool but long growing season, to the arid region east of the Cascade Mountains with hot summers and cold winters. At higher elevations in the interior, growing seasons can be quite short. Widely varying soil conditions add to the diversity to be addressed. For example, soil pH ranges from 4.0 to 6.0 in westside soils compared with 6.0 to 8.0 on the east side.

Several participants are involved in evaluating clones for the region, including the joint UW/WSU project and a USDA Forest Service project, both funded in part by the US Department of Energy. Also, the pulp and paper industry is testing and evaluating materials. Several firms have established commercial plantations, with James River and Boise-Cascade currently managing over 6,000 hectares, MacMillan Bloedel and Scott Paper. Ltd., a smaller but expanding acreage.

Evaluation of clones in the region is proceeding in two phases (Stettler and Heilman, 1984). Phase 1 consists of evaluating large numbers of new and relatively untested clones (at this point the clones may have been only pre-screened in the nursery). Tree numbers (ramets) per plot are small, ranging from 1 to 6 trees. Replications range from 3 to 6, depending upon the number of clones in the test and the size of the area

available for the experiment. Because of the close spacing and small numbers of plants per plot, the useful life of these tests is generally three or four years. Information developed in these tests includes survival, height, diameter, form, phenology, plus data on incidence of disease, insect and animal damage. In Phase 1 testing, clonal ranking for potential productivity is based on $\text{dbh}^2 \times \text{ht}$.

Several diseases have been identified in these trials. One of them occurs in cool and humid areas near the coasts of Oregon and Washington and north on Vancouver Island. The disease results in defoliation and shoot blight (caused by *Venturia*, but *Glomerella cingulata* has caused similar symptoms). In autumn 1991, two leaf rusts, caused by *Melampsora medusae* and *M. larici-populina*, occurred in scattered plantings of TxD clones in the region (Newcombe and Chastagner, 1992a and b). Considerable variation in susceptibility to these diseases was evident among the many clones developed in the program, and a concentrated effort to address these problems has been initiated under the direction of Dr. G. Chastagner (separate funding).

Most Phase 1 tests have single-tree plots arranged in the interlocked block design of Libby and Cockerham (1980). The design permits mechanical thinning by removing single replications while preserving the integrity of the experiment in the remaining trees. A drawback is the requirement for uniform test sites, since variation inherent in most sites cannot be blocked within the replications. As a consequence, sensitivity to test clonal differences is reduced. Despite this limitation, data from these tests form the basis for the current rankings of clonal performance. A reanalysis of the data is planned to statistically reduce effects of possible systematic site variation in these tests.

The Phase 2 trials are designed to evaluate productivity of the best clones identified in the Phase 1 trials. Larger plots are used in this phase, usually with unmeasured buffer trees in the borders. Consequently, numbers of clones tested are substantially reduced, ranging up to perhaps 50. The experimental design is a randomized complete block. Because of the large area required for these experiments, the number of test sites are limited. Information obtained from Phase 2 tests includes production data in dry weight and bole volume for individual trees and per hectare.

Phase 1 Trials

To date, most of the tests have been Phase 1 trials. Early results showed the most promising materials to be F_1 hybrids between *Populus trichocarpa* and *P. deltoides* (TxD hybrids) (Heilman and Stettler, 1985; Stettler, *et al.*, 1988). Other materials tested have included selected *P. trichocarpa*, backcrosses (TxTD and TDxT), three-species hybrids (MxTD, TDxM, and TDxN) and F_2 (TDxTD) hybrids. In two comparative trials at Pack Forest (WA) and Westport (OR) involving 691 and 381 clones, respectively, mean four-year volumes showed a rank order of $F_1 > B_1 > T > F_2 > D$. Volume ($\text{dbh}^2 \times \text{ht}$) of F_1 hybrids averaged 200 to 300 percent of the volume of *P. trichocarpa* clones whereas

height was only 9 to 25 percent greater. Clonal variation was abundant in the F_1 material, but even more so in the B_1 and F_2 generations (Stettler, *et al.*, 1988).

Superior production of TxD hybrids was also evident in a trial at Alger, Washington. This trial contained 379 clones of mostly UW/WSU TxD hybrids, but included selected *P. trichocarpa*, *P. deltoides*, and 12 NE hybrid clones, five of which were part of the 50 clones common to all sites in a North Central study (Hansen, *et al.*, 1991). Of the five NE clones common to both regions, the best performing NE clone at Alger (NE-42) ranked only 47th in mean diameter at six years; the other four NE clones ranked 149 to 310. Similar poor results had occurred with a few other hybrids, including "Robusta," that were planted in the trials at Pack Forest and Westport.

Examination of growth data for the best 25 TxD F_1 hybrids at five test sites showed rank changes by sources of *P. deltoides* (pollen) parentage across sites (Hansen, *et al.*, 1992). Northern sources performed better than southern sources at all sites, and in particular at the most northern site (Alger), where none of the clones with Texas parentage were present in the top 25. The other trend revealed was the relatively poor performance of the Mississippi parentage on the eastside site (Boardman). Such trends are not unexpected and are being studied more thoroughly with the expanded testing currently underway.

An overall assessment of 14 Phase 1 trials was conducted and reported in Hansen, *et al.* (1992). Westside Cascade trials (10 tests with a total of 1338 clones) were analyzed separately from eastside trials (4 tests, 263 clones). Apart from the general superiority of TxD F_1 hybrids, the most notable findings were (1) the skewed distribution, indicating exceptional performance by only a few clones, and (2) the relatively small number of site-stable, as opposed to site-specific, clones. Since clones were unevenly represented, the method devised to identify relatively site-specific clones was as follows: Clonal performance in each trial was rated by percentile based on 100 percent equalling the mean volume growth of the top five clones in each trial. The top 20 clones overall, combining both eastside and westside locations, were then identified. To qualify for the top 20, a clone had to be present in at least three separate trials. Those clones in the top 20 that performed above the 50 percentile in all field trials in which they were present were considered stable. A total of 6, or 30 percent, met this criterion.

The highest performer among the six stable clones (No. 184-411) was also the most stable based on this system of evaluation, since it scored above the 90th percentile in four of the five locations where it was planted. The other 14 clones in the top 20 performed below the 50th percentile at one or more locations and, thus, were considered site-specific. These results, indicating greater numbers of site-specific than stable clones, probably reflect the large soil and climatic differences that occur in the Pacific Northwest region.

Recent tests suggest great promise for some of the more than 400-new F_1 hybrids between *P. trichocarpa* and *P. maximowiczii* (TxM). Two-year diameter and height growth of TxM hybrids at Westport (OR) exceeded that of many selected TxD hybrids (Hansen, *et al.*, 1992). Many of the TxM clones surpassed the best TxD clones. This trend kept up in the third year. An important characteristic of TxM hybrids is their early bud break and leaf flushing which will preclude their use in areas with late spring frosts or short growing seasons.

The latest materials included in Phase 1 tests are *P. trichocarpa* x *P. nigra* (TxN) hybrids, primarily intended for east side growing conditions. First performance data from trials at Wallula (WA) by Boise-Cascade Corporation will be available by the end of 1993.

Phase 2 Trials

The results of one Phase 2 trial (Ceulemans, *et al.*, 1992) confirm the superiority of F_1 hybrids found in earlier work. However, because of heightened intracolonial competition in these monoclonal plots, the relative superiority was less pronounced than that measured earlier in field trials with single-tree plots. After 4 years, mean estimated stem volume of the hybrids was 1.5 times that of *P. trichocarpa* and 2.3 times that of *P. deltoides*. Corresponding height superiority was only 1.1 and 1.3, respectively. Clonal variation was the dominant theme in tree mortality, time of bud break and bud set, height and diameter growth, and number of branches. *Populus trichocarpa* had the highest number of sylleptic branches, *P. deltoides* had the lowest, and hybrids were intermediate. (Syllepsis and prolepsis are further discussed in the section below.) Significant clone by replicate interactions were observed in height, diameter, and volume growth. Spring and fall phenology and the length of growing period explained only in part the observed differences in growth between the TxD hybrids and the parental species.

Results of other Phase 2 trials have not been published although one incorporating fertilizer treatments is reported in the section on nutrition.

PHYSIOLOGICAL RESEARCH

Over the last decade, major findings in physiology have occurred in two different areas. First, studies in water relations, in particular, stomatal function, have been conducted from the scale of the tissue to that of entire canopies. Second, the role of canopy architecture on whole plant productivity has been extensively studied. Similar to the studies of stomatal function, canopy architecture studies have spanned from the cell to the stand levels of biological organization.

Stomatal Function: In contrast to the documented observations of most researchers, the stomata of well-watered *P. trichocarpa* were observed to be unresponsive to vapor density gradient, leaf water potential or light in initial field studies (Schulte, *et al.*, 1987). An examination of leaves excised from shoots of *P. trichocarpa* showed that the desiccation and subsequent wilting led to little or no change in leaf conductance. In contrast, the stomata of TxD hybrids closed rather rapidly as excised leaves were desiccated. The response of *P. deltoides* stomata was intermediate. Following these laboratory studies on excised leaves, the response of leaf conductance to gradual desiccation was studied using material grown in 5 liter pots under greenhouse conditions. Under such conditions, complete stomatal closure was observed in TxD hybrids and *P. deltoides* while only partial closure was noted in *P. trichocarpa*. In *P. trichocarpa*, the greatest reductions in conductance (to less than 20% of fully open values) were noted in young, expanding foliage while the conductance of mature leaves was 50% or greater than unstressed leaves. These results suggested that the stomatal behavior of *P. trichocarpa* was influenced by the rate of desiccation, by leaf age, and by the study of attached rather than excised leaves. Under field conditions in which periods of drought develop slowly, stomata of *P. trichocarpa* were found to be responsive to vapor density gradient and soil moisture (Pezeshki and Hinckley, 1988). Leaf water potentials had no apparent effect on stomatal closure and actually increased with closure.

In order to explain these observations, stomatal activity was examined in epidermal strips taken from leaves grown in the greenhouse (Schulte and Hinckley, 1987a & b). When clones of *P. trichocarpa* were well-watered, the stomata remained open in spite of plasmolysis of the guard cells and their loss of turgor. Even osmotic potentials of -7.2 MPa failed to close stomata of *P. trichocarpa* completely. While not entirely conclusive, these observations suggested that the maintenance of open stomata in spite of turgor loss in the guard cells was the result of an abnormal physical characteristic of the guard-cell wall, which was normalized to some degree by a period of water stress, especially if the stress occurred while leaves were still expanding. In contrast to the response of *P. trichocarpa*, stomata from epidermal peels of both *P. deltoides* and TxD hybrids closed prior to plasmolysis of guard cells or the turgor loss point of intact leaves.

The inability of *P. trichocarpa* stomata to close when the guard cells were plasmolyzed was not due to an inability of this species to produce an increase in foliar ABA (abscisic acid) in response to water stress, as is the case for "wilty" mutants of tomato and potato (Schulte and Hinckley, 1987a). Application of ABA to developing leaves of well-watered *P. trichocarpa* plants induced a normal state of stomatal function, though it was not clear if this condition would have persisted. On the other hand, stomata from completely mature leaves did *not* respond to exogenous applications of ABA. These results lead to the following conclusions:

- (a) The inability of *P. trichocarpa* stomata to respond, when plants are maintained in a well-watered condition, resulted from a low "resting" level of ABA in the foliage. Under unmanaged field conditions for trees greater than three or four years old, the

population of leaves developed under such conditions is probably rather small--this would not hold true for younger material or material grown under well-watered conditions.

- (b) ABA has a role in the development of stomatal function. A critical level is needed to induce changes in the guard cell walls that make stomata function normally, namely to close in response to low leaf water potentials. Interestingly, similar or partially dysfunctional stomatal responses have also been observed in micropropagated *Delphinium* (Santamaria, *et al.*, 1993). Exposure of immature *P. trichocarpa* leaves to periods of water stress apparently increases the level of ABA sufficiently to normalize stomatal function. The closer the leaves are to maturation, the less likely it is that stomatal function can be altered either by periods of water stress or exogenous application of ABA.
- (c) As studies increase in spatial scale (epidermal strips to whole plants in the field) and temporal scale (rapid aerial desiccation to longer-term soil desiccation), the more do *P. trichocarpa* stomata appear to approach more typical response patterns (Braatne, *et al.*, 1992; Pezeshki and Hinckley, 1988). However, studies at lower space-time scales were critical in elucidating genetic and developmentally unique aspects of the stomatal response of this species.
- (d) Stomatal responses to light and vapor pressure deficit appear to be strongly regulated by the soil environment (Pezeshki and Hinckley, 1988).

Perhaps the most interesting observation from this series of studies was the responsiveness of hybrid stomata to desiccation. Instead of being intermediate between the parents as in the case of many other hybrid features, stomatal response of hybrids either equaled or exceeded that of *P. deltoides*, possibly due to heterosis (Bate, *et al.*, 1988; Matyssek and Schulze, 1987; Stettler and Ceulemans, 1992). The exception to this trend was in hybrids with the more whitish undersurface leaves characteristic of *P. trichocarpa*. Molecular analysis showed them to be triploids (or aneuploids) containing two genomes of *P. trichocarpa* and one genome of *P. deltoides*, *i.e.*, TTD-hybrids (Bradshaw and Stettler, 1993a). Not surprisingly, their stomatal responsiveness was closer to that of *P. trichocarpa*, as were other leaf traits (Figliola, 1986; Braatne, unpublished).

Similar to the superior response noted in hybrids between *P. trichocarpa* and *P. deltoides*, hybrids with *P. maximowiczii* demonstrated superiority to parents. In all cases, the stomatal responses of *P. trichocarpa* were significantly lower than hybrid genotypes (Braatne, unpublished data). Bassman and Zwier (1991) observed similar results except that stomata of *P. trichocarpa* from eastern Washington (warm, dry continental climate) were more responsive than those of either a *P. trichocarpa* x *P. deltoides* hybrid or *P. deltoides*. Interestingly, stomata of intraspecific hybrids of *P. trichocarpa* (western x eastern Washington genotypes) were very responsive to low leaf water potentials (Braatne, unpublished data). Subsequent studies have revealed further differences in growth, and in photosynthetic and morphological variables between eastern and western Washington clones of *P. trichocarpa*. (Hinckley, *et al.*, 1992; Dunlap, *et al.*, 1993).

Most recently, Hinckley, *et al.*, (1993) have linked measurements of water loss from individual leaves to water transport of whole branches with water transport in the entire stem to stand-water loss in *Populus*. This study was performed during the fourth growing season on individual trees of a F_1 hybrid (clone No.). Towers and scaffolding were used to access the canopy; whole tree transpiration was measured at breast height using the methods of Cermak and Kucera (1990); branch transpiration was measured with Dynamax gauges (Gutierrez, *et al.*, 1993); and leaf conductances from leaves attached to measurement branches were recorded with a LiCor 1600 Steady State Porometer.

Leaf conductances were highest from leaves in the upper canopy and lowest from those in the lower canopy. Maximum conductances occurred before solar noon (1300 hours) and declined thereafter. Highest rates of branch water loss occurred from upper canopy branches and lowest from lower canopy branches. Water loss from middle and upper canopy branches was relatively constant from 1130 to almost 1930 hours despite considerable declines in leaf conductance (*e.g.*, from more than 400 to less than 300 $\text{mmol m}^{-2} \text{s}^{-1}$). The average leaf transpiration rates, estimated from porometer measurements, were 50 to 100% higher than values obtained at the branch level (Dynamax gauges). In addition, there was tendency for the highest porometer-based transpiration rates to be observed between 1500 and 1730 hours. These differences reflect the inability of the porometer-based measurements to properly account for the boundary layer conductance surrounding the leaf. Patterns of whole tree water loss demonstrated a plateau similar to the upper and middle crown branches; however, whole tree rates decreased after 1700 hours whereas they did not for these branches.

During the plateau of maximum transpiration from the middle and upper branches, average leaf conductance was calculated to be 409 $\text{mmol m}^{-2} \text{s}^{-1}$ (corresponds to an average from the porometer of approximately 312 $\text{mmol m}^{-2} \text{s}^{-1}$) while crown conductance was 181 $\text{mmol m}^{-2} \text{s}^{-1}$. These findings result in a boundary layer conductance of about 325 $\text{mmol m}^{-2} \text{s}^{-1}$ (*i.e.*, less than leaf conductance). Based upon a recent formulation from Jarvis and McNaughton (1986), we calculated Ω to be about 0.8, which indicates that a 10% change in leaf conductance would cause transpiration to change by only 2% (at constant vapor pressure deficit, etc.). *Populus* is thus very poorly coupled with the atmosphere when compared with a conifer (*i.e.*, $\Omega = 0.1$).

Perhaps our greatest surprise resulted from the comparison of stand water loss for this fast growing, deciduous hardwood located on an alluvial soil and with direct access to groundwater, to that from a stand of evergreen conifers growing on a droughty, upland, gravelly, glacial outwash soil. Maximum stand water loss for *Populus* was approximately 4.50 mm per day, *i.e.*, the same maximum as had been noted for *Pseudotsuga menziesii* (Fritschen, *et al.*, 1973). The conifer stand had a slightly smaller leaf area index (10 vs. 12), and a much lower maximum foliar conductance ($\sim 60 \text{ mmol m}^{-2} \text{s}^{-1}$) than did the *Populus* stand. However, the evergreen conifer is probably better coupled to the bulk

atmosphere than the deciduous hardwood. As a result, dramatic differences in stomatal morphology and conductance between these two species disappear when considering water loss from the whole canopy.

Had we used only leaf conductance measurements in this study, we would have had a sampling nightmare. On the other hand, if had we used only whole tree measures of water loss, we would not have identified important components of spatial and temporal heterogeneity in transpiration nor any of the unique genetic and developmental controls over water loss. It is a long way from studies of epidermal peels to stand level measures of water loss, but these studies with *Populus* and the work of other researchers (Meinzer, *et al.*, 1993) demonstrate the need to link physiological processes at different levels of structural organization. As stated by Meinzer (1993), plant water status and ecosystem water balance are governed largely by actual fluxes of water vapor rather than stomatal conductance. Therefore, measurements of stomatal conductance alone often do not provide enough information to evaluate the physiological and ecological consequences of contrasting patterns of stomatal activity in different species.

Canopy Architecture: The crown, whether viewed as a single leaf or a stand of trees, is the interface between the gaseous atmosphere and the liquid medium of the plant. It serves to intercept light and to convert this intercepted light into carbon products. It is the major sink for mineral nutrients and it is the only controlling point for water loss and water conservation. Our understanding of the role stomata, leaves, trees and stands on water loss was presented in the previous section. Here the accomplishments of the last decade in cell, leaf, branch and whole tree growth studies are reviewed.

Based upon studies at the level of the cell and the leaf, the following general points have been noted (Ridge, *et al.*, 1986; Isebrands, *et al.*, 1988; Roden, *et al.*, 1990; Dunlap 1991; Neuman, *et al.*, 1993). First, both cell division and cell expansion are affected by stress. Cell expansion is sensitive to changes in both water and nutrient concentrations and the presence of regulatory compounds originating in either the leaf or root. Cell division is responsive to changes in photosynthate availability.

Second, the interaction between cell division and cell expansion, as they determine individual leaf size, appears to be an adaptive response to the environmental conditions typical for the species under study (*e.g.*, *P. trichocarpa* vs. *P. deltoides*). Hybridization may uncouple this interaction and may result in advantageous combinations.

Third, in mesic or irrigated xeric environments, clones and hybrid clones with more horizontal lamina angle appear more productive than clones with more vertical angles. This advantage is most apparent under cloudy conditions. Finally, a significant correlation has been repeatedly observed between single leaf area, total leaf area and net primary productivity in young short-rotation material.

Ceulemans, *et al.*, (1990, 1992) and Hinckley, *et al.*, (1992) noted that the interaction between branch type (sylleptic vs. proleptic) and leaf area development were important components of how rapidly clones were able to occupy a site and initiate high rates of growth. The crown of poplars is frequently composed of a main stem and several different types of branches. Two alternative development processes, namely syllepsis and proleptis, are responsible for the types of branches we observe on most if not all of the clones examined. Scarascia-Mugnozza (1991) has observed that differences in development between sylleptic and proleptic branches are associated with differences in physiological function such as the timing, nature and extent of carbon export to the rest of the plant. Ceulemans (1990), Ceulemans, *et al.*, (1992) and McKevlin (1993) have found that genotype and spacing affect the relative expression of sylleptis and proleptis, thus determining how these two forms of branches contribute to the successful growth of an individual over time and in space.

In spite of the remarkable variation in crown architecture among and within natural populations of *Populus* spp., very different forms appear successful in apparently very similar environments (Ceulemans, 1990; Ceulemans, *et al.*, 1992). Sylleptis appears to be a favorable, but certainly not the only, morphological attribute that determines final yield (Hinckley, *et al.*, 1989). Perhaps its relatively early expression in some clones confers an initial advantage to these clones. Under short-rotation management such an advantage may be an especially desirable feature. However, as rotation intervals increase, other morphological attributes may be more important. Unfortunately, little work has been conducted on older material.

Understanding the linkage between crown structure and productivity and how the various components of crown structure are genetically determined vs. environmentally plastic are important research goals of short-rotation culture. For example following a four-year study of clones of *Populus* spp. and their hybrids, Ceulemans, *et al.*, (1990) concluded that the following branch characteristics should be included in the list of productivity components for poplar ideotypes used in short-rotation intensive culture: (1) acute branch angle, (2) many small branches, combined with a high proportion of sylleptic branches, and (3) high leaf area density on the branches, though there should be limits because of leaf clumping and self-shading effects. Differences in plantation density and rotation length may affect these recommendations. Because of the moderate to high genotypic control over branch characteristics, natural populations of *Populus* offer an interesting reservoir of diversity from which such characteristics can be selected.

Three critical future research needs exist. First, an understanding of the regulation of whole tree carbon distribution is important and remains a major limitation in material selection, model building and cultural prescription. Second, the knowledge base in genetics and, in particular, molecular genetics, needs to be coupled more closely with studies of physiology and morphology. Third, physiological studies must address issues of

scale--which lower level processes are necessary to address questions placed at higher levels--and must identify useful indicators of whole tree or stand processes.

NUTRITION AND PLANTATION MANAGEMENT

Nutrition

Most of the cultural requirements for success with short rotation *Populus trichocarpa* X *P. deltoides* (TxD) hybrid plantations in the Pacific Northwest (PNW) are known. An area of remaining uncertainty is nutrition, particularly diagnosing and predicting the needs for fertilizer, especially nitrogen.

Studies with other trees show complex relationships between nitrogen, canopy leaf area and efficiency of foliage in stemwood production. The effect of nitrogen in increasing the area and/or mass of foliage is well documented (Heilman and Gessel, 1963; Brix, 1983; Binkley and Reed, 1984; Vose and Allen, 1988). In his study of Douglas-fir, Brix (1983) reported that 63% of the stemwood response to nitrogen fertilization could be attributed to an increase in foliage biomass and 37% to increased efficiency of the foliage in production of biomass. On the other hand, increasing the leaf area of stands that are already high in leaf area can reduce efficiency of the foliage and can, in the extreme, result in no increase in production of stemwood (Waring 1983). Information on such effects of nitrogen on the TxD hybrids is needed.

The purpose of the study by Heilman and Xie (1993a) was to examine the nitrogen response by *Populus* TxD hybrids on a moist alluvial soil with moderate fertility. The following aspects of nitrogen response were examined: 1) effect on individual tree growth and form; 2) influence on production of above-ground biomass and bole volume; and 3) effect on foliar nitrogen concentrations. Also investigated were soil nitrogen concentrations to enable comparison of soil nitrogen status in this study with that of prior and future nitrogen response studies. The effects of N fertilization on soil pH and N levels were also examined since fertilization with ammonium fertilizer in particular is known to reduce soil pH, an effect that at some point could reduce *Populus* growth. Also of interest in this study was determination of differences in responses of clones to nitrogen fertilizer. Such differences are important to our efforts to identify and quantify variation among hybrid clones in nitrogen-use efficiency (Heilman and Stettler 1986).

The study by Heilman and Xie (1993b) examined response of trees to nitrogen in terms of leaf properties, development of leaf area and changes in efficiency of the canopy in both intercepting light and in producing stemwood and biomass. The same six TxD hybrids were investigated as in the nitrogen fertilization experiment described above. The study was especially designed to detect clonal differences in nitrogen response.

Effect of N on Growth (Heilman and Xie, 1993a). The N-treated trees received a total of 500 kg N ha⁻¹ in three equal applications at the beginning of the second, third and fourth growing seasons. Annual growth in height and diameter were significantly increased by nitrogen only in the third growing season. Mean stem dimensions and productivity after four growing seasons for fertilized and control plots, respectively, were: total height, 13.2 vs 12.6 m; DBH, 11.2 vs 10.1 cm; total basal area, 20.1 vs 16.6 m² ha⁻¹; mean annual volume growth (inside bark) 22.2 vs 18.4 m³ ha⁻¹ y⁻¹; and mean annual woody biomass production (above-ground) 25.3 vs 20.9 Mg ha⁻¹ y⁻¹. Nitrogen increased yield at the end of 4 years by 21 percent. All values except total height were significantly different according to an analysis of variance. Judging by the lack of fertilizer response in the fourth year, two annual applications of N in the second and third growing seasons might have been enough to obtain the measured growth response. Competition among trees at the relatively close spacing (1.5 x 3 m) may have limited the ability of these fast growing trees to respond to the third nitrogen application. Nitrogen appeared to shorten the time required to reach culmination of periodic annual increment. At the wider spacing now recommended for these plantations (2.1 x 3 m) nitrogen responses may be of longer duration. Clones differed significantly in all measures of growth except annual height growth, but no significant clone x nitrogen interactions were found for most parameters studied.

Effect of N on Light Interception, Leaf Area and Productivity (Heilman and Xie, 1993b). Fertilized canopies captured more light (PAR) as measured close to zenith with the Sunfleck Ceptometer. At this time of day, over 98% of available light (PAR) was captured by the densest canopies in this experiment. In year three, the difference between fertilized and unfertilized plots in light capture was comparatively smaller than the difference in productivity. In view of the significantly higher vertical canopy densities (leaf area index/canopy length) on fertilized plots, the differences in light capture between control and fertilized plots may be at a minimum when the sun is overhead. Measurement of the light capture at other heights in the canopy and at other sun angles is needed to better evaluate the relationship of light capture by canopies to productivity.

Canopy efficiency of these trees was very high with means between 5 and 6 Mg dry biomass ha⁻¹ y⁻¹ per unit leaf area index (L). Means varied little between years but significantly between clones (at least in year three) with the most productive clone having the highest canopy efficiency, and the least productive the lowest.

Specific leaf area (leaf area divided by leaf weight) increased from year three to year four, indicating an average decrease in thickness of leaves; but clones clearly differed in the degree of change. Whether decreased leaf thickness resulted from an increase in relative abundance of early (thinner) leaves, or from a reaction of leaves to increased shading (more numerous "shade" leaves), or both is unclear. Nor do we know how the increase in specific leaf area affects the efficiency of the canopy in capturing light and

producing biomass. More study of these relationships is needed. Patterns of leaf fall varied only slightly among these six clones but independently of nitrogen treatment.

The 21% increase in biomass production due to N treatment (Heilman and Xie 1993a) came primarily in the third year when production was about 40% greater on fertilized plots. In sharp contrast, the increase in the fourth year was not significant and averaged only about 8%. The greatest increase in mean leaf area index (L) of fertilized plots occurred in year three when L on fertilized plots averaged 6.1, or 61% more than on control plots, which had a mean L of 3.8. In year four, L on control plots averaged 6.5 - even more than the fertilized plots had in year three. With equal or larger L than the fertilized plots had in year three, the production on most of the control plots (four out of six) in year four was equal or larger than it was on fertilized plots in year three. Consequently, with high L on control plots and lowered canopy efficiency at the high L levels on fertilized plots, the difference in biomass increment between treated and control plots in year four was relatively slight.

This study showed large increases in leaf area index (L) due to nitrogen treatment. It also showed reduced efficiency of the canopy in producing biomass at high levels of L. The combined effect manifested itself especially in those canopies at the highest levels of L, which were the fertilized plots of the higher yielding clones. Although data indicated that productivity of biomass had not peaked at L below 13, that level of L may be close to the value where maximum biomass production will occur.

Short Rotation Coppice

The capacity to resprout after harvest was one of several reasons for considering poplar species, among them *P. trichocarpa*, as prime candidates for short-rotation intensive culture. However, little information was available on the genetic variation of coppicing ability in black cottonwood and how this translates into coppice productivity in different clones from different sources. Of equal interest was the comparison in this trait between black cottonwood and its TxD hybrids, which had shown superior first-rotation performance at four years (Stettler, *et al.*, 1988).

In a paper by Heilman and Stettler (1990), we reported on the second-rotation performance of an experiment initiated in 1979 (Heilman and Stettler, 1985), in which trees, after a first-rotation harvest at four years, were allowed to resprout. The study examined resprouting in 50 clones of *P. trichocarpa* from 10 major river drainages in western British Columbia, Washington, and Oregon. Additionally, three TxD hybrids, a 'Robusta' clone, and a previously selected *P. trichocarpa* clone from Cottage Grove, Oregon, were included in the experiment. Mean dry wt yield of *P. trichocarpa* clones was 11.3 Mg ha⁻¹ yr⁻¹ (CV among clones = 61%) in the coppice rotation vs. 12.6 Mg ha⁻¹ yr⁻¹ (CV among clones = 40%) in the initial rotation; a difference that may, in part, be due to the use of less fertilizer in the coppice rotation. Nevertheless, a number of

clones including the hybrids had higher yields in the coppice than in the initial rotation. Yield of clones in the coppice rotation was only moderately correlated with yield in the initial harvest ($r^2 = 0.48$), but the same two clones, TxD hybrids 11-11 and 11-05, were the top producers in both rotations. Cumulative mortality of *P. trichocarpa* at the end of the experiment, including failed cuttings, averaged 36%, but was highly variable (range = 7-66% among clones). Yield in both harvests differed significantly among sources of *P. trichocarpa*. As with other components of productivity reported earlier, performance in coppice varied significantly among the 50 *P. trichocarpa* clones, and more so than in the first rotation. *P. trichocarpa* clones identified as superior in coppicing in this experiment may be considered as potential parents for new hybrids intended for coppice culture.

PROPAGATION/PRESERVATION

Production of cuttings for propagation in experimental plantings has again constituted a major activity of this project. Most of the cuttings are produced in stool bed nurseries which also serve as clone banks for preservation of the materials. Included in the four established nursery bed/clone banks are the hybrids produced in the project, clones of natural species collected from various sources and a variety of research materials. Early clone banks contained 25 plants of each hybrid clone. Later clone banks hold two plants of each pure-species clone and five of each hybrid.

First cuts in the selection process are done in the clone banks. Successful propagation from cuttings and successful resprouting are the first selection criteria. Other selection criteria applied in the clone banks include disease and insect susceptibility, animal (rabbit) damage, relative growth rate and early age specific gravity (although not used to date). Apart from serving as repository of production clones, the clone banks also provide important research materials for studies conducted in-house as well as by collaborating scientists elsewhere.

The Clone Arboretum contains selected *P. trichocarpa*, *P. deltoides* and *P. maximowiczii*. and some hybrid clones that are being grown for future breeding purposes. Over 60 clones in four copies each have been planted with the oldest now 6 years old.

DATABASE MANAGEMENT

The database management system adopted in this project has had to serve two broad purposes: (1) to expedite implementation of project activities, and (2) to coordinate information access and exchange among project scientists, outside collaborators and the DOE Short Rotation Woody Crops Program Technical Database. As the program has expanded, so has the database, and so has the sophistication of the hardware and personnel. Today, field data are entered directly in a TMDR field computer and then

transferred to hard disk in the lab in Seattle (486/33 CPU with 211 MB hard drive and 8 MB RAM, connected to the university's network of mainframes and workstations). Thus, the efficiency of data analysis and subsequent storage has been greatly improved.

Access to this information will be broadened by the development of a **Poplar Molecular Network (PMN)**. Through funding from a new program on Collaborative Research in Plant Biology, jointly sponsored by DOE/NSF/USDA, we have been able to acquire a full-time database manager and a Sun SPARC station LX computer to expand the scope of the database. With the rapid development of the *Populus* genome map and the availability of informative pedigree material, the moment seemed ripe for a broader sharing of information, materials, techniques and protocols on poplar biology. PMN makes use of Internet, a worldwide collection of networks, to achieve this. The long-term benefits of PMN will be a better integration of new information on *Populus* through the organizing principle of a genome map. The immediate benefits to this project from initiating the PMN have been the availability of added computer expertise in the person of the new database manager, Carl Riches, and the resulting improvement of the existing database.

During the past five years, several important reports were printed and distributed among all scientists and industrial collaborators. They include:

- **Clone Register.** A list of all clones collected or generated in the UW/WSU Poplar Research Program and accessions from other programs. It contains the ID number of each clone, its taxon, origin or parentage, and the parents' geographic source. Originally issued in 1987, an updated register was printed in 1991, containing information on more than 7700 clones. Addenda have given supplemental information on gender and ploidy of some clones.
- **Pedigree Clone Register.** A 1992 supplement to the Clone Register which focuses on advanced-generation pedigrees. It is primarily intended for researchers who conduct detailed studies on large families from select pedigrees (*e.g.*, genome mapping).
- **Plantation Register.** A list of all plantations in which clones of the UW/WSU Poplar Research Program are being tested. It permits tracing a clone from stoolbed to all plantations in which it is represented (including the number of copies). Issued in 1989 in ring-book format, it permits periodic updating.

TECHNOLOGY TRANSFER

Information and advice about cottonwood hybrids and their culture has been provided to individuals and companies through a variety of means. By far the most time is taken up by one-on-one conversations over the phone. Referrals come from a variety of sources with numbers of calls significantly increasing following news media publicity (mostly newspaper articles). With publication of an extension bulletin (Heilman, *et al.*, 1991), it

has been easier to address these requests for information since callers can purchase and read the bulletin. Publication of that bulletin has also allowed county extension agents to provide the information and a number of these individuals have become competent to do that.

Field tours have also been effective in providing information about the culture of the hybrids. A number of field tours were held over the last five years with the latest in the fall of 1992 involving a tour of James River plantations at Clatskanie, Oregon.

Annual workshops have also been helpful for exchanging information with industrial cooperators and university officials. Such a workshop was held over two days in February 1993 at Pack Forest and was attended by over 40 participants.

Several formal seminar series under the auspices of the University of Washington have provided an ongoing outlet for research findings and industry reports. These have been held since 1988 and have been well attended by faculty, graduate students, technicians and industry representatives.

Scientists in the poplar research group have also participated in annual presentations to the Washington State Legislature for review of projects funded by the Washington Technology Center.

CONCLUDING OBSERVATIONS

It seems appropriate to conclude this report with a few observations of a general nature. They pertain to several broader issues of research for which this project offers a unique perspective.

Continuity. The importance of the continual support offered to this project by the US Department of Energy over a period of 15 years cannot be overestimated. The prospect of funding continuity has provided the incentive for a long-term research approach and an incremental program development. Sustained support has been responsible for the continuity of personnel, the systematic accumulation of technical experience, the gradual build-up of pedigreed plant material, and the maintenance of long-term research records. These assets, in turn, have enabled us to attract other scientists into collaborative agreements. Thus, funding from DOE has had a multiplier effect through many collaborative grants it made possible from other agencies, such as the National Science Foundation, the Department of Agriculture's Competitive Grants program, the US Forest Service, the Washington Technology Center, and from industry.

Collaboration. From its outset, this project has been multi-disciplinary and multi-institutional, combining the complementary features of a land-grant institution and a major research university. Both elements have provided for intellectual enrichment and

programmatic enhancement. Over the years, the number of interactive researchers and institutions has significantly increased and today constitutes an impressive international network (Table 1). A good example of the power of collaborative research is the recent adoption of a three-generation TxD hybrid pedigree as focal material for the genetic linkage map and for all associated studies on productivity traits. It called for agreements on joint protocols among biochemists, geneticists, pathologists and physiologists from four institutions (UW, WSU, Iowa State University and the US Forest Service North Central Experiment Station). It demanded a massive expansion of pedigree material (620 genotypes) to generate more than 10,000 plants for two replicated plantations of identical design to be installed east and west of the Cascades, totalling 11.2 acres. This was only possible through major contributions in personnel, land and material, made by three companies (Boise Cascade Corporation, James River Corporation, and Mt. Jefferson Farms, Salem OR) and through their willingness to maintain these plantations for the next six years. Existing precedents in corn and tomato attest to the value of such plantations in tracing important commercial traits to molecular markers. This concentrated collaborative effort will test the concept's relevance to poplar. What can be safely predicted is that the numerous data to be collected by many researchers on this material will be more easily integrated into a coherent model of poplar growth and development and its underlying genetics.

Education. Many graduate students, postdoctoral research associates and visiting scientists have been associated with this project and the broader poplar research program. Clearly they have been both contributors to and beneficiaries of its educational function. Probably the most effective educational mechanism has been the Poplar Seminar Series held every two weeks during Fall and Winter Quarters. While typically meeting on the UW campus, it has also convened one or twice per quarter at Puyallup and has been generally attended also by scientists and graduate students from other research areas. The seminar has been devoted to current research reports by graduate students and faculty, presentations by visiting scientists, and regular updates by industrial collaborators on poplar culture in the region. The unusual range of topics, spanning from the molecular level to that of the field plantation or natural population, has forced a broadening of perspectives and understanding in all participants. The positive experience from this exercise has been instrumental in coalescing in 1989 a broader community on the UW campus into the "Plant Molecular Integration and Function" (PMIF) Committee of the Graduate School. Through Graduate School funds, PMIF has provided graduate fellowships to participating students from several departments and has helped to offer new graduate courses in plant biochemistry, plant genetics and plant-microbe interactions.

Innovation. What may be the most rewarding and encouraging aspect of the poplar research project is the element of innovation that manifests itself in all phases of the program. All people involved, from the biochemist at the lab bench to the industrial manager of fiber plantations, display an unusual predilection to chart a new course. It

derives in good part from the novelty of short rotation culture, the lack of historical precedence in the region, and, as a consequence, the absence of constraints by tradition. It also feeds on the rapid results, the high degree of control and the high repeatability of working with clonal material of a fast growing tree. At the recent Poplar Retreat held at Pack Forest, bringing together all researchers and industrial collaborators, the most persistent element throughout all sessions seemed to be the shared excitement by all in being part of a new venture that demands new thinking, new methods, and new commitments. The momentum generated by this spirit may well be the most effective antidote against the recessionary economic forces that currently set limits to the funding of this project.

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