Agroforestry Systems **32**: 29–44, 1995.
© 1995 *Kluwer Academic Publishers. Printed in the Netherlands.*

The potential of biotechnology in temperate agroforestry practices*

N. B. KLOPFENSTEIN and J. G. KERL
USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, National Agroforestry Center, East Campus, University of Nebraska, Lincoln, NE 68583-0822, USA

Key words: genetic engineering, marker-aided selection, molecular diagnostics, plant tissue culture

Abstract. Technologies in forest molecular biology and tissue culture could play an increasing role in the choice of genotypes for successful establishment of agroforestry practices. Research areas such as micropropagation, somatic embryogenesis, genetic engineering, marker-aided selection, and molecular diagnostics are merging with traditional forest biological studies to help identify and produce better-suited trees for agroforestry plantings. A combination of classical and molecular biological research could be used to improve pest and stress resistance of selected genotypes, modify structure and function, and monitor pests of trees. This merger of approaches, as well as continued technological development, could accelerate the production and selection of suitable tree genotypes for agroforestry plantings.

Introduction

Agroforestry technology has evolved since agriculture began [King, 1989]. Recently, molecular biology has shown potential to accelerate forest technological development. Current studies in molecular biology are providing what could be the basis for significant advances toward improving agroforestry practices.

Common to all agroforestry practices is the need to provide the right tree for the right purpose on a given site. At present, we lack sufficient information to produce or select the best genotypes. Full benefits of agroforestry are sacrificed when mal-adapted trees are used, due to pest attack and environmental stresses. Mal-adapted genotypes also fail to adequately function as a windbreak, soil stabilizer, shade provider, riparian buffer, living snowfence, etc. Establishment of agroforests is both time consuming and expensive. Tree sites are typically of low quality, and tree maintenance is often neglected when maintenance priorities are given to herbaceous crops. To survive and grow over their optimal life span, trees must tolerate changing environmental stresses and resist continual threats from changing insect and pathogen populations.

Hence, molecular biological techniques and other biotechnologies are being

* The US Government right to retain a non-exclusive, royalty free licence in and to any copyright is acknowledged.

developed to accelerate the production and selection of optimally adapted genotypes. These genotypes will be selected for their ability to grow and perform specific functions within a defined agroforestry system. Because such developments require multidisciplinary interactions, biotechnological methods are beginning to be utilized in several different areas. This paper briefly describes the status of these methods.

Plant tissue culture

Plant tissue culture is the best known of the established biotechnological tools for woody plant propagation [Haissig et al., 1987] (Fig. 1). With tissue culture techniques, a variety of organs, tissues, or cells can serve as source material for the propagation and regeneration of trees on a chemically defined culture medium. Through the culture process, this tool provides a means to clonally propagate large numbers of desirable trees for research studies and outplanting. The genetically identical plant material developed by cloning can be used to evaluate genetic and environmental effects on tree growth. Once elite trees are identified, tissue culture propagation can rapidly provide large quantities of planting stock via axillary bud multiplication [Chun, 1993; Lakshmi Sita, 1993], adventitious shoot regeneration [Louis, 1994], somatic embryogenesis [Aitken-Christie et al., 1994; Cervelli et al., 1994], or synthetic seeds produced via somatic embryogenesis [Attree and Fowke, 1993; Gupta and Kreitinger, 1993]. Clonal plantings must be considered carefully, however, to assure that adequate genetic variability is maintained [Libby, 1988; Libby and Ahuja, 1993; Maynard, 1986; Muhs, 1992; Zsuffa, 1992]. Tissue culture methods may also provide a means to screen and select for resistance to physical and chemical stresses such as herbicides [Michler and Haissig, 1988], pathogen toxins [Dorion et al., 1994; Kruger and Manion, 1993], or environmental toxins [Chung and Chun, 1990]. The production or propagation of trees with altered chromosome numbers, such as haploid or polyploid, for tree improvement programs may also be facilitated by tissue culture [Chun and Hall, 1992; Von Aderkas and Dawkins, 1993; Son et al., 1993; Wann et al., 1988; Winton, 1970]. Disease-free trees for use as planting stock or international germplasm exchange also can be produced via tissue culture manipulation [McNabb et al., 1986], and cold storage of tissue cultures can be used to preserve germplasm of numerous trees in a relatively small space [Ahuja, 1991; Find et al., 1993; Son et al., 1991]. Furthermore, tissue culture systems are necessary to efficiently produce and regenerate genetically engineered trees.

Plant tissue culture technologies have contributed numerous examples of how improved genotypes can be selected for agroforestry practices [Ahuja, 1988, 1993]. In ongoing studies at the USDA Forest Service, Rocky Mountain Station, National Agroforestry Center (NAC) tissue culture techniques are used to produce and multiply clonal material of green ash (*Fraxinus pennsylvanica* Marsh.) for studies to identify sources and mechanisms of drought

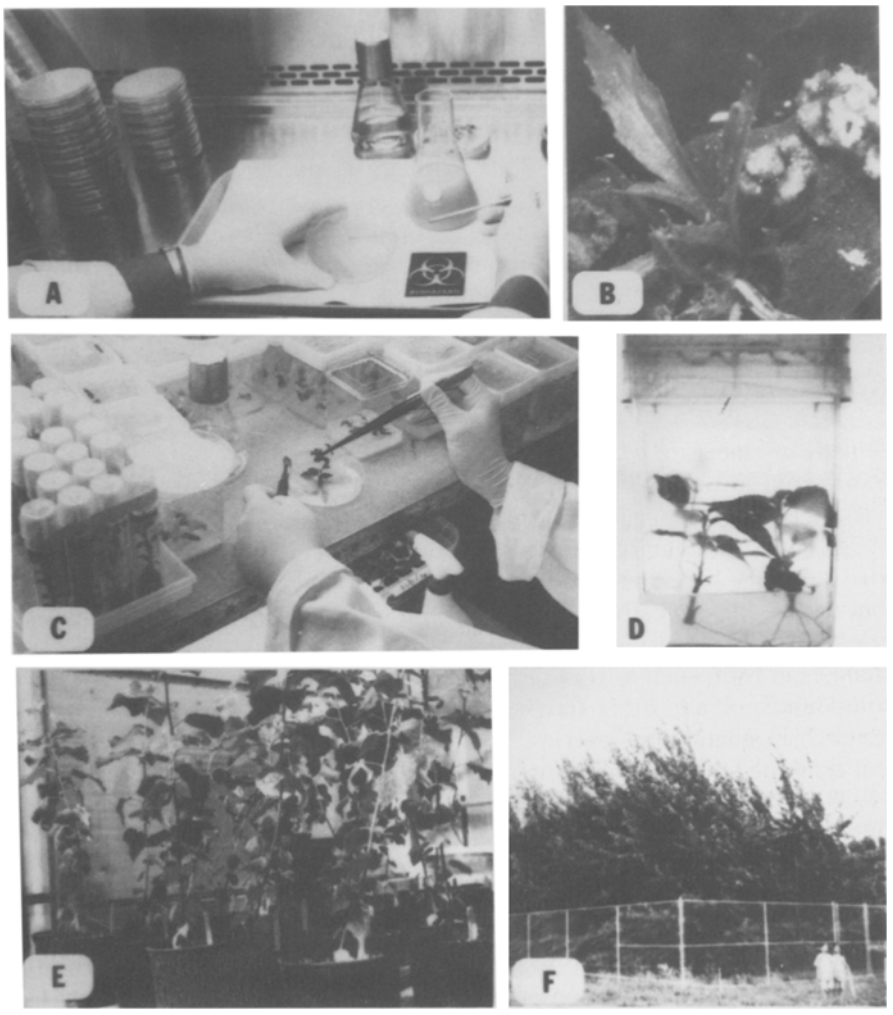


Fig. 1. Genetic engineering and tissue culture. (A) Foreign genes from any organism are replicated in bacteria and transferred to tree cells. (B) Tissue culture techniques are used to regenerate genetically engineered trees from transformed cells on a chemically defined growth medium. (C) Tree shoots or other tissues are subcultured onto new culture media using aseptic techniques. (D) Selected genotypes are propagated in tissue culture. (E) Tissue culture-derived trees are adapted to lower humidity conditions and established in pots for greenhouse growth. (F) Genetically engineered, tissue culture-derived trees can be grown in the field when government regulated biosafety guidelines are followed so as to minimize risks of gene flow to native germplasm and reduce impacts on other components of the ecosystem. Pictured is a field plot of transgenic poplar established in 1989 by Iowa State University in accordance with USDA APHIS guidelines.

stress resistance [Kim et al., 1994]. The NAC also conducts cooperative research with North Dakota State University using tissue culture to propagate ponderosa pine (*Pinus ponderosa* Doug. ex Law.) and to identify sources and mechanisms of resistance to western gall rust [Cheng et al., 1993]. Research conducted by the USDA Forest Service in St. Paul, MN, uses tissue culture methods to generate *Populus* material that is resistant to Septoria canker [Ostry et al., 1990, 1994]. Parallel work at Rhinelander, WI, was aimed at generating herbicide-resistant *Populus* plants [Michler et al., 1989]. Tissue culture methods could play a prominent role in the future development of suitable trees for agroforestry systems.

Genetic engineering

Genetic engineering techniques allow a specific gene from any species to be transferred to an individual tree species using various methods, including bacterial vector or 'gene gun' methods [Jouanin et al., 1993; Manders et al., 1992] (Fig. 1). Each transferred gene can then code for a specific enzyme that in theory can alter or augment the physiology of a tree. Desired traits that can be theoretically incorporated into genotypes of various tree species via genetic engineering include pest resistance, stress resistance, specific changes in form such as root or branch structure, the ability to degrade toxic compounds, or any other trait that is influenced by a single gene. Current research in genetic engineering of trees is directed toward designing genes that function only in specific tissue [Ellis et al., 1991; Hauth and Beiderbeck, 1992; Wilde et al., 1992], at specific developmental stages [Ellis et al., 1991, 1994], or in response to certain stimuli such as wounding [Ellis et al., 1994; Klopfenstein et al., 1991], flooding [Ellis et al., 1991], plant growth regulator application [Aronen et al., 1994; Duchesne and Charest, 1992; Ellis et al., 1991; Newton et al., 1992], increased temperature [Ellis et al., 1991], or light exposure [Ellis et al., 1991]. Genetic engineering also provides a tool to better understand the function of specific genes, thereby allowing trees to be selected that carry genes that code for desirable functions. Presently, the inability to transfer more than a few genes at once is a major limitation in the potential contributions of genetic engineering to agroforestry. Additional transgenes could perhaps be incorporated into selected genomes by sequential transformation processes or through cross breeding of transgenic selections, but such processes would be time consuming. Because multigenic systems are yet to be characterized at the molecular level in woody plants, it remains uncertain whether multigenic traits can be manipulated with genetic engineering.

Examples of how genetic engineering might contribute to agroforestry are increasing. Workers at NAC and Iowa State University have transformed *Populus* hybrids with potato proteinase inhibitor II (*pin2*) genes in an attempt

to study and improve resistance to insects and disease [Klopfenstein et al., 1991, 1993]. It is hoped that proteinase inhibitors will interfere with nutrition-deriving processes of pests, and thereby slow growth and development without placing a strong selection pressure on the pest. At the University of Wisconsin, *Populus* sp. and white spruce (*Picea glauca*) were transformed with genes encoding endotoxin proteins from *Bacillus thuringiensis* (Bt) to control insect damage [Ellis et al., 1993; McCown et al., 1991], and a similar approach is being used with larch (*Larix decidua* Mill.) at Michigan Technical University [Shin et al., 1994]. Strategies are being developed to improve the stability of resistance imparted by Bt endotoxin gene. Such strategies may include adding an inducible promoter or regulatory region to the gene so that the gene is active only at set times such as when a specific damage threshold has been reached. Other strategies involve incorporating the gene into trees with other resistance factors, interplanting with trees that lack the specific resistance gene, and developing complementary biological control strategies [Raffa, 1989; Strauss et al., 1991]. Other examples of genetic engineering studies include cooperative work with the USDA Forest Service in Rhinelander, WI in which genetic engineering was used to produce genotypes of *Populus* sp. that are herbicide resistant [Donahue et al., 1994; Fillatti et al., 1987]. In a novel approach to bioremediation, molecular research at the University of Washington is directed toward detoxifying environmental toxins such as trichloroethylene (TCE) with genetically engineered *Populus* spp. [Stomp et al., 1994].

Environmental risks associated with field use of genetically engineered trees are a major concern that must be thoroughly addressed before genetically engineered trees can be utilized in agroforestry systems. Such risks are being evaluated at Iowa State University and the University of Wisconsin to help ensure environmentally safe planting of genetically engineered trees [McNabb et al., 1991; Raffa, 1989]. Field tests have been conducted with genetically engineered poplar (*Populus* spp.) [DeBlock, 1990; Ellis et al., 1994; Klopfenstein et al., 1991], white spruce (*Picea glauca* (Moench) Voss) [Ellis et al., 1994], apple (*Malus pumila* Mill.) [James et al., 1993] and walnut (*Juglans regia* L.) [McGranahan et al., 1988; Wrubel et al., 1992]. Field tests of genetically engineered trees must be conducted in a manner that minimizes risks to native germplasm and other ecosystem components [Ellis et al., 1994; McNabb et al., 1991; Strauss et al., 1994, 1995]. Protection of native germplasm can perhaps be approached by deploying selections in areas outside of their native range, using reproductively sterile selections, or removing genetically engineered trees before flowering. In addition, the potential effects of introduced genes on other organisms within the ecosystem should be thoroughly evaluated before deployment of genetically engineered trees. Nevertheless, genetic engineering should contribute to our understanding of gene function in trees, and more field plantings of genetically engineered trees will likely occur after environmental risks are adequately addressed.

Marker-aided selection

Marker-aided selection is being developed to provide a system to select trees based on correlations between DNA characteristics and the 'trait of interest' [Neale et al., 1992] (Fig. 2). In theory, it will become possible to identify quantitative trait loci (QTLs) in seedling tissue DNA to predict performance traits of a genotype as a future mature tree. In an ongoing QTL mapping project with hybrid *Populus*, it was found that 1–5 QTLs were responsible for a large portion of genetic variance in many traits of interest. For example, two QTLs controlled 44.7% of the genetic variance in stem volume after 2-years' growth [Bradshaw and Stettler, 1995]. In addition, specific genes are being identified that may be related to resistance or susceptibility to pests and stresses. Examples of genes identified from trees include two chitinase genes, a proteinase inhibitor-like gene, and phenylalanine ammonia-lyase genes from *Populus* spp. [Bradshaw et al., 1989; Davis et al., 1991; Hollick and Gordon, 1993; Subramaniam et al., 1993]. Alcohol dehydrogenase genes, superoxide dismutase genes, a chalcone synthase gene, a dihydropinosylvin synthase gene, and a stilbene synthase gene also have been identified from *Pinus* spp. [Fliegmann et al., 1992; Karpinski et al., 1992; Kinlaw et al., 1990; Schwekendiek et al., 1992]. Continued development of molecular genetic technologies for trees could dramatically improve trees planted for agroforestry by eliminating genotypes that are 'predestined' to a shortened life span because of a predetermined genetic susceptibility to pests and environmental stresses.

With most woody plants, genes that contribute to desired traits are yet to be identified. Genetic mapping projects have begun to focus on the genetic basis of desired traits in trees. To begin a mapping project, pedigreed plant material is needed that possesses a range in traits of interest. After mapping studies have identified a large set of molecular markers that are dispersed throughout the chromosomal DNA, researchers look for correlations between the occurrence of a specific trait and the occurrence of DNA markers. Current examples of genetic mapping projects with woody species include studies of (a) loblolly pine (*Pinus taeda* L.) at the USDA Forest Service Institute of Forest Genetics in Berkeley, CA [Williams and Neale, 1992], and North Carolina State University [Amerson et al., 1994; Wilcox et al., 1992]; (b) slash pine (*Pinus elliottii*) by the USDA Forest Service in Gulfport, MS [Nelson et al., 1993]; (c) *Populus* spp. at the University of Washington [Bradshaw and Stettler, 1995]; and (d) *Eucalyptus grandis* in a joint effort between North Carolina State University and Aracruz Florestal in Brazil [Grattapaglia et al., 1994]. As genetic mapping technology continues to improve, genetic mapping of more diverse woody plant species should become more practical. Researchers at Oklahoma State University are using similar genetic technologies in an attempt to identify molecular markers that are associated with drought stress resistance in woody plants [Tauer et al., 1992]. Additional genetic mapping of diverse tree species can be anticipated when more multi-

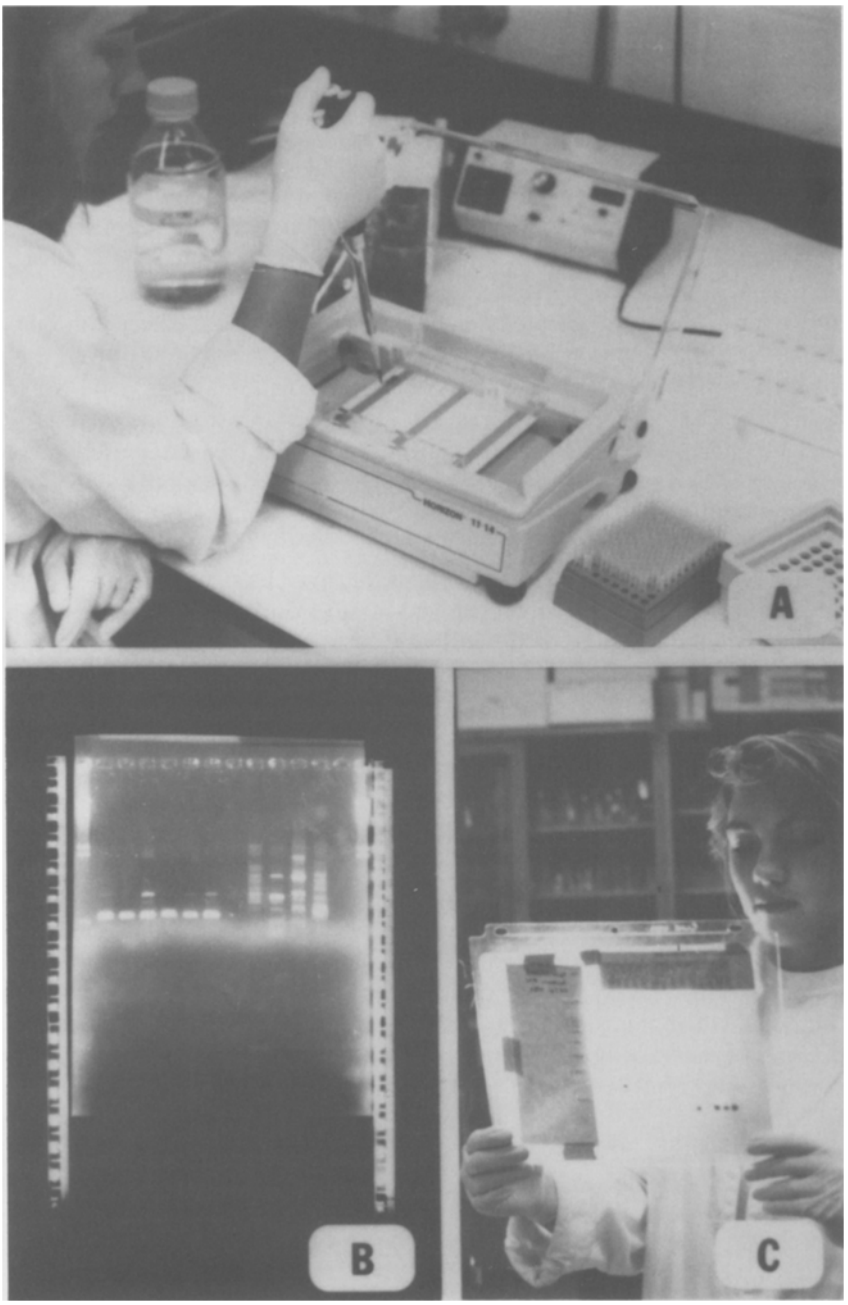


Fig. 2. DNA analysis. (A) DNA samples are loaded in an agarose gel in preparation for electrophoresis. (B) Patterns of DNA migration are visualized under UV light after staining. (C) Specific DNA sequences are identified using specific probes and autoradiography.

generation pedigrees become available from breeding programs and mapping technologies become more efficient.

Molecular diagnostics

Molecular diagnostics use DNA or protein analyses to detect, identify, or characterize specific genes, specific proteins, phylogenetic relationships, and correlations between DNA or protein characteristics and physical traits (Figs. 2 and 3). Such molecular tools can thus assist agroforestry practices by ensuring the genetic purity of labeled planting stock. The technique of random amplified polymorphic DNA (RAPD) fingerprinting has proved useful in the differentiation of poplar (*Populus* spp.), willow (*Salix* spp.), and Norway spruce (*Picea abies* L. Karst) clones [Heinze, 1994; Castiglione et al., 1993; Lin et al., 1994]. Other DNA fingerprinting techniques have been used to characterize dogwood (*Cornus florida*) genotypes at the University of Tennessee [Culpepper et al., 1991] and box elder (*Acer negundo* L.) genotypes at Washington University [Nybom and Rogstad, 1990]. Continued development of such techniques in genetic fingerprinting may help prevent future misidentification of specific genotypes used in agroforestry plantings.

Molecular probes, such as an antibody or specific DNA sequence, could also be specifically useful for identifying difficult-to-detect pathogens such as viruses, mycoplasma-like organisms, or other obligate parasites on agroforest trees. Such diagnostics also are useful for tracking geographic movement of pests, so that new pest strains can be better managed. Among current examples of molecular diagnostics are molecular markers developed for (a) western gall rust pathogens (North Dakota State) [Tuskan et al., 1991]; (b) fusiform rust pathogens (USDA Forest Service at Gulfport, MS) [Doudrick et al., 1993a,b]; (c) the causal agents of ash yellows, elm yellows, and chestnut blight (Cornell University) [Hibbin et al., 1991; Lee et al., 1993; Milgroom et al., 1992; Sinclair et al., 1992]; (d) bacteria in association with scorch diseases of elm and sycamore [Sherald and Lei, 1991] with subsequent development of diagnostic kits (Agdia, Inc., Elkart, IN); (e) pathogens that cause walnut witches' broom (University of Georgia) [Chen et al., 1992]; (f) butternut canker pathogens and poplar pathogens that cause Septoria canker and leaf spot (cooperative research at the USDA Forest Service in St. Paul, MN) [Furnier et al., 1994; Ward and Ostry, 1992, 1994]; and (g) Diplodia tip blight and canker pathogens of conifers (University of Wisconsin-Madison) [Smith and Stanosz, 1995]. USDA Forest Service research at Research Triangle Park, NC, is using molecular methods to characterize populations of forest insects such as Nantucket pine tip moth, pine coneworm species, sawfly species, and the balsam wooley adelgid [Richmond et al., 1992]. As molecular diagnostic tools become more common, we should gain improved biological understanding of how specific tree species/genotypes interact with pests in agroforestry systems.

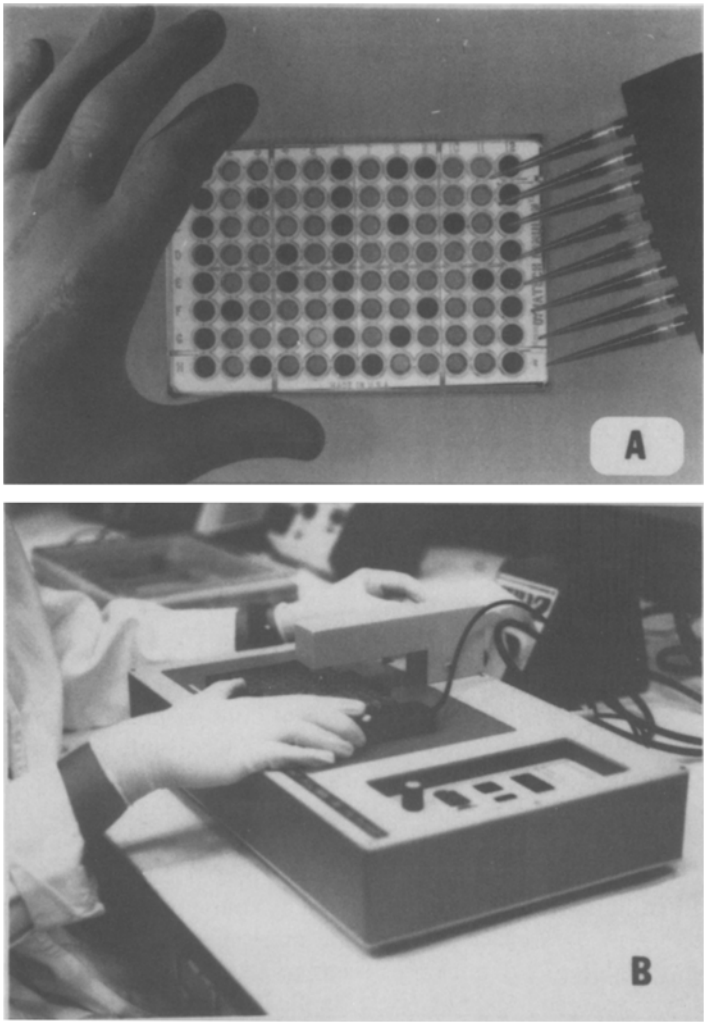


Fig. 3. Protein analysis. ELISA based systems can be used to identify tree species, insects, or pathogens. (A) Enzyme-linked immunosorbant assays (ELISAs) use antibodies to detect specific proteins in tissue extracts. (B) The colorimetric ELISA reaction can be quantified using a microplate reader.

The future

Applications of biotechnologies to complement classical tree improvement processes must be designed to assure adequate genetic diversity and counteract the negative effects of future selection pressures exerted by changing pest populations and physical environments. Traditional improvement programs will also continue to provide the basis for future biotechnological

developments by providing tree genotypes with a known genetic background along with breeding and growth (progeny trial) records.

Currently, agroforest biotechnological research is limited by several factors. First, relatively few researchers are active in the field, yet there are hundreds of tree species that are useful for agroforestry. In the past, rapid research gains have occurred only when numerous researchers across a wide range of disciplines focus on a single species. The multi-year generation time of agroforestry tree species and the current shortage of research positions in forest genetics also limit the available genetic information that is needed for further biological research. Finally, molecular biological research is limited by a lack of multi-generation, genetic pedigrees of agroforestry species. To help overcome these inherent difficulties, researchers have adopted model woody species on which to focus molecular biological research. Consequently, biotechnological research tends to focus on poplar (*Populus* spp.) and *Eucalyptus* spp. as a model species for deciduous woody plants and loblolly pine (*Pinus taeda* L.) and radiata pine (*Pinus radiata* D. Don) as a model species for coniferous woody plants. Information and technologies that are developed for model species can then be used to more efficiently develop similar information and technologies for other woody species.

Molecular technologies are currently addressing most areas of forest research, and agroforestry applications of biotechnology are becoming more evident as research products become available to the public. Biotechnological contributions to agroforestry ecosystems also hold promise to limit the need for pesticides and increase biodiversity by helping to provide pest-resistant and stress-resistant trees. Continued studies are necessary, however, to assure that sources of pest resistance are selected and deployed in a manner that maximizes stability, a feature that is paramount for long-lived tree species. In addition, molecular diagnostic monitoring of pest populations will increasingly facilitate integrated pest management systems. In the future, agroforest biotechnological research should provide more opportunities to incorporate innovative technologies from other areas of biological research. However, agroforestry researchers cannot wait complacently for other research disciplines to provide needed technologies and information. Nonforestry research will not address the unique structure, function, and ecological interactions of trees in agroforestry systems. Specialized research in all aspects of forest biology, including research at the molecular biology level, is required before the unique features and ecological interactions of woody plants are fully understood. Such information is essential to efficiently select and provide the right genotypes for the specific sites and purposes in agroforestry systems.

Acknowledgements

The authors wish to thank Jimmy Martinez for providing the photographs in this paper. Thanks are also extended to Drs Mary Ellen Dix and Young Woo

Chun for critical reviews of this manuscript. This paper was supported in part by the USDA Forest Service and funds from contract #DOE OR22072-17 with the Consortium for Biotechnology Research. Use of trade names in this paper does not constitute endorsement by the USDA Forest Service.

References

Ahuja MR (ed) (1988) Somatic Cell Genetics of Woody Plants. Kluwer Academic Publishers, Dordrecht, The Netherlands, 225 pp

Ahuja MR (ed) (1993) Micropropagation of Woody Plants. Kluwer Academic Publishers, Dordrecht, The Netherlands, 507 pp

Ahuja MR (1991) Application of biotechnology to preservation of forest tree germplasm. In: Ahuja MR (ed) Woody Plant Biotechnology, pp 307–313. Plenum Press, New York

Aitken-Christie J, Gough D, Maddocks D, Sigley M, Burger F and Carter PCS (1994) Towards commercialization of conifer embryogenesis. In: Michler CH, Becwar MR, Cullen D, Nance WL, Sederoff RR and Slavicek JM (eds) Applications of Biotechnology to Tree Culture, Protection, and Utilization, pp 181–190. USDA Forest Service, North Central Forest Experimental Station, Gen Tech Rep NC-175

Amerson HV, Campbell MM, Crane BS, Goldfarb B, Gratapaglia D, Liu B-L, McCord S, McKeand SE, O'Malley DM, Plomion C, Sederoff RR, Suries S, Whetten R and Wilcox PL (1994) Mapping of economic traits in forest trees. In: Proc 4th Inter Congr Plant Mol Biol, Section 10–2 Marker Assisted Breeding and Hybrid Seed Production, 19–24 June 1994, Amsterdam, The Netherlands (Abstract #1843)

Aronen R and Haggman H (1994) Transient B-glucuronidase expression in Scots pine tissues derived from mature trees. Can J For Res 24: 2006–2011

Attree SM and Fowke LC (1993) Embryogeny of gymnosperms: advances in synthetic seed technology of conifers. Plant Cell, Tissue and Organ Culture 35: 1–35

Bradshaw HD and Stettler RF (1995) Molecular genetics of growth and development in *Populus*. IV. Mapping QTLs with large effects on growth, form, and phenology traits in a forest tree. Genetics 139: 963–973

Bradshaw HD, Jr, Hollick JB, Parsons TJ, Clarke HRG and Gordon MP (1989) Systemically wound-responsive genes in poplar trees encode proteins similar to sweet potato sporamins and legume Kunitz trypsin inhibitors. Plant Mol Biol 14: 51–59

Castiglione S, Wang G, Damiani G, Bandi C, Bisoffi S and Sala F (1993) RAPD fingerprints for identification and for taxonomic studies of elite poplar (*Populus* spp.) clones. Theor Appl Genet 87: 54–59

Cervelli RL, Webster F, Edmonds T, Sutton B and Scott M (1994) Pilot production and scale-up delivery of spruce somatic seedlings. In: Michler CH, Becwar MR, Cullen D, Nance WL, Sederoff RR and Slavicek JM (eds) Applications of Biotechnology to Tree Culture, Protection, and Utilization, pp 191–197. USDA Forest Service, North Central Forest Experimental Station, Gen Tech Rep NC-175

Chen J, Chang CJ and Jarret RL (1992) DNA probes as molecular markers to monitor the seasonal occurrence of walnut witches' broom mycoplasmalike organism. Plant Dis 76: 1116–1119

Chun YW (1993) Clonal propagation of non-aspen poplar hybrids. In: Ahuja MR (ed) Micropropagation of Woody Plants, pp 209–222. Kluwer Academic Publishers, Dordrecht, The Netherlands

Chun YW and Hall RB (1992) Polyploidy induction in the Crandon clone of *Populus alba* × *P. grandidentata* by colchicine treatment. Korean J Breeding 24: 141–150

Cheng Z-M, Shi NQ, Schnurr J and Lu JZ-X (1993) Development of tissue culture and genetic transformation systems for elms, hackberry, birches and ponderosa pine. In: Mohn CA

(compiler) Proc 2nd North For Gen Assoc Conf, p 181, 29–30 July 1993. University of Minnesota, St. Paul, MN

Chung KH and Chun YW (1990) Variation in aluminum tolerance among 5 species of *in vitro* cultured *Populus*. J Korean For Soc 79: 26–32

Culpepper JH, Sayavedra-Soto LA, Bassam BJ and Gresshoff PM (1991) Characterization of *Cornus* (dogwood) genotypes using DNA fingerprinting. J Am Soc Hortic Sci 116: 1103–1107

Davis JM, Clarke HRG, Bradshaw HD, Jr and Gordon MG (1991) *Populus* chitinase genes: structure, organization, and similarity of translated sequences to herbaceous plant chitinases. Plant Mol Biol 17: 631–639

DeBlock M (1990) Factors influencing the tissue culture and the *Agrobacterium tumefaciens*-mediated transformation of hybrid aspen and poplar clones. Plant Physiol 93: 1110–1116

Donahue RA, Davis TD, Michler CH, Riemenschneider DE, Carter DR, Marquardt PE, Sankhla N, Sankhla D, Haissig BE and Isebrands JG (1994) Growth, photosynthesis, and herbicide tolerance of genetically modified hybrid poplar. Can J For Res 24: 2377–2383

Dorion N, Bigot C and Neumann P (1994) Evaluation of Dutch elm disease susceptibility and pathogenicity of *Ophiostoma ulmi* using micropropagated elm shoots. Eur J For Path 24: 112–122

Doudrick RL, Nance WL, Nelson CD, Snow GA and Hamelin RC (1993a) Detection of DNA polymorphisms in a single urediniospore-derived culture of *Cronartium quercuum* f. sp. *fusiforme*. Phytopathology 83: 388–392

Doudrick RL, Nelson CD and Nance WL (1993b) Genetic analysis of a single urediniospore culture of *Cronartium quercuum* f. sp. *fusiforme*, using random amplified polymorphic DNA markers. Mycologia 85: 902–911

Duchesne LC and Charest PJ (1992) Effect of promoter sequence on transient expression of the B-glucuronidase gene in embryogenic calli of *Larix* × *eurolepis* and *Picea mariana* following microprojection. Can J Bot 70: 175–180

Ellis DD, McCabe DE, McInnis S, Ramachandran R, Russell DR, Wallace KM, Marinell BJ, Roberts DR, Raffa KF and McCown BH (1993) Stable transformation of *Picea glauca* by particle acceleration. Bio/Technology 11: 84–89

Ellis DD, McCabe D, Russell D, Martinell B and McCown BH (1991) Expression of inducible angiosperm promoters in a gymnosperm, *Picea glauca* (white spruce). Plant Mol Biol 17: 19–27

Ellis DD, Rintamaki-Strait JA, Wraith SJ, McCown BH, Kleiner KW and Raffa KF (1994) Expression of introduced genes in trees: practical and environmental considerations. In: Proc TAPPI 1994 Biol Sci Symp, 3–6 October 1994, pp 139–146. TAPPI Press, Atlanta, GA

Fillatti JJ, Sellmer J, McCown B, Haissig B and Comai L (1987) *Agrobacterium* mediated transformation and regeneration of *Populus*. Mol Gen Genet 206: 192–199

Find JI, Floto F, Krogstrup P, Moller JD, Norgaard JV and Kristensen MMH (1993) Cryopreservation of an embryogenic suspension culture of *Picea sitchensis* and subsequent plant regeneration. Scand J For Res 8: 156–162.

Fliegmann J, Schroeder G, Schanz S, Britsch L and Schoeder J (1992) Molecular analysis of chalcone and dihydopinosylvin synthase from Scots pine (*Pinus sylvestris*), and differential regulation of these and related enzyme activities in stress plants. Plant Mol Biol 18: 489–503

Furnier GR, Stolz AM and Ostry ME (1994) Variacion genetica en elfitopatogeno *Sirococcus clavigignenti-juglandacearum*. Proc El VI Congresso Latinamericano de Botanica. 2–8 October 1994, Mar del Plata, Argentina (Abstract)

Grattapaglia D, Bertolucci FL, Penchel R and Sederoff R (1994) Molecular genetic mapping of economically important traits in *Eucalyptus grandis*. In: Proc TAPPI 1994 Biol Sci Symp, 3–6 October 1994, pp 133–138. TAPPI Press, Atlanta, GA

Gupta PK and Kreitinger M (1993) Synthetic seeds in forest trees. In: Ahuja MR (ed) Micropropagation of Woody Plants, pp 107–119. Kluwer Academic Publishers, Dordrecht, The Netherlands

Haissig BE, Nelson ND and Kidd GH (1987) Trends in the use of tissue culture in forest improvement. Bio/Technology 5: 52–59

Hauth S and Beiderbeck R (1992) *In vitro* culture of *Agrobacterium rhizogenes*-induced hairy roots of *Salix alba* L. Silvae Genet 41: 46–48

Heinze B (1994) Distinguishing individuals and clones of Norway spruce using RAPD fingerprinting. In: Michler CH, Becwar MR, Cullen D, Nance WL, Sederoff RR, and Slavicek JM (eds) Applications of Biotechnology to Tree Culture, Protection, and Utilization, p 40 (Abstract). USDA Forest Service, North Central Forest Experiment Station, Gen Tech Rep NC-175

Hibben CR, Sinclair WA, Davis RE and Alexander JH, III (1991) Relatedness of mycoplasmalike organisms associated with ash yellows and lilac witches' broom. Plant Dis 75: 1227–1230

Hollick JB and Gordon MP (1993) A poplar tree proteinase inhibitor-like gene promoter is responsive to wounding in transgenic tobacco. Plant Mol Biol 22: 561–572

James DJ, Passey AJ, Webster AD, Barbara DJ, Viss P, Dandekar AM and Uratsu SL (1993) Transgenic apples and strawberries: advances in transformation, introduction of genes for insect resistance and field studies of tissue cultured plants. Acta Hort 336: 179–184

Jouanin L, Brasileiro ACM, Leple JC, Pilate G and Cornu D (1993) Genetic transformation: a short review of methods and their applications, results and perspectives for forest trees. Ann Sci For 50: 325–336

Karpinski S, Wingsle G, Olsson O and Hallgren J-E (1992) Characterization of cDNAs encoding CuZn-superoxide dismutases in Scots pine. Plant Mol Biol 18: 545–555

Kim M-S, Schumann CM, Cregg BM and Klopfenstein NB (1994) Micropropagation and regeneration systems for green ash (*Fraxinus pennsylvanica* MARSH). In: Michler CH, Becwar MR, Cullen D, Nance WL, Sederoff RR and Slavicek JM (eds) Applications of Biotechnology to Tree Culture, Protection, and Utilization, p 74 (Abstract). USDA Forest Service, North Central Forest Experiment Station, Gen Tech Rep NC-175

King KFS (1989) The history of agroforestry. In: Nair PKR (ed) Agroforestry Systems in the Tropics, pp 3–11. Kluwer Academic Publishers, Dordrecht, The Netherlands

Kinlaw CS, Harry DE and Sederoff RR (1990) Isolation and characterization of alcohol dehydrogenase cDNAs from *Pinus radiata*. Can J For Res 20: 1343–1350

Klopfenstein NB, McNabb HS, Jr, Hart ER, Hall RB, Hanna RD, Heuchelin SA, Allen KK, Shi N-Q and Thornburg RW (1993) Transformation of *Populus* hybrids to study and improve pest resistance. Silvae Genet 42: 86–90

Klopfenstein NB, Shi NQ, Kernan A, McNabb HS, Jr, Hall RB, Hart ER and Thornburg RW (1991) Transgenic *Populus* hybrid expresses a wound-inducible potato proteinase inhibitor II – CAT gene fusion. Can J For Res 21: 1321–1328

Kruger BM and Manion PD (1993) Sensitivity of *Populus tremuloides* to toxic metabolites of *Hypoxylon mammatum* and susceptibility to *H. mammatum* infection. Can J Bot 71: 1298–1303

Lakshmi Sita G (1993) Micropropagation of *Eucalyptus*. In: Ahuja MR (ed) Micropropagation of Woody Plants, pp 263–280. Kluwer Academic Publishers, Dordrecht, The Netherlands

Lee I-M, Davis RE, Sinclair WA, DeWitt ND and Conti M (1993) Genetic relatedness of mycoplasmalike organisms detected in *Ulmus* spp. in the United States and Italy by means of DNA probes and polymerase chain reactions. Phytopathology 83: 829–833

Libby WJ (1988) Testing and deployment of brave new plantings. In: Ahuja MR (ed) Somatic Cell Genetics of Woody Plants, pp 201–209. Kluwer Academic Publishers, Dordrecht, The Netherlands

Libby WJ and Ahuja MR (1993) Micropropagation and clonal options in forestry. In: Ahuja MR (ed) Micropropagation of Woody Plants, pp 425–442. Kluwer Academic Publishers, Dordrecht, The Netherlands

Lin D, Hubbes M and Zsuffa L (1994) Differentiation of poplar and willow clones using RAPD fingerprints. Tree Physiol 14: 1097–1105

Louis K (1994) Propagation of *Populus tremuloides* × *P. tremula* via leaf micro-cross section technology. In: Proc TAPPI 1994 Biol Sci Symp, 3–6 October 1994, pp 41–45. TAPPI Press, Atlanta, GA

Manders G, Davey MR and Power JB (1992) New genes for old trees. Exp Bot 43: 1181–1190

Maynard CA (1986) Population genetics of forest trees: implications for the application of *in vitro* techniques. In Vitro Cell Dev Biol 22: 231–233

McGranahan GH, Leslie CA, Uratsu SL, Martin LA and Dandekar AM (1988) Agrobacterium-mediated transformation of walnut somatic embryos and regeneration of transgenic plants. Bio/Technology 6: 800–804

McNabb HS, Jr, Klopfenstein NB, Hanna RD, Hall RB, Hart ER, Heuchelin SA and Thornburg RW (1991) A field trial of transgenic hybrid poplar trees: establishment and growth through a second season. In: MacKenzie DR and Henry SC (eds) Biological Monitoring of Genetically Engineered Plants and Microbes, pp 155–159. Agric Res Inst, Bethesda, MD

McNabb HS, Jr, Serres RA, Cram MM and Klopfenstein NB (1986) biological constraints in the exchange of planting material for energy production. In: Joint Proc IEA For Energy Programme and FAO/Cooperative Network of Rural Energy Forest Energy Conference and Workshops on Research in forestry for Energy, pp 96–97.Rungstedgaard, Denmark, 28 October–1 November 1985, Swedish University of Agricultural Science, no. 49/1986

McCown BH, McCabe DE, Russell DR, Robison DJ, Barton KA and Raffa KF 1991) Stable transformation of *Populus* and incorporation of pest resistance by electric discharge particle acceleration. Plant Cell Rep 9: 590–594

Michler CH and Haissig BE (1988) Increased herbicide tolerance of *in vitro* selected hybrid poplar. In: Ahuja MR (ed) Somatic Cell Genetics of Woody Plants, pp 183–189. Kluwer Academic Publishers, Dordrecht, The Netherlands

Michler CH, Voelker TM and Bauer EO (1989) Production of putative herbicide tolerant plants from somatic embryos regenerated from *in vitro* selected callus. In Vitro 25: 62 (Abstract)

Milgroom MG, Lipari SE and Powell WA (1992) DNA fingerprinting and analysis of population structure in the chestnut blight fungus, *Cryphonectria parasitica*. Genetics 131: 297–306

Muhs H-J (1992) Macro and microvegetative propagation as a tool in tree breeding as demonstrated by case studies of aspen and cashew and regulations for marketing clonal material. In: Baker FWG (ed) Rapid Propagation of Fast-growing Woody Species, pp 71–85. CAB International, Wallingford, UK

Neale DB, Devey ME, Jermstad KD, Ahuja MR, Alosi MC and Marshall KA (1992) Use of DNA markers in forest tree improvement research. New For 6: 391–407

Nelson CD, Nance WL and Doudrick RL (1993) A partial linkage map of slash pine (*Pinus elliottii* Engelm var. *elliottii*) based on random amplified polymorphic DNAs. Theor Appl Genet 87: 145–151

Newton RJ, Yibrah HS, Dong N, Clapham DH and von Arnold S (1992) Expression of an abscisic acid responsive promoter in *Picea abies* (L.) Karst. following bombardment from an electric discharge particle accelerator. Plant Cell Rep 11: 188–191

Nybom H and Rogstad SH (1990) DNA 'fingerprints' detect genetic variation in *Acer negundo* (Aceraceae). Plant Syst Evol 173: 49–56

Ostry ME, Hackett W, Michler C, Serres R and McCown B (1994) Influence of regeneration method and tissue source on the frequency of somatic variation in *Populus* to infection by *Septoria musiva*. Plant Sci 97: 209–215

Ostry ME, Skilling DD, Lee-Stadelmann OY and Hackett WP (1990) Recovery of somatic variation in resistance of *Populus* to *Septoria musiva*. In: Rodriguez R (ed) Plant Aging: Basic and Applied Approaches, pp 113–116. ASI Ser. A Life Sci, Vol 186. Plenum Press, New York

Raffa KF (1989) Genetic engineering of trees to enhance resistance to insects. BioScience 39: 524–534

Richmond JA, Page M, Drooz AT and Davis EJ (1992) Looking at forest insects by contemporary methods. In: Proc Soc Am For 1992 National Convention, 25–28 October 1992, pp 519–521. Richmond, VA

Schwekendiek A, Pfeffer G and Kindl H (1992) Pine stilbene synthase cDNA, a tool for probing environmental stress. FEBS Lett 301: 41–44

Sherald JL and Lei JD (1991) Evaluation of a rapid ELISA test kit for detection of *Xylella fastidiosa* in landscape trees. Plant Dis 75: 200–203

Shin D-I, Podila GK, Huang Y and Karnosky DF (1994) Transgenic larch expression genes for herbicide and insect resistance. Can J For Res 24: 2059–2067

Sinclair WA, Griffiths HM, Davis RE and Lee I-M (1992) Detection of ash yellows mycoplasmalike organisms in different tree organs and in chemically preserved specimens by a DNA probe vs. DAPI. Plant Dis 76: 154–158

Smith DR and Stanosz GR (1995) Confirmation of two distinct populations of *Sphaeropsis sapinea* in the north central United States using RAPDs. Phytopathology 83: 699–704

Son SH, Chun YW and Hall RB (1991) Cold storage of *in vitro* cultures of hybrid poplar shoots (*Populus alba* L. × *P. grandidentata* Michx.). Plant Cell, Tissue and Organ Culture 27: 161–168

Son SH, Moon HK and Hall RB (1993) Somaclonal variation in plants regenerated from callus culture of hybrid aspen (*Populus alba* L. × *P. grandidentata* Michx.). Plant Sci 90: 89–94

Stomp A-M, Han K, Wilbert SM, Gordon MP and Cunningham SD (1994) Genetic strategies for enhancing phytoremediation. Recombinant DNA Technology II, Ann N Y Acad Sci 721: 481–491

Strauss SH, Howe GT and Goldfarb B (1991) Prospects for genetic engineering of insect resistance in forest trees. For Ecol Manage 43: 181–209

Strauss SH, Rottmann WH, Brunner AM and Sheppard LA (1994) Genetic engineering of sterility in forest trees. In: Proc TAPPI 1994 Biol Sci Symp, 3–6 October 1994, pp 117–131. TAPPI Press, Atlanta, GA

Strauss SH, Rottmann WH, Brunner AM and Sheppard LA (1995) Genetic engineering of reproductive sterility in forest trees. Molecular Breeding 1: 5–26

Subramaniam R, Reinold S, Molitor EK and Douglas CJ (1993) Structure, inheritance, and expression of hybrid poplar (*Populus trichocarpa* × *Populus deltoides*) phenylalanine ammonia-lyase genes. Plant Physiol 102: 71–83

Tauer CG, Hallgren SW and Martin B (1992) Using marker-aided selection to improve tree growth response to abiotic stress. Can J For Res 22: 1018–1030

Tuskan G.A, Walla JA, Lundquist JE, Wang CG and McClean P (1991) The use of molecular markers in studying ponderosa pine-*Peridermium harknessii* interactions. In: Hiratsuka Y, Samoil JK, Blenis PV, Crane PE, Laishley BL (eds) Rusts of Pine. Proc IUFRO Rusts of Pine Working Party Conf, 18–22 September 1989, pp 178–187. Banff, Alberta, Canada. Information Report NOR-X-317, Forestry Canada, Northwest Region, Northern Forestry Centre, Edmonton, Alberta, Canada

Von Aderkas P and Dawkins MD (1993) Haploid embryogenesis in trees. In: Ahuja MR (ed) Micropropagation of Woody Plants, pp 57–65. Kluwer Academic Publishers, Dordrecht, The Netherlands

Wann SR, Wyckoff GW and Wyckoff JL (1988) A tissue culture solution to a forestry problem – the propagation of a tetraploid European aspen. Tree Planters' Notes Summer: 28–30

Ward KT and Ostry ME (1992) Genetic and pathogenic variability of *Septoria musiva*. In: Colombo SJ, Hogan G and Wearn V (eds) Proc Twelfth North Am For Biol Workshop, 17–20 August 1992, p 62. Sault Ste. Marie, Ontario, Canada

Ward KT and Ostry ME (1994) Morphological and molecular genetic variation among isolates of *Septoria musiva*. Proc American Phytopathological Society, 6–12 August 1994 (Abstract). Albuquerque, NM. Phytopathology 84: 1145

Wilcox PL, Sederoff RR, Chaparro J, Grattapaglia D, Wier BS, Zeng ZB and O'Malley DO (1992) A proposed method for mapping quantitative trait loci in conifer clones using half sib families. Proc Sixth Int Conifer Biotechnol Working Group, 23–28 April 1992 (Abstract). NC State University, Research Triangle Park, NC

Wilde HD, Meagher RB and SA Merkle (1992) Expression of foreign genes in transgenic yellow-poplar plants. Plant Physiol 98: 114–120

Williams CG and Neale DB (1992) Conifer wood quality and marker-aided selection: a case study. Can J For Res 22: 1009–1017

Winton LL (1970) Shoot and tree production from aspen tissue cultures. Am J Bot 57: 904–909

Wrubel RP, Krimsky S and Wetzler RE (1992) Field testing transgenic plants: An analysis of the US Department of Agriculture's environmental assessments. BioScience 42: 280–289

Zsuffa L (1992) Experiences in vegetative propagation of *Populus* and *Salix* and problems related to clonal strategies. In: Baker FWG (ed) Rapid Propagation of Fast-Growing Woody Species, pp 86–97. CAB International, Wallingford, UK