REVIEW

Research Gap Analysis for Application of Biotechnology to Sustaining US Forests

Ross W. Whetten and Robert Kellison

ABSTRACT

The expanding human population of the world is placing greater demand on forest resources, both natural forests and plantations. Both types of forests are being adversely affected in North America as well as in other parts of the world, due to the globalization of trade and to climate change and associated changes in pest and disease incidence. Biotechnology may help to accelerate the progress of breeding programs working to develop trees with increased pest and disease resistance; better productivity and form; improved wood properties for pulp, solid wood, and bioenergy products; increased tolerance to adverse sites; and greater carbon sequestration. Key gaps in current scientific understanding limit these developments, but social acceptance of transgenic trees is also a major limitation. The process of acceptance of genetically engineered trees for use in commercial forests will require the coordinated effort of all parties, including forest biotechnologists, forest ecologists, regulatory agencies, and landowners.

Keywords: invasive pests, disease resistance, wood quality, phytoremediation, abiotic stress

Forests in the United States are facing challenges on a variety of fronts. The risk of introduction of exotic plants, animals, and microbes is increasing because of the increase in global commerce, and regulatory agencies are unable to keep pace with the risk (Anonymous 2001). Damage from indigenous pests and diseases and from abiotic stress is also increasing in some areas because of climate change (Kurz et al. 2008, van Mantgem et al. 2009) and the reduction of prescribed fire and timber harvesting on federal lands (Bonnicksen 2006). Fragmentation and conversion to other uses are additional threats to US forestlands. From 1953 to 1997, the southeastern United States lost 16 million ac of pine timberlands (South and Buckner 2003), and from 1992 to 2001, the United States as a whole lost 11.6 million ac by conversion to other uses (Wickham et al. 2008).

The shrinking forestland base and increasing biotic and abiotic stresses on forest productivity are accompanied by intensifying demand for goods and services from forests because of the growing human population and the demand for forest products from the developing world. Fenning and Gershenzon (2002) argued that accelerated development of plantation forestry, aided by biotechnological tools to increase plantation productivity, is essential to meet the growing demand for forest products and reduce the degradation of natural forests. The area of plantation forests (defined as planted by human intervention rather than by natural regeneration) is increasing worldwide, while the area of naturally regenerated forest is decreasing (Food and Agriculture Organization [FAO] 2006). This trend implies that plantation forests may have to substitute, to some degree, in providing other goods and services formerly provided by natural forests, in addition to meeting the timber production goals that are often the primary motivation for plantation forestry. Although plantations are often dismissed as "biodiversity deserts," the reality is more complex—depending on the species of interest and the style of plantation management, forest plantations can contribute in meaningful ways to preservation of biodiversity (Carnus et al. 2006, Wehenkel et al. 2009). The objective of this analysis is to identify gaps where ad-

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ditional research is needed, to help meet the objective of satisfying world demand for forest products while protecting natural forests from further degradation.

Areas of Forest Biotechnology Research

Forest biotechnology has been defined in FAO documents as encompassing three major areas: the use of molecular genetic markers to gain information about genetic characteristics of populations and the genetic basis of traits, the use of advanced propagation technologies for cost-effective production of uniform high-quality planting stock, and genetic engineering of trees to introduce new characteristics of economic or ecological value (Yanchuk 2001). Technological advances over the past 10 years have greatly increased the amount of data that can be obtained from molecular marker studies, so that genetics, or the study of genes and how they affect characteristics of an organism, has expanded into genomics, or the study of entire genomes of organism—all the genes and the complex network of interactions among genes that give rise to the characteristics of an individual. Propagation technologies have also advanced, particularly with respect to loblolly pine (*Pinus taeda*) in the United States, and somatic embryogenesis is now an established component in the marketplace of pine planting stock. Genetic engineering of forest tree species in the United States is an area of active research; 473 applications for field tests of transgenic forest tree species (not including horticultural tree crops such as apple, pear, papaya, or walnut) are pending, and 80 permits for tests are currently active (Information Systems for Biotechnology 2009).

Quantitative Genetics and Genomics

Traditional tree breeding approaches are firmly based in quantitative genetics methods (White et al. 2007). A combination of genetically improved planting stock and improved silviculture techniques has increased forest productivity in the southeastern United States over the past 20 years, providing better value to landowners as well as providing an increasing proportion of the raw material for the forest products industry (McKeand et al. 2006a, 2006b). During the same time, advances in molecular biology and biochemistry have given rise to the new field of genomic science, broadly defined as investigation of all the genes in an organism in a systematic way rather than one or a few at a time. A key component of the transition from quantitative genetics to genomics has been the dramatic increase in DNA sequencing technology as a result of the Human Genome Project and subsequent investment by federal funding agencies (Service 2006). The ability to obtain the complete DNA sequence of all genes in an organism provides researchers with a powerful set of tools to understand how genes work together to control tree growth and development. A project to determine the DNA sequence of the black cottonwood genome has deposited an initial draft sequence in public databases (Tuskan et al. 2006), and DNA sequencing of the *Eucalyptus grandis* genome is underway, with deposit of a draft sequence in public databases expected within a year (Department of Energy 2009).

Statistical methods similar to those used in quantitative genetics are also required in genomic research, to help organize and structure the massive amounts of data obtained from the new high-throughput technologies. It is likely that genomics and quantitative genetics will become more closely allied in many tree breeding programs as new methods are developed and gain credence among tree breeders (Grattapaglia et al. 2009).

Advanced Propagation Technologies. The history of asexual propagation dates to 6,000 years before present, for food crops such as olive (*Olea europaea*; Burdon and Libby 2006). Because of the common use of grafting and rooting throughout history, few people would consider these types of asexual propagation as biotechnology. Somatic embryogenesis, however, is a more advanced form of asexual propagation that has long been considered a part of forest biotechnology (Haines 1994). Somatic embryogenesis consists of culturing somatic tissue (as opposed to germ cells such as pollen or ova) in the laboratory, under conditions that allow differentiation of embryos similar to those formed by fertilization and differentiation in plant reproductive tissues. In loblolly pine, tissue explants are typically made from immature embryos, during a narrow window of time after fertilization occurs. Successful initiation of an embryogenic culture allows production of multiple embryos, all genetically identical, which can then be germinated and grown into genetically identical plants (Nehra et al. 2005). A key advantage of somatic embryogenesis as a means of asexual propagation is that embryogenic cultures can be cryopreserved, or stored frozen in liquid nitrogen, and recovered after field testing of the corresponding plants has established the value of a particular culture (Park et al. 1998). This testing step is essential for loblolly pine somatic embryogenesis programs, because the embryogenic cultures are initiated from untested immature seeds, and so the value of any particular culture may vary according to the value of the parents of the cross and the random segregation of genes within the progeny of the cross.

Genetic Engineering

Genetic engineering refers to the addition of a gene or set of genes to the genome (nuclear or organellar) of an organism, using laboratory methods rather than traditional breeding approaches. A common method for gene transfer into plants is based on the biology of a soil-borne bacterium, *Agrobacterium tumefaciens*, the causative agent of crown gall disease in a variety of plants. Virulent isolates of this bacterium can transfer a segment of DNA, called T-DNA for "transferred DNA," to the nuclear genome of plants (Chilton et al. 1977). Sophisticated modifications have been made to the bacterial DNA to modify the bacterial disease process into an industrial-scale process for introducing new genes into many plant species, including species such as maize and conifers (Gelvin 2003).

Genetic engineering of resistance to insects, viral diseases, and herbicides, in addition to other characteristics, has been applied to a variety of agronomic crops including corn, soybeans, wheat, rice, cotton and canola, and the area planted to genetically engineered or "transgenic" crops has grown from about 4 million ac in 1996 to over 128 million ac in 2001 (James 2003) and was estimated to exceed 270 million ac by 2007 (Herring 2008). Similar gene transfer has also been successfully accomplished in several forest tree species for insect resistance, herbicide tolerance, cold tolerance, and soil remediation (Fladung and Ewald 2006). Resistance to application of genetic engineering technologies in agriculture and forestry has been strong, while applications of similar technologies in the pharmaceutical and biomedical sectors have been well received. Some critics of plant biotechnology have argued on grounds of ethical or moral reasoning that genetic engineering of plants is fundamentally wrong, but an objective council of ethicists convened to consider this argument concluded that any moral

wrongdoing in plant genetic engineering would be outweighed by the moral good of reducing human suffering and increasing standards of living worldwide (Anonymous 1999). Other observers argue that the disparity in public awareness of plant genetic engineering versus biomedical applications of the same technology was created by a deliberate campaign to frame the debate over "genetically modified organisms" in terms of ecological safety, driven by a hidden agenda of economic self-interest (Herring 2008).

As the human population and development pressures on forest resources continue to increase, however, the potential contributions to human welfare of agricultural and forest biotechnology, in general, and genetic engineering, in particular, may come to be more highly valued than they have been over the past 20 years. The cumulative experience of over 10 years of genetic engineering experiments with trees has been summarized by Strauss et al. (2004). This and other experimentally supported demonstrations of the specific steps to take in responsible applications of biotechnology in forestry have the potential to move the public awareness into an appreciation of the value of using all available scientific tools to meet societal needs for forest products and ecosystem services. Those tools increasingly include practical applications of genetic engineering as well as traditional methods of forest management and tree breeding.

Potential Uses of Biotechnology in Forestry

Most types of gain in forest productivity from biotechnology are similar to those obtained from tree improvement programs. The hope, still not fully realized, is that integration of biotechnology into tree improvement programs will increase the pace of improvement and, perhaps, also the range of characteristics that can be improved. Current tree improvement program goals typically focus on improving rates of growth; uniformity in size, form, and wood properties; adaptability to a range of sites and environments; and resistance to insects and diseases. Secondary benefits may include tolerance to changing climatic conditions, increased sequestration of atmospheric carbon dioxide, amelioration of contaminated soil, and increased suitability of woody biomass as feedstock for integrated biorefineries.

Enhancing Forest Productivity

Forest productivity is best measured in comparison with a standard. The standard for genetic improvement in the southeastern United States is the unimproved local provenance, used for plantation establishment of southern pines until commercial quantities of seed from seed orchards became available in the late 1960s. Current plantation establishment in the region uses open-pollinated families from second-generation orchards and is making a transition to using full-sibling families produced by mass-controlled pollinations (MCPs; McKeand et al. 2008). The MCPs result from crosses made between two parent trees that have been proven superior for traits of interest, such as growth rate, form, disease resistance, and wood properties. Over 94 million MCP loblolly pine seedlings were planted in the southeastern United States between 2000 and 2007 (McKeand et al. 2008), and that number is now estimated to exceed 150 million seedlings (S.E. McKeand, pers. comm., North Carolina State University, Nov. 16, 2009).

Such controlled crosses are often also used as the source of immature embryos for initiation of somatic embryogenic cultures. The difference is that the plantlets derived from somatic embryogenesis are genetically identical, produced from only one or a few cultures from a particular full-sibling family, whereas the MCP seedlings contain the full range of genetic variation within the full siblings produced from a controlled cross. The benefit gained from reducing the genetic diversity within the population of trees planted in plantations is found in the increasing uniformity of the resulting industrial raw material, along with the ability to capture increasing amounts of genetic improvement by using only the very best trees for planting stock. The relative improvements in volume production from the different stages in tree improvement are shown in Table 1 (Li et al. 1999, Kellison 2006).

The development of somatic embryogenesis for commercial use is being done almost exclusively by private firms such as CellFor and ArborGen. Those firms are marketing the plant material to a range of forest owners across the South, as well as in some foreign countries such as Brazil, Chile, Australia, and New Zealand (Pait 2004). Somatic embryogenesis–derived pine planting stock accounts for about 23 million plants over the past 6 years, versus a total of about five billion pine seedlings planted during that time (S.E. McKeand, pers. comm., North Carolina State University, Nov. 4, 2009). The small number is caused by (1) high cost of the plantlets, which are about eight times that of open-pollinated seedlings from second-generation seed orchards and about four times that of seedlings from MCPs (Dougherty and Wright 2009); and (2) lack of harvest-age data to support the performance expectations.

Table 1. Genetic improvement of loblolly pine in the southern United States compared with the commercial check.

Genetic origin	Volume gain (over commercial check) (%)
First generation	7–8
1.5 Generation	10–12
Second generation	20–25
Mass-controlled pollinations	35–40
Third generation[a]	35–40
Clonal lines[b]	60–70

[a] Seeds from third-generation orchards are just coming into commercial production.
[b] The clonal lines in Table 1 are from rooted cuttings. Those being regenerated from somatic embryos will have a projected additional gain, amounting to more than 100% improvement over the unimproved check (Dougherty and Wright 2009).
Source: From Li et al. 1999 and Kellison 2006.

Most growth-and-yield models for loblolly pine do not explicitly account for increased productivity due to genetic improvement, so modeling efforts are often based on the assumption that genetic improvement of planting stock is equivalent to an increase in the site index (SI). [1] One such modeling study (Dougherty and Wright 2009) estimated relative gains of 58 and 135% for mass control–pollinated family plantings or somatic embryo–derived clonal plantings, respectively, over open-pollinated seedlings. The authors emphasize that actual gains will depend on the geographic area where the tests are conducted and on the intensity of land management and that the landowner should make independent decisions on the use of the planting stock and the management intensity based on personal circumstances.

Averting Catastrophic Loss of Forest Ecosystems—Insects and Diseases

Numerous examples exist where local and regional ecosystems are suffering degradation and mortality from a combination of insects and diseases.

Insects

Among the insects either currently causing, or with potential to cause, tree mortality on a large scale in North America are mountain pine beetle (*Dendroctonus ponderosae*), Asian longhorned beetle (*Anoplophora glabripennis*), emerald ash borer (*Agrilus planipennis*), sirex wasp (*Sirex noctilio*), gypsy moth (*Lymantria dispar*), hemlock wooly adelgid (*Adelges tsugae*) (Koch et al. 2006), and ambrosia beetle (*Xyleborus glabratus*). With the exception of the mountain pine beetle, all these insects are exotic rather than native to North America, and researchers are actively searching for ways to manage these invasive species (Brockerhoff et al. 2006). A number of manufactured chemicals are effective in controlling some of the insects (e.g., Doccola et al. 2007), but application of insecticidal chemicals over large forest areas is restricted by potential negative effects on flora, fauna, water and air quality, and food supplies, as well as by cost considerations.

One alternative to chemical use is a naturally occurring, soil dwelling bacterium, *Bacillus thuringiensis*, which produces protein toxins that kill some insects. Different isolates of this bacterium produce toxins specific to different orders of insects, including the Lepidoptera (butterflies and moths), Coleoptera (beetles), and Diptera (flies) (Feitelson et al. 1992). Lysed bacterial cultures, containing bacterial spores and free crystalline toxin, are approved for use in the United States as insecticidal sprays. The bacterial genes that encode several of these specific protein toxins have been isolated from the bacterial DNA and transferred to the DNA of several crop and tree species, conferring resistance to the family of insect pests against which the toxin is active (James 2003, van Frankenhuyzen and Beardmore 2004). Two lines of transgenic poplar, a *Populus nigra* clone and a triploid hybrid, have been released for widespread planting in northern China, where insect attacks limit the growth of noninsect-resistant poplar genotypes (Hu et al. 2001).

The confirmed presence of another exotic pest, the sirex wood wasp (*S. noctilio*), in New York state in 2006 and its more recent spread to surrounding states has raised concerns of the potential damage to native pine stands, in contrast to other areas of the world where infestations have been confined to plantations of exotic pine species. Biological control in plantations is largely achieved by introducing nematodes (*Beddingia siricidola*) and parasitic wasps (*Ibalis leucospodes, Megarhyssa nortoni, Ryssa* spp.) in combination with good silvicultural practices (Carnegie et al. 2005). Because of the random location of natural stands and their interspersion with trees of different species over wide geographic areas, control of the wasp offers a huge challenge. A strain of *B. thuringiensis* that makes toxins active against Hymenopteran pests (the taxonomic order that includes wasps, bees, and sawflies) has been described, and genetic engineering of pines for resistance to *S. noctilio* is, in principle, possible (Garcia-Robles et al. 2001). In the event of a catastrophic outbreak of *S. noctilio*, reforestation with resistant trees might be a means of restoring pine forests.

Diseases

Among the major fatal diseases affecting America's forests are fusiform rust disease of southern yellow pines (caused by *Cronartium quercuum* [Berk] Miyabe ex Shirai f.sp. *fusiforme*; Nelson et al. 2009), sudden oak death (*Phytophthora ramorum*; Grunwald et al. 2008), oak wilt (*Ceratocystis fagacearum*; Juzwik et al. 2008), pitch canker (*Fusarium circinatum*; Wingfield et al. 2008), and newly identified redbay wilt (*Raffaelea* spp.; Fraedrich et al. 2008). The presence of pitch canker of North American origin in Chile, South Africa, and perhaps elsewhere on Monterey pine (*Pinus radiata*) is evidence that diseases and insects flow both to and from the United States.

Genetic engineering of resistance to disease caused by fungal or bacterial pathogens has been much less successful, to date, than engineering resistance to viral diseases, herbicides, or insects (Collinge et al. 2008), although molecular genetic markers have proven valuable in crop breeding programs for combining multiple disease-resistance alleles into a single crop variety (Langridge et al. 2001). Such marker-assisted breeding is within reach for resistance to fusiform rust disease in loblolly pine (Nelson et al. 2009), but, to date, little is known of potential genetic resistance to the other pathogens described previously. An alternative approach to host resistance is to use biological control of the pathogen, an approach that is under investigation using a hypovirus that is lethal to the chestnut blight pathogen, *Cryphonectria parasitica*. Other species of chestnut (*Castanea* sp.), specifically Chinese (*Castanea mollissima),* Japanese (*Castanea crenata*), and European (*Castanea sativa*) are also susceptible to chestnut blight, but not as susceptible as American chestnut (*Castanea dentata*). One or more of the hypovirus strains have been effective in controlling *C. parasitica* on European chestnut in Europe. The hypovirus has been introduced into the United States on American chestnut trees infected with the disease. It is effective in controlling the disease, but remains localized on inoculated trees, as distinct from the European situation where the virus spreads naturally among diseased trees. Research is in progress to modify the hypovirus for colonization and self-perpetuation on the pathogen of American chestnut but, to date, no breakthroughs have been achieved (Peever et al. 2000).

Genetically Engineered Trees for Adverse Sites

Forest genetics, including biotechnology, can provide a better understanding of the mechanisms underlying adaptability of forest tree species to different environments. The components of such a program will include selection for species tolerant to the target conditions, hybridization or breeding of selected genotypes, and genetic engineering of forest trees to tolerate specific conditions. Examples of preliminary success in genetically engineering trees to tolerate adverse environmental conditions include poplars (*Populus* spp.) engineered with an enzyme from mammals for detoxification of trichloroethylene (TCE; Doty et al. 2000, 2007), and *Eucalyptus* hybrids genetically engineered for cold tolerance (Information Systems for Biotechnology 2009).

Phytoremediation involves the detoxification of contaminated soils. Plants, such as those from the *Populus* genus bioengineered with a mammalian gene, have been effective in degrading the toxic chemical TCE, which is found worldwide as a pollutant because of its wide use as a dry cleaning solvent. Transgenic plants can metabolize a range of compounds including TCE, ethylene dibromide, carbon tetrachloride, benezene, styrene, chloroform, and various metal ions to remediate contaminated soils (Dowling and Doty 2009). Phytoremediation trials with transgenic poplars are in progress in various places in the United States in the attempt to clean up toxic waste sites.

Attempts to introduce species of *Eucalyptus* into the southern United States for commercial purposes have been in progress for more than 50 years (Hunt and Zobel

1978). Relative to indigenous hardwood species, the eucalypts have highly desired wood properties for paper manufacture (high fiber count) and rapid tree growth. Until recently, the effort has been a failure largely because the species of choice are intolerant of freezing temperatures. ArborGen has introduced a gene conferring cold tolerance into *E. grandis* and a hybrid eucalyptus (*E. grandis* × *Eucalyptus urophylla*) clone, and field trials have been underway for several years (Information Systems for Biotechnology 2009). An application has been filed to allow the transgenic hybrid clone to flower, which would allow growing the field trials to rotation age of 7–9 years (Animal and Plant Health Inspection Service 2009a, 2009b).

Fiber Quality and Products

Genetically Engineered Trees for Pulp and Paper

A significant amount of effort has been invested in testing the idea that wood properties in trees can be altered by genetic engineering, in ways that will allow for more cost-effective, efficient, or environmentally friendly production of pulp and paper products. Space constraints prevent a comprehensive review of this extensive literature, which has been summarized relatively recently in a book (Fladung and Ewald 2006). The published results can be summarized briefly by noting that many authors have reported modification of wood properties in both hardwood and softwood tree species by genetic engineering, including changes in one or more aspects of lignin content, composition, or subunit bonding patterns, or in cellulose or hemicellulose content or composition. A relatively small proportion of the published reports, however, describe field experiments indicating that the transgenic trees show adequate growth under field conditions to be considered a viable substitute for available planting materials. Even fewer publications describe an economic model under which transgenic planting stock could be sold for a price high enough to compensate the effort required to develop and adequately test it but low enough that landowners could profitably grow the material, and pulp and paper producers could buy the resulting roundwood at a cost that makes the entire value proposition feasible. Future changes in the cost of industrial roundwood may make the value proposition more clearly beneficial, and alternative business models, such as a vertically integrated supply chain in which a single business entity grows the raw material and processes it into value-added products, would clearly affect the economic feasibility as well. Several currently active field trials of transgenic trees underway in the United States list "altered lignin biosynthesis" among the traits modified (Information Systems for Biotechnology 2009).

Using Trees for Bioenergy and Biomaterials

The Energy Independence and Security Act of 2007 contained language that requires the use of 36 billion gal of alternative fuels by 2022, up from 7 billion gal at the time of the introduced legislation. Of the 36 billion gal, 21 billion gal are to be derived from biomass (corn, agronomic crop residues, switch gras, and woody biomass). Such biofuels will probably be produced from biomass as one of several products from an integrated biorefinery, which would also produce heat, energy, and other value-added biomaterials in addition to transportation fuels (Ragauskas et al. 2006).

Great potential lies in the discovery and engineering of enzymes or inorganic catalysts that allow for conversion of wood components to feedstocks for efficient bioenergy use as well as for production of biomaterials. Advances in the understanding of microbial mechanisms for using cellulose for growth may provide insights into the most efficient approaches for capturing the maximum value from biomass for conversion to energy or to transportation fuels and other products (Zhang and Lynd 2005). Chemical as well as biological processes for production of transportation fuels from biomass are an active area of research, and continued improvements in the efficiency of the process are likely to emerge in the coming years (Huber et al. 2006).

Societal Acceptance of Genetically Engineered Trees

Regulatory agencies in virtually all countries prohibit the release of transgenic trees until a lengthy and costly evaluation is made to assure that the genetically modified trees pose no risk to man or the environment. Transgenic field crops were planted on over 222 million ac worldwide in 2007, and after 15 years of experience, the ecological effects of transgenic crop cultivation seem to be comparable with the effects of nontransgenic crop cultivation (Thies and Devare 2007). Two horticultural tree crops, papaya and plum, have been engineered for virus resistance and approved for commercial planting. Two transgenic insect-resistant poplar lines have been approved for commercial planting in China, the only documented approval of transgenic forest trees in the world (Ewald et al. 2006.

There is need for an example transgenic forest tree to work through the regulatory process, to pave the way for commercial use of other transgenic tree species. This process should include scientists, government forestry agencies, regulatory agencies, and advocates as well as skeptics and opposition leaders. One candidate tree for this exercise is American chestnut, which has great social value as well as environmental and economic importance. Research in progress has developed a tree of 15/16 American chestnut and 1/16 Chinese chestnut, which confers the growth, form, and nut quality of American chestnut and has high resistance to chestnut blight (Sisco 2008). Research is also in progress to identify the two or three genes in Chinese chestnut that convey resistance to the fungal attack. The combination of these approaches offers two options for commercial application: (1) the use of genetic markers that allow identification of American-Chinese hybrid offspring with the desired gene combination for resistance, and (2) genetic engineering of pure American chestnut to produce plants containing the genes for resistance.

The Institute of Forest Biotechnology, a nonprofit organization in Raleigh, North Carolina, has organized the Responsible Use Initiative with the objective of engaging collaborators throughout the world to help establish principles for the eventual release of transgenic forest trees. The collaborators recognize that the potential concerns are global rather than country specific and that a biological misstep in one area might negatively affect forestry worldwide. More information about the Responsible Use Initiative is available from the Institute of Forest Biotechnology (Institute of Forest Biotechnology 2009).

Critical Research Gaps

Despite the progress that has been made in the three major areas of forest biotechnology research, more remains to be done to assure that the potential of biotechnology to contribute to the economic and ecological stability of forests is realized. The following

sections address specific areas of research needs within each of the three major topics.

Quantitative Genetics and Genomics

Costs of DNA sequencing are decreasing, and it is likely that additional forest tree species will be subjected to genome sequencing in the next few years. The two forest tree species chosen for genome DNA sequence determination, to date, are both hardwood species, with relatively small genome sizes compared with conifers. The genome of *Populus trichocarpa* contains about 550 million base pairs of DNA (Bradshaw and Stettler 1993), and that of *E. grandis* contains about 640 million base pairs (Grattapaglia and Bradshaw 1994). The loblolly pine genome, by contrast, contains about 23 billion base pairs of DNA (Wakamiya et al. 1993). Until recently, the time and cost of sequencing a conifer were prohibitive, considering that hundreds of millions of dollars and 5 years of effort were required to sequence the human genome, which is about sevenfold smaller. These constraints have been largely overcome by the development of massively parallel DNA sequencing technologies (Service 2006). These technological developments have led to the recent formation of the Pine Genome Initiative, which advocates loblolly pine as the next forest tree for genome sequencing. Loblolly pine was chosen because of the extensive genetic resources available, including a cytological map (Islam-Faridi et al. 2007), and extensive collections of DNA sequences already in the public databases. Legislation to accomplish the task was included in the 2007 Farm Bill, passed into law in May 2008, and the National Institute of Food and Agriculture (NIFA) is currently inviting proposals for research projects to accelerate the completion of a reference genome sequence for loblolly pine (NIFA 2010a). It is critical to recognize that the genome sequence, although fundamental to understanding the functions of the genome, is only the beginning. Years of research will be required to uncover the networks of interactions among genes and between genes and environmental and developmental stimuli that affect growth and productivity traits of forest trees.

Areas in which gaps in current understanding exist and where a fusion of genomics and quantitative genetics may contribute new understanding include the genetic basis of inbreeding depression, genotype-by-environment interactions, and response to biotic and abiotic stresses. A deeper understanding of plant physiology, integrated with genomic information, could provide a key enabling technology for advances in all these fields (Nelson and Johnsen 2008). An understanding of the basis of inbreeding depression, and a strategy to avoid the problems that this can pose, will become essential as tree breeding programs in the United States move into advanced generations. Coancestry, or the degree of relatedness among trees in a breeding program, increases over multiple cycles of breeding with the same population, and it will be increasingly important for breeding programs to manage this coancestry so that it contributes to meeting the breeding program objectives, rather than causing inbreeding depression and decreased performance. Genotype-by-environment interaction, observed as a difference in relative ranks of performance among the same set of families or clones of trees when grown in different environments, has been reported to be a minor concern for loblolly pine (McKeand et al. 2006a). Those authors reported results from what seemed to be a random sample of clones. A recent report that compared two clones of contrasting ideotypes, however, found that the clones did show differential responses to variation in site preparation and nutrient availability (Tyree et al. 2009). A better understanding of the extent and degree of genetic differences in performance across different types of sites will become increasingly important as landowners begin to consider the balance of risks and benefits from planting full-sibling families or clonal varieties to maximize plantation productivity. Douglas-fir, another major commercial forest plantation species in the United States, has been reported to have relatively low levels of genotype-by-environment interaction within coastal regions of western Washington State (Dean 2007, 2008), but those experiments were conducted with relatively diverse families of trees. It remains to be seen whether clonal testing would identify more extreme interactions between tree genotypes and sites in Douglas-fir, as seems to be the case in loblolly pine.

Risk management to ameliorate the potential hazards from biotic stresses such as exotic pests and diseases or abiotic stresses due to climate change would benefit greatly from a better understanding of the signaling pathways and response mechanisms that allow forest trees to cope with these factors. Genomics and quantitative genetics can contribute an understanding of how much genetic variation exists in current populations, both in the wild and in the breeding programs, so that appropriate management strategies can be implemented to deal with the risks posed by changing temperature and precipitation patterns over the coming decades (Savolainen et al. 2007). Common garden tests of trees from across the natural range of a species have been used in the past to study genetic variation in adaptability; such tests in the future could include a much more detailed investigation of physiological and molecular genetic mechanisms to provide a mechanistic understanding of the basis of the observed differences (Matyas 1996, St. Clair and Howe 2007). Adaptation to biotic stresses caused by exotic pests or diseases can sometimes be accelerated by sampling the genetic diversity of a related species from the native range of the introduced pest or pathogen. The breeding program to introduce resistance to chestnut blight from Chinese chestnut to American chestnut is an example of such an effort; this program began as a traditional breeding effort but has since gained a genomics component (Wheeler and Sederoff 2009).

Closer collaborations between field researchers and laboratory scientists will be essential to capture all the benefits from genomics research and make them available in a form useful to forest managers and tree breeding programs. The USDA Coordinated Agricultural Project grant program has the objective of moving genomics research from the basic research phase into application and is currently supporting a project, along with the US Forest Service, to move genetic-marker technologies into application in loblolly pine and Douglas-fir breeding programs in the United States (NIFA 2007). A valuable contribution to meeting societal needs from forest resources could be made by a similar funding program aimed at synthesizing the basic research disciplines of tree physiology and forest ecology with the applied discipline of silviculture and integration of those fields of research with the quantitative genetics and genomics components now being integrated into breeding programs. Such a funding program was announced by NIFA in March 2010 as part of an initiative to support research into mitigation of and adaptation to climate change (NIFA 2010b). Recent changes in timberland ownership patterns, notably a shift from vertically integrated forest products companies to nonindustrial landowners and investment orga-

nizations, add additional motivation to existing calls to reconsider the funding model for cooperative research programs (Byram et al. 2005).

Propagation Technologies

The rate at which embryogenic cultures can be successfully initiated from immature seeds is a constraint on the cost-effectiveness of somatic embryogenesis in pines, and research has shown that there is a strong genetic component to the variation in culture initiation rates (Mackay et al. 2006). This suggests that a breeding program could improve the rate of somatic embryogenesis culture initiation, by selecting and crossing parents that have the desired characteristic of high rates of successful culture initiation.

One important question that should be addressed, before such a breeding program is initiated, is whether there is any correlation (positive or negative) between the traits already selected in breeding programs (such as productivity, form, or disease and pest resistance) and the trait of high somatic embryogenesis induction efficiency. The worst-case scenario would be a negative correlation between somatic embryogenesis and one or more other desired traits, so that efficient propagation would come at the cost of progress toward other breeding objectives.

Genetic Engineering

Worldwide concerns about adverse environmental effects from transgenic forest trees still have not been laid to rest, and additional research in the area of ecology and population genetics of transgenic trees is sorely needed (Farnum et al. 2007, McCord 2008). The risk entailed in release of a particular transgenic tree in a particular environment is a function of the specific engineered genes in the tree and a range of environmental factors related to the site where the transgenic tree is to be planted. It is therefore likely that ecological risk assessment will continue to take place on a case-by-case basis.

Conclusion

Forest biotechnology, in concert with tree breeding and silviculture programs, is crucial to meet industrial demands for forest products while allowing conservation of remaining natural forests. Genetic modification of trees can result in products and ecosystem services that are extensions of those from existing plantations, but could also result in completely new products as yet unanticipated. As the science progresses, however, caution must be exercised to avoid disturbances to natural ecosystems. Society must be involved with the application of these emerging technologies to assure that the needs of the world are met rather than the benefits falling to a selected few.

Endnote

[1] SI is commonly used in forestry to measure the productivity of a site. The measure is the total height of the dominant and codominant trees in the stand at a given age. For plantation-grown loblolly pine, the base age is commonly set at 25 years.

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