Technical overview of Forest Biotechnology Research in the U.S.

T. Kent Kirk
U.S.D.A. Forest Service
Forest Products Laboratory
One Gifford Pinchot Drive
Madison, Wisconsin 53705

SUMMARY

My purpose in this brief presentation is to provide a framework in which recent research in forest biotechnology can be viewed, as a foundation for subsequent papers in this conference. A schematic (Figure 1) depicts the gross biochemical activities of trees, and how trees interact with other biological systems and the environment; also included is the fate of harvested trees, because bioprocessing is playing an increasing role in tree utilization. Research can be divided into three. categories: (i) Tree culture; (ii) tree protection and (iii) tree utilization. Major goaIs of tree culture biotechnology research are the following: (a) developing and improving vegetative propagation procedures, including tissue culture procedures; (b) identifying and mapping genes of interest (c) genetically engineering trees; and (d) developing molecular markers to aid in progeny selection. Biotechnology research in tree protection is aimed at mitigating the damage caused by insects, diseases, and environmental stressors via two avenues: (a) direct protection through control of insects and diseases, and (b) indirect protection through development of resistant tree varieties. Whereas most of the potentials of biotechnology in tree culture and tree protection have yet to be realized, several biotechnology applications in wood utilization have been commercialized in the past five years. The total U.S. research effort in forest tree biotechnology is estimated to be on the order of $25-31 million per year.

INTRODUCTION

Trees are complex biological systems, and arc part of an even more complex system--the forest ecosystem. Trees grow and reproduce, and they also interact in many ways with other biological systems and with the environment. The biochemical activities of trees and the other forest organisms are quite numerous, and the genes that encode the responsible enzymes are even more numerous. In recent years, as scientists have begun to focus on how the new discoveries in biology (mainly the techniques of biotechnology) might be used in forestry, they have cosequently had a large assortment of targets from which to choose. My purpose in this brief presentation is to provide a framework in which recent research in forest biology can be viewed as a foundation for subsequent papers in this conference. I have constructed a schematic that incorporates the various applications and potential applications of biological research. Brief descriptions of each of those potentials address the objectives of current research, the level of support and whether the research is being done by industry, government, or universities. My focus is on research in the United States, although research is progressing in many other countries as well.

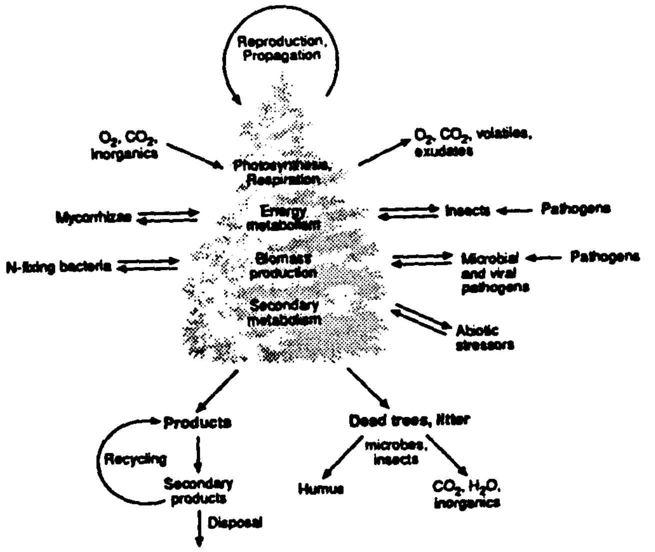

Fig. 1 Gross biochemical activities of trees

The schematic (Figure 1) depicts the gross biochemical activities of trees, their interaction with other biological systems and the environment, and the fate of harvested trees, for bioprocessing is playing an increasing role in tree utilization. Trees assimilate O_2, CO_2 and nutrients, and they produce O_2,CO_2, and various volatile and non-volatile products such as terpenes and root exudates. They photosynthesize and respire, and they biosynthesize in connection with growth, teproduction, energy metabolism, and the various other activities. They also biosynthesize secondary metabolites such as heartwood extractives. They respond to insects, microbial pathogens, mycorrhizal fungi and nitrogen-fixing bacteria, and environmental stessors. Trees and parts of trees die and the tissues are biodegraded by microbes and insects to humus, CO_2, and H_20, with release of inorganic nutrients. Trees are harvested and converted into many different primary and secondary products, which may be reused and recycled, and which ultimately are returned to the environment through biodegradation, incineration, landfill, etc. All of the biological processes contain targets for biotechnological alteration and improvement, and the utilization processes offer potential targets for biotechnology as well. We can consider the potential applications of biotechnology to forestry in three broad categories: Tree culture, tree protection, and tree utilization.

FOREST BIOTECHNOLOGY RESEARCH

Tree Culture

Forest trees are the single most valuable crop in the U.S.; the harvested value of the crop in 1992 was approximately $19 billion. The value per unit of land can be increased greatly by improvements in tree culture.

Major goals of tree cultute research are the following: (a) developing and improving vegetative propagation procedures, including tissue culture procedure (b) identifying and mapping genes of interest (c) genetically engineering trees and (d) developing molecular markers to aid in progeny selection.

Vegetative propagation. Learning how to vegetatively propagate commercial forest tree species is a major research and development goal. Vegetative propagation allows cloning of superior lines, and avoids the loss of desirable traits and the uncertainties associated with sexual reproduction. In addition, the ability to propagate transformed cells (cells to which DNA has been introduced via the techniques of biotechnology) and to regenerate plants from cultured cells is prerequisite to genetic engineering. Research in this area is focused on the near-term goal of improving rooting procedures for cuttings, particularly of conifers, and on the longer-term goal of micropropagation through organogenesis and somatic embryogenesis. Somatic embryogenesis is of particular interest in the long term because it has the potential for producing, inexpensively, large numbers of clones propagated as artificial seeds. Although good progress has been achieved with somatic embryogenesis in coniferous species and especially hardwoods, further work is needed to improve rate of germination. Research on the various aspects of vegetative propagation of trees is being conducted in industry, university, and Forest Service laboratories, and collaboration among these groups is common. The total U.S. effort is probably on the order of $7-9 million.

Gene identification. Considerable research is focused on identifying genes in trees that are responsible for traits of interest, including stress responses, growth characteristics, wood quality, flowering, etc. Examples of traits for which genes are being sought in commercial forest trees include lignin composition, disease and insect resistance, dormancy, cold hardness, and growth rate. Genes for some traits are much more easily identified than those for others, depending on knowledge of the underlying biochemistry. For example, because the biochemical pathway from glucose to lignin is known, the genes encoding key enzymes--and thus lignin composition--have been identified By cotrast, the biochemistry controlling wood density is largely unknown, making it difficult to identify the responsible genes. Indirect and innovative procedures are being developed to locate and identify genes of interest. The total U.S. research effort in this difficult area is probably $3-4 million, and is being done primarily in Forest Service and university laboratories.

Genetic engineering. A limited amount of research is already aimed at genetically engineering trees. Transformation of a number of tree species has been reported; in the U. S., *Populus* spp. serve as the model for much of the research because techniques for genetically engineering and micropropagating are relatively advanced. Even so, research in this area is modest, because technical problems have to be solved, as has been mentioned. However, the transformation system itself is not viewed as the major bottleneck to genetic engineering of forest trees at this time, even though it is still inefficient, especially for conifers. Promising results have recently been reported with the use of DNA-coated microprojectiles, and successes have beenreported with other procedures as well. Limitations to the broad use of genetic engineering to improve forest trees include primarily the facts that only a few genes for traits of commercial interest have been identified, that culturing cells of many tree species is difficult, and that regenerating whole plants from cultured cells of commercial tree species has met with only limited success.

Those biochemical activities that are specific to woody plants, or even to single species or varieties of trees, such as wood fiber formation and resistance (or susceptibility) to specific insects or diseases, often must be studied in the tree species of interest. It is important to note, however, that much of the research on non-forest plants is relevant to commercial forest trees. For example, research underway on cellulose biosynthesis in cotton plants is uncovering enzymes and genes for which similar counterparts can be anticipated in trees. The same is true for photosynthesis research on spinach. Major research efforts are underway in many laboratories on the small plant *Arabidopsis thaliana,* because it completes its life cycle in just 6 weeks. Funds for much basic biochemical research is more effectively spent on *A. thaliana* than on forest trees.

Molecular markers. The application of biotechnology that is likely to pay off in the nearest term in tree culture is the development of molecular markers as aids in the selection of desired progeny. A serious problem today is identifying desired progeny before they get Too old to propagate vegetatively--by rooting cuttings, for example. Molecular markers allow rapid identification of the gene or genes of interest in minute samples. Research aimed at developing molecular markers for screening is accelerating, and is underway primarily in industrial and Forest Service laboratories. Molecular marker research for commercial tree species is being funded in the U.S. at a level of perhaps $1-2 million yearly.

Tree Protection

Biotechnology offers a powerful approach to mitigating the damage caused by tinsects and diseases, as well as by environmental stressors. Insects and diseases probably cause in excess of $1 billion damage annually to U.S. forests; environmental stressors exact an additional toll, probably quite substantial. Forest trees are affeted by a great many different insect pests and diseases, the latter caused by fungi, bacteria and viruses. Among insect pests, the gypsy moth and southern pine bark beetle probably are the most damaging, costing in excess of $100 million annually. Among the many diseases of forest trees, fusiform rust and white pine blister rust probably are the most destructive, being responsible for losses in saw timber alone of more than $50 million yearly.

Research is focused mainly on the gypsy moth, Southern pine bark beetle, fusiform and white pine blister rusts, and various environmental stressors such as drought. The research falls into two catcgoties: (a) direct protection through control of insects and diseases, and (b) indirect protection through development of resistant tree varieties.

Direct protection. Researchers in universities, government, and industry are working toward developing strains of the gypsy moth virus, LdMNPV, with enhanced killing speed, potency, and production attributes. In addition, research is aimed at scaling up the production of the virus for application. Forest Service research on the gypsy moth virus has recently resulted in improved virus strains for production and with increased killing speed; approximately $1.3 million is being spent annually on these efforts against the gypsy moth. In addition, probably $10 million is being invested in the U.S. in research and development on other insect viruses for use in agriculture, some of which might be useful in forestry as well.

The biotechnology product “Bt toxin” is already being used commercially in agriculture and forestry. This toxin is produced by the bacterium *Bacillus thuringiensis,* and is effective against lepidopterous insects, including gypsy moth. New and improved Bt toxins are being developed in university, Forest Service and, mainly, industry laboratories. The research effort is perhaps on the order of $2 million per year.

Whereas the gypsy moth is exposed while it defoliates trees, and is thus accessible to sprays, etc., bark beetles reside under the bark, and are difficult to control with applications of chemicals or biological control agents. One biological approach is to use pheromones to attract the insects to a central point for control. Techniques of molecular biology apparently have not yet been applied to these pests.

Direct biocontrol of the rusts and other fungal, bacterial, and viral diseases of tree is labor-intensive and costly at best. A small Forest Service research effort is directed at identifying antifungal agents produced by bacteriaI endophytes.

Indirect protection. Trees have a wide varitey of natural defenses against insects and diseases, which is why most microbes, insects, and viruses do not cause tree diseases. Considerable research is underway to identify genes that confer resistance to fusiform rust and white pine blister rust in resistant varieties and species of conifers. When these genes have been identified, they can theoretically be inserted into susceptible trees to provide resistance. This is fairly long-range research. In the near term, the identification of markers for these resistance genes will allow marker-assisted tree breeding and asexual progeny selection to hasten the development of resistant lines of trees. Stress tolerance genes are also being sought in Forest Service laboratories for eventual development of improved trees; those programs are probably under $1 million per year.

Another biotechnological approach to indirectly controlling insects and diseases in trees is to insert foreign genes that confer resistance. Thus, several tree species have been transformed with insect-controlling genes such as the Bt toxin gene. The transformed trees have not been released for commercialization, however, due to debate over the likelihood of selecting for resistant insects.

Forest Service activities probably account for the greatest share of total forest protection biotechnology research, being funded at the level of approximately $5 million per year. Universities and industry probably spend on the order of $2-3 million per year.

Tree utilization

Whereas most of the potentials of biotechnology in tree culture and tree protection have yet to be realized, several biotechnology applications in wood processing have been commercialized in the past five years. Xylanases for prebleaching kraft pulp (“bleach boosting”) was discovered in Finland, and developed there and elsewhere. By hydrolyzing the xylans in pulp, the enzymes apparently release entrapped lignin, reducing the requirement for bleaching chemicals. Xylanases are used commercially by mills in Scandinavia and Canada, and are under extensive testing in U.S. mills. Research in our laboratory and in a number of industrial laboratories is aimed at developing improved xylanases. A mixture of cellulases and hemicelulases for improving the drainage of pulp slurries for papermaking is commercial in France, where this use of the enzymes was developed. The enzymes convert fines and soluble hemicellulases to soluble sugars, reducing the solution viscosity. Lipase for pitch control in mechanical pulp mills was developed in Japan, where it is commercial. The enzyme hydrolyzes triglycerides to fatty acids and glycerol; triglycerides are the most problematic component of wood-derived pitch. (Pitch refers to the mixture of hydrophobic materials that cause a number of problems in pulp and paper manufacture, including downtime for cleaning, breakage of paper on the paper machine, and holes in the paper.) Another pitch control product has been developed in the U. S., where it is now commercial. It is a fungus inoculum that is introduced into wood chips as they are piled for storage at pulp mills. The fungus covers the chips and penetrates the chip interiors, deriving its nourishment from the pitch components.

Other biotechnology processes for the forest-based industries are being researched. Included is the use of enzymes in recycling fibers, primarily for deinking. Another is biopulping, the use of lignin-degrading fungi to “soften” wood chips prior to pulping. The fungus treatment reduces energy consumption in mechanical pulping, improves paper strength properties, and as a natural process, is compatible with the environment. Lignin-degrading fungi have also been found to decompose an impressive number of. manmade chemicals, including creosote components, pentachlorophenol, DDT, TNT, and some chlorinated dioxins use of the fungi in bioremediation is now being researched in a number of laboratories. Finally, considerable research has been conducted on the use of wood as a feedstock for the production of ethanol and other chemicals by fermentation. In the U. S., ethanol is commercially produced almost entirely by fermentation of cornstarch-derived glucose. Enzymes can be used to hydrolyze isolated wood cellulose and hemicelluloses--or these components in wood if the wood has been pretreated to make them accessible. Sugars derived from wood include xylose and other 5-carbon sugars in addition to glucose and other 6-carbon sugars; because fermentation of 5-carbon sugars cannot yet be done economically, research is focused on improving the rates and yields in their fermentation. The research effort on converting wood to ethanol has decreased dramatically in the last few years; current programs are in U.S. Department of Energy and U.S. Department of Agriculture laboratories.

Overall, funding for the research effort on biotechnology applications in tree utilization is probably on the order of $4-5 million per year. The research is being conducted primarily by industry, by our laboratory, and to a lesser extent by universities,

CONCLUSIONS

U.S. research investment in forest biotechnology is on the order of $25-31 million per year, and is in three areas: tree culture ($11-15 million), tree protection ($10-11 million), and tree utilization ($4-5 million). The most likely near-term payoff in tree culture research is is the development of molecular markers as aids in the selection of desired progeny. In the longer term, it is in artificial seeds produced by somatic embryogenesis, and in genetically engineered trees. In the tree protection area, the most likely near-term payoff is in development of viral pathogens of insects. Over a longer term, it is in trees genetically engineered for resistance to insects, pathogens, and environmental stressors. Enzyme and microbial technology-based products are already commercial in the pulp and paper industry. Near-term research will improve those products and produce new ones. Longer-term, research could result in commercialization of biopulping and in new bioremediation strategies. The amount of research to be done to realize the potentials of biotechnology in the forest industries is great compared to the investment. Coordination among the university, government. and industy scientists is essential. The presentations to follow at this conference will no doubt shed new light on advances being made in these areas.

ACKNOWLEDGEMENTS

I thank the following for providing valuable input to this presentation: Brent McGown (University of Wisconsin); Ronald Sederoff (North Carolina State University} James Slavicek, James Stewart, and Richard Smith (U.S. Forest Servie); Nick Wheeler (Weyerhaeuser); and Dave Canavera (Westvaco).

1994
Biological Sciences Symposium

Airport Marriott
Minneapolis, Minnesota
October 3-6

Proceedings

TAPPI
Technology Park/Atlanta
P. O. Box 105113
Atlanta, GA 30348-5113, USA

TAPPI PRESS
1994

on recycled paper