

# **HERITAGE TREES<sup>®</sup> PROGRAM VISION AND IMPLEMENTATION PLAN**

**Prepared by Heritage Trees<sup>®</sup> Committee  
Institute of Forest Biotechnology**

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## **INTRODUCTION**

Heritage Trees<sup>®</sup> are species and individual trees that have special significance for cultural, ecological or historic reasons. They are found in landscapes around the world. Many are threatened by stress factors such as deforestation and development, exotic insect and disease organisms, and climate change.

The Heritage Trees<sup>®</sup> Program (HTP) is one of four cornerstones of the Institute of Forest Biotechnology (Appendix 1). The purpose of the HTP is to promote and integrate the application of emerging biotechnology to rescue threatened species or ameliorate threats to individual trees of special significance in natural or controlled environments. The program is directed to strengthening traditional approaches with new technology or encouraging new approaches for the protection and restoration of Heritage Trees<sup>®</sup>.

## **PRIORITIES**

American chestnut, American elm, English elm, Fraser fir and other Heritage Trees<sup>®</sup> have been devastated by exotic (non-native) disease organisms and insect pests (Appendix 2). Forest biotechnology has great potential to assist ongoing efforts to conserve and restore these species by developing new approaches to research and management of tree diseases and insect pests.

There are several key obstacles to accelerating forest biotechnology research on tree diseases and insect pests. Biotech research is expensive and requires specialized tools and expertise. Technical success is uncertain and there are many unanswered questions about the potential market for Heritage Trees<sup>®</sup> that are resistant to insects or diseases. There are also issues and uncertainties related to ecological effects, public acceptance, and regulation of some biotech approaches to managing insects and diseases (e.g., inserting resistance genes in trees).

Nevertheless, research and applications in agriculture clearly demonstrate the great potential for biotechnology to yield new and effective approaches to managing insect pests and diseases (Appendix 3). Protecting and restoring Heritage Trees<sup>®</sup> devastated by exotic insects and diseases is a great scientific and social challenge that offers a proving ground for promising new research methods and technologies. Success could result in a wide range of ecological, economic, and cultural benefits associated with conservation and restoration of Heritage Trees<sup>®</sup> and development of more effective management strategies for tree diseases and insect pests.

The first priority of the HTP is to develop and implement detailed plans for bringing the tools of biotechnology to the task of developing resistant varieties of American chestnut, English and American elms, and Fraser fir. The HTP is working in collaboration with diverse interested parties with relevant expertise in scientific, legal, regulatory, social, and ecological issues. The HTP recognizes and appreciates the important ongoing work of its partners and envisions productive cooperative relationships that fully respect the independence, interests, and intellectual property of all parties.

## INITIAL PROGRESS

The Institute of Forest Biotechnology has taken several important steps toward establishing the HTP and will augment individual efforts in a way that is thoughtful, practical, useful, and non-competitive to all parties. Steps already taken include:

- *A Heritage Trees® Workshop: Developing Partnerships, Strategies and a Practical Program.* This workshop focused on American chestnut and was held November 1-2, 2001. Participants included representatives from The American Chestnut Foundation and the American Chestnut Cooperators Foundation, USDA Forest Service, university and industry scientists, and environmental public interest groups. Scientific progress, potential new technologies and innovative approaches were discussed. A workshop report and abstracts of presentations are available at the IFB website ([www.forestbiotech.org](http://www.forestbiotech.org)).
- *Fraser Fir Heritage Trees® Workshop.* This meeting was held December 11, 2002. Attendees from universities, industry and USDA Forest Service gathered to address the environmental and economic impact of insects and diseases affecting Fraser fir; the status of research programs; and opportunities for collaborative intervention. A meeting report is available at the IFB website. The meeting led to IFB's ongoing collaboration in efforts to form a Center for Adelgid Research at North Carolina State University and organize a broad-based initiative to save Fraser fir.
- *Forest Biotechnology in Europe: Impending Barriers, Policy and Implications.* This meeting was held September 12-13, 2002 and was directed broadly at forest biotechnology issues in Europe. It was significant for the HTP because it strengthened IFB's international outlook and confirmed IFB's commitment to a mode of operation that is collaborative, constructive, pragmatic and professional. A meeting report and other materials are available at the IFB website.

IFB has also been collaborating with ArborGen and The American Chestnut Foundation (TACF) in developing an approach to supporting and strengthening ongoing research on fungal resistance mechanisms in American chestnut. A good understanding of key opportunities and challenges has been developed among the cooperators. There are excellent opportunities to accelerate ongoing biotech research at the University of Georgia, the State University of New York's College of Environmental Science and Forestry, and other institutions. It is clear that coordination with ongoing selection and breeding programs is critical to success. The main near-term challenges are to develop and implement effective strategies for (a) developing new sources of research funding support, and (b) accessing and managing intellectual property.

## PROGRAM VISION

The Heritage Trees® Program will rescue threatened species and individual trees of special significance by:

- ***Communicating*** the need for accelerated and well-coordinated efforts to sustain and restore Heritage Trees®, and the potential value of forest biotechnologies in combination with traditional approaches.
- ***Promoting development of compelling science plans*** for biotechnology for conserving and restoring Heritage Trees® in collaboration with leading research institutions and funding agencies around the world.
- ***Promoting collaboration and information exchange*** on Heritage Trees® worldwide by organizing conferences and by maintaining a web-based directory of relevant research programs and publications.
- ***Facilitating access to intellectual property*** necessary for progress in biotechnology research on insect and disease resistance in Heritage Trees®.

- *Addressing the social, ecological, regulatory and economic issues* related to Heritage Trees® conservation and restoration generally and biotechnology approaches in particular.
- *Developing new sources of federally and privately sponsored competitive funding* for biotechnology research on resistance to insect pests and disease organisms by tree species of special significance for ecological or cultural reasons.

### **GOVERNANCE AND SCOPE OF THE HERITAGE TREES® PROGRAM**

The Institute of Forest Biotechnology (IFB) has established a Heritage Trees® Committee (HTC) to direct the Heritage Trees® Program. Current members are listed in Appendix 4.

The HTC will have two categories of members. A majority will be members of the IFB Board and will be appointed to the HTC by the Board. The remaining members of the HTC will be invited to serve on the committee by the HTC itself. These “invited members” will be selected to provide expertise needed to accomplish specific tasks. The HTC may form subcommittees at its discretion.

The HTC and the IFB President are responsible for the routine operations of the Heritage Trees® Program including allocation of available staff time and other resources in a manner consistent with this Implementation Plan and priorities established by the IFB Board in its annual program / budget plan.

Significant changes in the scope, budget plans, and strategic directions of the Heritage Trees® Program must be approved by the IFB Board. It is the responsibility of the IFB President and HTC Chair to maintain excellent communications with the Board and to ensure that significant changes proposed by the HTC are reviewed and approved by the Board. The annual budget and annual report of the HTC will be parts of the IFB annual budget and annual report, and are thus subject to review and approval by the IFB Board.

The scope of the Heritage Trees® Program will include biological research and development, intellectual property management, investigations of ecological and societal issues, and regulatory process management. It is understood that active participation in intellectual property management, ecological risk assessment, and regulatory processes for Heritage Trees® will test the limits of IFB’s objectivity and effectiveness. However, it would be disingenuous of IFB to support Heritage Trees® research for noble purposes while eschewing difficult and essential tasks. The IFB, HTC and the Heritage Trees® Program will preserve their objectivity and effectiveness by adhering to the highest standards of scientific and business integrity in all their activities.

### **STAFF AND OTHER RESOURCES FOR THE HERITAGE TREES® PROGRAM**

The IFB Board will allocate IFB staff time and other resources to the Heritage Trees® Program it deems necessary in its annual budget plan. The IFB President and HTC will be responsible for utilizing these resources in a manner consistent with this Implementation Plan and the IFB’s annual budget plan.

Day to day operations of the Heritage Trees® Program will be managed by a Program Manager supervised by the IFB President. Initially, IFB’s Project Manager (Susan McCord) will serve as Heritage Trees® Program Manager on a part-time basis.

The IFB will seek donations of funding, intellectual property, and professional services from public and private sources for the Heritage Trees® Program. Fundraising will fall into two categories: general support for IFB to administer the Heritage Trees® Program and targeted support directed to specific projects or species. The IFB President, Heritage Trees® Program Manager, and the HTC will ensure that fund raising strategies and activities for the Heritage Trees® Program are coordinated with IFB’s overall fund raising efforts. Initial fundraising efforts for the Heritage Trees® Program are described in Appendix 5.

**RESEARCH AND DEVELOPMENT PLANS FOR HERITAGE TREES®**

The Heritage Trees® Program will collaborate with other organizations to develop and implement compelling R&D plans with the goal of restoring Heritage Trees®. The plans will address all foreseeable aspects of the R&D process that are critical to success. In general, critical aspects will include biological research and development, and investigations of ecological and societal issues.

Biological research and development will emphasize biotech methods including genomics and mapping approaches but will be guided by the view that traditional tree selection and breeding are foundations for progress. Topics to be considered in biological R&D plans will include:

Gene Delivery Track

- Identification of Suitable Starting Germplasm
- Establishment of Initial Germplasm into Culture
- Propagation Methods
  - Micropropagation
  - Somatic Embryogenesis
- Identification of Gene Transfer Approaches
  - Agrobacterium tumefaciens* and *A. rhizogenes*
  - Biolistics
  - Appropriate Selection Systems

Genomics Track

- Early & Rapid Throughput Disease Resistance Screening Assay Systems
- Genetic Mapping
- Physical Mapping
- Genome Sequencing
- Functional Assignment

The Gene Delivery Track and the Genomics Track converge at this point.

- Identification of Suitable Novel Genes
- Assaying of Promoters for use with Target Tree Species
- Screening Primary Transformants and Demonstrating Novel Gene Expression
- GM Tree Regeneration
- Challenge with Pathogen
- Characterization of Anti-Pathogen Effects
- Pilot Scale Plantings
- Mass Propagation of Elite GM Materials
- Nursery Strategy
- Regulatory Strategy
- Selection of Suitable Reintroduction Sites
- Pilot Scale Reintroduction
- Deployment Scale Reintroduction

Ecological investigations will emphasize identifying knowledge gaps and outlining ways to fill these. Close attention will be paid to monitoring ongoing research and regulatory activities with implications for the **Heritage Trees® Program**. Topics to be considered will include:

- Identify ecological concerns and pertinent current research.
- Risks associated with transgenic trees becoming weedy and unintended adverse effects on growth characteristics and wood properties.
- Rates of gene flow within and across taxonomic boundaries.
- Risk of transgenes causing adverse effects in non-target species. This might include adverse impacts from tissues of transgenic trees, decrease in palatability to herbivores, and producing forest litter with unusual properties that affect decay rates and nutrient cycling.
- Risks and benefits associated with flowering control as a strategy to restrict gene flow.
- Identify knowledge gaps.
- Convene a diverse group of stakeholders, including regulatory agencies, to discuss the ecological issues, research and knowledge gaps relative to transgenic trees.
- Work in concert with APHIS and EPA in the United States and their counterparts internationally to develop language and criteria for assessment of genetically engineered trees.

The ability to place social imperatives at the forefront of a developing technology is a rare opportunity and one that will be folded into the Heritage Trees Program. Topics to be considered will include:

- Development of educational and informational materials on the Heritage Trees Program.
- Including social scientists in program meetings and engaging them in specific aspects of the project as determined by the HTC.
- Utilizing the IFB website to disseminate information and progress to reach a larger audience.
- Publication of materials in conjunction with HTP collaborating partners, particularly those with public outreach programs and constituents.
- Serving as a bridge between developing science, policy and a wider audience, enabling a transparent process.

Investigations of ecological and societal issues will proceed in coordination with biological R&D but with sufficient separation to ensure independence and credibility. As a guiding principle, the Heritage Trees® Program will be prepared to terminate or redirect projects in which risks appear to outweigh benefits or impose unacceptable burdens on ecosystems or society. However, where research indicates good potential for significant ecological and social benefits with acceptable risk, IFB will seek partnerships with third parties willing to undertake restoration efforts<sup>1</sup>. Restoration partners will have primary responsibility for compliance with all regulatory requirements associated with field testing and deployment of Heritage Trees® that have been genetically engineered for enhanced resistance to exotic insects and disease organisms. IFB may

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<sup>1</sup> Restoration efforts are defined as reintroduction of the insect or disease resistant variety through arboretums, parks and private homes with a long-term goal of well-planned and monitored deployment in collaboration with government agencies, NGOs and others.

provide monetary support and guidance to the regulatory efforts of the restoration partners as part of efforts to monitor and evaluate the efforts of restoration partners.

## **INTELLECTUAL PROPERTY**

Intellectual property issues are often significant barriers to progress in plant biotechnology research where expected benefits are primarily ecological and social rather than economic. A strategy for identifying and addressing intellectual property issues is essential to the success of the Heritage Trees® Program.

The critical intellectual property issue for the Heritage Trees® Program is ensuring that scientists working on biological R&D plans have access to enabling technologies and disease resistance genes such as:

- Transformation technology such as from DuPont or Syngenta
- Promoters and selectable markers such as from Monsanto
- GUS and selectable markers such as from CAMBIA
- Binary vectors such as from Syngenta
- Transcription factors such as from Mendel
- Anti-fungal proteins such as from Bayer, Syngenta, or Monsanto
- Propagation technologies such as from CellFor, ArborGen, and others

As a general strategy, IFB will develop capabilities needed to facilitate access to intellectual property for Heritage Trees® research. For example, necessary capabilities for developing disease-resistant varieties of Heritage Trees® include:

- Obtain and hold licenses for technologies needed to develop disease resistance in Heritage Trees®.
- Bundle technologies into a “package” so that technologies from multiple donors can be “stacked” together in developing the disease resistant trees.
- Make the technology package available for Heritage Trees® research through sublicenses to public and private research institutions.

Although this strategy is simple in concept, its implementation will be complex. IFB will need specialized legal assistance for licensing and sublicensing activities. Legal assistance must be supported by donations of funding and / or professional services. Moreover, technology donors will expect IFB to have credible and specific plans for enforcing sublicenses and protecting donors’ intellectual property throughout the R&D and deployment process from early research stages through successful restoration of Heritage Trees®. Such plans need to be drafted before starting negotiations with potential donors of technology licenses (see Appendix 6).

Many universities and foundations have substantial experience in addressing intellectual property challenges similar to those facing the Heritage Trees® Program. Relevant models include the Cystic Fibrosis Foundation ([www.cff.org](http://www.cff.org)) and the Rockefeller Foundation’s initiatives *Public Intellectual Property Resource for Agriculture (PIPRA)* ([www.pipra.org](http://www.pipra.org)) and *African Agricultural Technology Foundation (AATF)* ([www.aftechfound.org](http://www.aftechfound.org))

## **Next Steps**

This document was approved unanimously by the IFB Board of Directors on February 5, 2004. Next steps include:

1. Develop a comprehensive Heritage Trees® R&D plan for American chestnut that encompasses ongoing work as well as the research strategy outlined by Professor Sederoff for transferring specific resistance genes from Chinese chestnut to American chestnut (Attachment 7).
2. Review and refine draft strategy for protecting the intellectual property of technology donors (see Attachment 6).
3. Develop a proposal to prospective donors of technology, funding and professional services based on a complete Heritage Trees® R&D plan for American chestnut and the HTC's strategy for protecting the intellectual property of technology donors.
4. Present the proposal to prospective donors at a Heritage Trees® Symposium during early summer 2004.

## APPENDIX 1 THE INSTITUTE OF FOREST BIOTECHNOLOGY

The Heritage Trees® Program is one of the four cornerstone programs of the Institute of Forest Biotechnology (IFB). Founded in 2001, the IFB is a non-profit organization that works for societal, ecological and economic benefits from appropriate uses of biotechnology in forestry worldwide. For more information on IFB, visit our website at [www.forestbiotech.org](http://www.forestbiotech.org)

## APPENDIX 2 INITIAL PRIORITIES OF THE HERITAGE TREES® PROGRAM

**American chestnut.** The American chestnut (*Castanea dentata*) was an overstory tree of the Appalachian Mountains ranging from southern Maine to Alabama and Mississippi. It was a massive tree with wide spreading branches and occupied 25 percent of the Eastern hardwood forest in 1900. In the Southern Appalachians it grew to heights of 100 feet and could grow to 20 feet in diameter. It flowered in early summer and produced spectacular light yellow flowers. The chestnuts were abundant and desirable food for people and wildlife. It was a graceful shade tree in towns and cities. The wood from the American chestnut was light, straight grained, with excellent dimensional stability and highly resistant to decay. It was widely used for fences, posts, pilings, railroad ties, log cabins, and furniture. The bark and branch tips were a major commercial source of tannins for the leather industry.

Very early in the 20<sup>th</sup> century a fungal blight (*Cryphonectria parasitica*) was introduced from Asia. The fungus infects trees and spreads from the point of infection. Mature trees can be killed in only a few years. Most trees sprout back, only to be killed by later infection. By the middle of the century, most of the American chestnut in the eastern US had been destroyed.

Efforts to protect and restore the American chestnut have been underway for many years. For example, The American Chestnut Foundation and the American Chestnut Cooperators Foundation coordinate an extensive volunteer network that identifies and protects remaining trees; supports breeding and research programs; and distributes seedlings and educational materials.

Many different approaches to control chestnut blight have been tested. These include applying mud plasters to the stem infection; isolation of healthy trees in environments free from the blight fungus; deployment of hypovirulent strains of the fungus; and genetic selection and breeding for resistance. Chinese and Japanese chestnuts that grow well in the United States have shown resistance to the chestnut blight. Efforts to extend resistance to the American chestnut by backcrossing have yielded some potential candidates for testing, but a tree with demonstrated good growth, form and resistance is not yet available.

Biotechnology has excellent potential to complement the ongoing breeding and research efforts of The American Chestnut Foundation and others working to restore the species. Important exploratory research is already underway at the University of Georgia, the New York College of Environmental Science and Forestry, and other leading institutions. Current research priorities include development of new technology for gene introduction; genetic mapping of disease resistance factors; and methods for vegetative (clonal) propagation of specific genotypes.

**English and American Elm.** Elms are one of the world's major tree groups, with more than 40 species found both naturally and cultivated, throughout the temperate world. Among the most important species is the American elm (*Ulmus americana*) while in Europe the dominant forms are the English elm (*U. procera*), the field elm (*U. minor*) and the wych elm (*U. glabra*). Through the centuries, elms have found many uses, including the provision of shade, use in parks and

other landscape settings, as well as for timber purposes. Timber durability, strength, water resistance, flowing grain and landscape value are all highly prized features of elms.

Dutch elm disease has decimated the stately elm trees in recent decades, to the extent that the few remaining mature elms are highly valued for conservation and biodiversity reasons, with citizens groups and charitable bodies working to ensure their survival. Dutch elm disease spreads by the transmission of the *Ophiostoma* vascular wilt fungi by Scolytid elm bark beetles. More than a billion elms have been lost since the disease was first reported in the early 1900s, throughout the Northern Hemisphere and New Zealand.

Two pandemic disease waves have been identified, firstly, due to *O. ulmi* in NW Europe around 1910, which destroyed >40% of elm populations in Europe and N America, before declining in Europe in the 1940s. No such decline occurred in N America, due to the greater susceptibility of *U. americana*. The second, continuing pandemic is due to the North American and Eurasian races of *O. novo-ulmi*, destroying >90% of elms in many countries. The range of these two highly aggressive subspecies overlap in Europe, causing worry about the potential to produce hybrid fungi with even more lethal properties. Elms are also under attack to a lesser extent, from viral elm yellows, and elm leaf beetles.

Traditional breeding has been seen as a way of identifying trees able to resist Dutch elm disease. Extensive European and North American programs have, until recently, failed to provide any continuing resistance to the fungal pathogen, leading to a reliance on expensive and frequent chemical prevention measures such as Alamo®, Arbortect 20-S® and fungal extract based treatments including Dutch Trig® and ElmGuard® to individual trees. Unfortunately, each of these measures is at best, only partially effective. USDA and Morton Arboretum breeding programs have led to ‘disease tolerant’ American and hybrid elm selections, but their stability in use remains largely untested.

Forest biotechnology offers two highly promising routes to combat the fungal pathogen. Genetic modification to prevent fungal establishment and spread within elite elm trees is being investigated at the University of Abertay Dundee, Scotland, in collaboration with the UK Forestry Commission. This allows any gene to be inserted into an elm and trees regenerated avoiding the need to go through lengthy sexual crossing steps. Hundreds of genetically modified English elms have been produced and are now being tested for their ability to resist fungal challenge. This approach can be extended to other elm species, such as the American elm and the wych elm, which are also under attack. A second possible approach is to use d-factor RNA viruses, as biological control agents to debilitate the disease causing fungus. This could lead to reduced fungal pathogenicity. Genetic modification and d-factors each use the tools of forest biotechnology to potentially address the threat of Dutch elm disease to the remaining elms and provide excellent opportunities to return the stately elms to their dominant landscape position.

**Fraser fir (*Abies fraseri*).** This species ostensibly represents the southern end of the wide-ranging balsam fir (*A. balsamea*). It was isolated from the parent species by a gap in the Appalachian Mountains following the Ice Age of about 10,000 years ago. Genetic selection within the separate populations has resulted in morphological differences that clearly justify species distinction between *A. fraseri* and *A. balsamea*.

The range of the southern species (*A. fraseri*) is restricted to seven populations at the highest land elevations in the Southern Appalachians: one in Virginia, two in Tennessee, and four in North Carolina. The companion species at those locations is red spruce (*Picea rubra*), which also finds its optimal distribution in the north temperate and boreal latitudes, far removed from the Southern Appalachians.

In the mid-1950s, the balsam wooly adelgid from Europe was found at the Roan Mountain location in North Carolina. By about 1970 that pest had invaded each of the other isolated populations. Despite efforts to control the pest by spraying, the effects were devastating. All the overstory trees were killed after repeated attacks of three or more years running. Only the skeletons of trees remained. Early observation showed that the insects did not attack trees less than about 20 years old (less than 20 feet tall). The landscape today is replete with a background of dead trees under which young stands of Fraser fir trees have naturally regenerated. It is uncertain whether the regenerated trees will be subjected to a reinvasion of the pest as they age.

In the mid-1960, interest developed for using Fraser fir as Christmas trees. A selection program was started for creation of an orchard from which seeds would be obtained for establishment of Christmas tree plantations. That effort also resulted in a genetic variation study of the seven natural populations of Fraser fir. It entailed the collection of seeds from selected trees and establishment of progeny tests that are located at various places at elevations about 1,000 feet lower than the elevation of the natural stands. As the trees in the genetic tests developed, they were evaluated for growth rate, tree form, needle color, and resistance to pests. Fortuitously, the trees in the genetic tests as well as those in Christmas tree plantations, which were also planted at 2,500 to 3,500 feet elevation, were relatively immune to attack from the wooly adelgid.

The Christmas tree plantations were primarily located on old-field sites. That location was beneficial because land did not have to be cleared to plant the trees. Before long, however, a root rot (*Phytophthora cinnimonium*) began to invade the planted stands. That plague is uncommon to rare in the natural stands of Fraser fir because it is incompatible with the acid soils at the high elevations. Conversely, balsam wooly adelgid is of minor concern in the Christmas tree plantations.

Efforts are in progress to control the wooly adelgids by genetic selection and breeding, by biological controls, and eventually by biotechnology. The latter might be accomplished by inserting a gene into the tree that will either be lethal or will repel the insect. An alternative is to insert a gene into the insect that would prevent it from asexually propagating, which is the common form of reproduction.

**Other Priorities.** Invasive insect and disease organisms are causing significant damage to many important tree species. Biotechnology research on insect and disease resistance may have applications in many species (Table 1).

Research on tissue culture and whole-plant regeneration is essential to the success of traditional breeding and biotech approaches to developing resistant varieties of Heritage Tree species. Progress in tissue culture and regeneration research may be useful in conserving and propagating the germplasm of rare or unique trees for scientific and cultural purposes and for the benefit of generations to come. Some bristlecone pines (*P. aristata*) in the US Southwest are 5000 years old and among the oldest living plants on Earth. In Scotland, a large and ancient oak is believed to be the only remnant of Birnam Wood, the great oak forest described by Shakespeare in *Macbeth*. Also in Scotland, the last surviving Dunkeld larches have unique historical and ecological significance as progenitors of offspring planted throughout Europe over the past three centuries.

| <b>Species</b>                              | <b>Pest</b>                 |
|---|-----------------------------|
| Eastern hemlock ( <i>Tsuga Canadensis</i> ) | Hemlock wooly adelgid       |
| American beech ( <i>Fagus grandifolia</i> ) | Beech scale                 |
| White ash ( <i>Fraxinus americana</i> )     | Ambrosia beetle             |
| California oaks ( <i>Quercus</i> spp.)      | <i>Phytophthora romorum</i> |
| Butternut ( <i>Juglans cineria</i> )        | Fungal disease              |

|                             |            |
|-----------------------------|------------|
| Oaks ( <i>Quercus</i> spp.) | Gypsy moth |
| - partial list – add more   |            |

### APPENDIX 3 AN OVERVIEW OF BIOTECHNOLOGY R&D ON PLANT RESISTANCE TO INSECT PESTS AND DISEASE ORGANISMS

Biotechnology has largely developed for the production of new crops and molecular products. There is, however, great potential for biotechnology to contribute to solving problems of forestry and ecology that are not necessarily market driven.

Several indigenous tree species are under threat from exotic (i.e., non-indigenous) insect and disease organisms. Indigenous tree species have evolved with resistance to their indigenous insect pests and disease organisms. Unfortunately, indigenous trees sometimes lack effective resistance to exotic insects and disease organisms.

Traditional agronomic approaches to insect and disease management such as breeding for resistance and Integrated Pest Management have generally met with limited success when applied to forest trees. Forest trees are inherently difficult to work with because of their slow growth, long reproductive times, large size and usually, a lack of extensive research history. Biotechnology can circumvent some of these limitations and has considerable potential for the benefit of Heritage Trees®. Below is a brief summary of biotechnology approaches that have been demonstrated to have potential to control insect pests and disease organisms.

**Insect Pests.** There are many examples of controlling lepidopteran and coleopteran insect pests via expression of *Bacillus thuringiensis* (Bt) endotoxin genes in plants. Each Bt strain has a different complement of Bt endotoxins, and each endotoxin has a different activity spectrum against different insects. Therefore there is significant diversity in Bt endotoxins, which is useful in finding genes that have activity against insect pests. In addition to endotoxins, *Bacillus thuringiensis* also produces vegetative insecticidal proteins (VIPs) that are active in controlling different insect pests.

Several commercial crop biotech products (Bt corn, Bt cotton, Bt potato) have been successfully introduced and adopted by farmers because the products provide excellent protection against target insects and allow farmers to reduce their use of expensive insecticides. These products are heavily protected by patents held by major agricultural products companies (Syngenta, Monsanto, Dow-Elanco).

Bt endotoxins and proteins have not shown commercial level activity against piercing and sucking insects such as the balsam woolly adelgid that afflicts Fraser fir. There is another class of insecticidal genes from the bacterium *Photorabdus luminescens* that shows promise. Patents for this technology appear to be dominated by Syngenta.

Lectins are another group of plant-derived proteins that have insecticidal activity. The lectins have the common property of binding to specific carbohydrate structures found in insect body tissues. Work over the last 25 years has shown that lectins can play a role in resistance to insect and nematode pests in the plant, and that they are potentially important in crop protection. Potatoes transformed with plant lectins have shown resistance to insects (e.g., snowdrop lectin confers resistance to weevils).

Managing insect resistance is critical to the long-term success of insect control strategies. Elements of resistance management strategies can include: inserting multiple insecticidal genes

with different modes of action; monitoring for insect resistance development; maintaining non-transgenic refugia where targeted insects can reproduce without any selection pressure; and continuing limited use of insecticides to diversify the selection pressure and slow development of insect resistance to plant gene products.

**Fungal and Bacterial Diseases.** To date, no transgenic crops have been commercialized with fungal resistance. However, there is significant ongoing research and development in this area by academics and agricultural products companies.

An important current priority is understanding and applying genes that are involved in Systemic Acquired Resistance (SAR). In essence, SAR theory holds that a plant resistance gene (R) encodes proteins that both recognize specific pathogen-derived avirulence (*Avr*) proteins and initiate signal transduction pathways leading to complex defense responses. Most R genes are in the nucleotide binding (NB) leucine rich repeat (LRR) class. These are subdivided into the TIR family and the CC:NB:LRR family.

R genes conferring resistance to bacterial, viral and fungal pathogens use multiple signaling pathways. Manipulation of R genes and their signaling pathways by transgenic expression may be a useful approach to improve disease resistance in plants. Examples of successful demonstration of R genes conferring resistance in transgenic plants include: Xa21 (*Xanthomonas*/rice), and the *Pto* protein kinase, *Prf*, LRR genes (*Pseudomonas*/tomato). Over expression of *Prf* in tomato led to increased resistance to three bacterial and one viral pathogen, without detrimental effects on plant growth or fruit production, although this broad spectrum resistance has yet to be tested in field conditions.

R proteins have shared motifs that suggest that their downstream signaling pathways contain similar and/or compatible components. If this is the case, the R genes isolated from one species might be used to engineer new disease resistance specificities into other species. However, it appears that R genes only perform well in plants that are members of the same plant family (e.g., R genes from tomato can function in tobacco, and vice versa; but *Arabidopsis* R genes do not appear to function in Solanaceous species and vice versa). Whether this restricted cross-taxa function is due to the absence or incompatibility of other essential recognition or signaling components has yet to be determined. This could be a limitation to applying R gene transgenic technology to trees without utilizing a gene from a closely related plant species. Completion of the *Arabidopsis* genome sequence will enable identification of a nearly complete R-gene set in any flowering plant.

Another approach is to work at a point further downstream, with genes that are intermediate between R genes and the final effector molecules. Some pathways may be shared by multiple and different defense responses in plants. These pathways could be activated by many stimuli including R genes, non-specific elicitors, wounding and salicylic acid. Expression of the NPR1 gene in *Arabidopsis* was manipulated to increase its protein levels, and increased resistance was demonstrated to two pathogens (*Pseudomonas* and *Peronospora*). Defense related genes were also expressed. This type of specific induction of only a certain defense pathway might avoid agronomic problems associated with constitutive activation of some R gene-mediated pathways such as those leading to the hypersensitive response (HR).

Mendel Biotechnology has identified the complete set of *Arabidopsis* transcription factors. This is an important milestone towards identifying transcriptional regulators that are a part of the signal transduction pathway for disease resistance. Both Mendel Biotechnology and university researchers have demonstrated that Npr1, a transcriptional regulator, enhanced resistance to a diverse array of pathogens. In addition, they have identified several other transcription factors that provide resistance to multiple disease organisms. Additional genes involved downstream of

the R genes in the signaling pathway are the *Arabidopsis* mutations at the ndr1 and eds1 loci and are effective in resistance to both bacterial and fungal pathogens mediated by several different R genes.

Another class of genes is the MAPKs, which can be activated by R gene pathways. These kinases appear to be convergence points for many different defense responses in plants. Examples of these are the SIPK and WIPK genes in tobacco. This class may be shared with general stress responsive pathways, because they can be activated by many stimuli including non-specific elicitors, wounding, and salicylic acid.

The genes in the signal transduction pathway stimulate the production of pathogenesis-related (PR) proteins. PR proteins constitute a point where the various response networks intersect by reacting with different inducers such as salicylic acid, jasmonic acid, systemin, and ethylene. PR proteins have been classified into 12 major groups or families, some of which are antifungal: B-1, 3-glucanases PR-2, chitinases (PR-3) and fungal membrane permeabilizers (PR-5). These can be introduced singularly or in combinations into plants. A transgenic example is the Rice PR-5 protein gene in wheat, which delays the infection of the wheat scab pathogen fungus.

Plant defensin is one of the pathogenesis-related proteins. These have antimicrobial properties and are characteristically small, basic, and cysteine rich. Disease resistance in rice is an example of the use of this type of protein. In order to develop rice with broad spectrum disease resistance, transgenic rice plants were made that expressed one of the defensin genes from the *Brassicaceae* constitutively throughout the plant tissues. These were then tested for disease reaction to *Magnaporthe grisea* and *Xanthomonas oryzae*. Transgenic plants with high resistance to *M. grisea* and *X. oryzae* showed high resistance to other seed-transmitted phytopathogenic bacteria, such as *Burkholderia plantarii* and *B. glumae*. It was also demonstrated that highly enhanced resistance to each race of rice blast was obtained by introduction of defensin gene. These results indicate that the Brassicaceae defensin effectively works in rice plants against a broad spectrum of pathogen attack.

There are other antifungal transgenic approaches that are based on different proteins. For example, Syngenta has demonstrated that engineering plants with genes that produce natural plant antifungal compounds, such as pyrrolnitrin, can create disease resistance. Syngenta also has achieved Fusarium control through gene expression that degrades the production of mycotoxins produced by Fusarium.

**Other antifungal and antibacterial approaches.** Antimicrobial peptides from a variety of organisms are known to inhibit the growth of pathogens. One approach to control bacterial disease is to introduce lytic proteins that have cytotoxic activity to disease causing organisms. Examples of lytic proteins are Cecropin B and Attacin A. These are naturally occurring lytic proteins from *Hyalophora cecropia*, the Giant Silk Moth.

Two 38-amino acid peptides, SB-37 and Shiva-1, have been designed and chemically synthesized as substitution analogs of Cecropin B. SB-37 is a close analog of the natural peptide, whereas Shiva-1 has only 46% amino acid homology.

Attacin A, SB-37, and Shiva-1 are cytotoxic *in vitro* to a number of different species of plant pathogenic bacteria at micro molar concentrations, with Shiva-1 having the greatest activity. Shiva-1 is also highly cytotoxic to *Erwinia amylovora*. The transgenic use of the wound inducible proteinase II promoter from potato with Shiva-1 in apple against the fire blight bacterial pathogen *Pseudomonas solanacearum* resulted in delayed symptoms and reduced disease severity.

Oxalate oxidase is a new class of proteins that respond to pathogen attack. This may be mediated through the production of hydrogen peroxide or by degradation of oxalic acid produced by an invading pathogen. Transformation of soybean with the wheat germin gene (OxO), which encodes for oxalate oxidase (OxO), an enzyme that targets oxalic acid secreting pathogens, has resulted in resistance to the white mold disease. The most advanced transgenic line, 80-30-1, has undergone two years of confined field trials at a total of 7 different sites in Ontario and Quebec and has shown superior resistance to white mold without any loss in yield. This line shows a variable level of oxalate oxidase activity, which may explain its incomplete resistance. There are other transgenic lines with consistently higher levels of resistance.

Some other proteins that have provided disease resistance in transgenic plants are chitinases and Harpin. The chitinase gene from *Trichoderma viride* confers resistance against rice sheath blight pathogen. Harpin is a protein produced by *Erwinia amylovora*, the pathogen that causes fire blight, and it can elicit resistance to pathogens and insects in transgenic plants. Antibodies that target specific pathogens, especially viruses, have also been shown to be effective in transgenic plants.

**Viral diseases.** The same SAR pathway genes that confer fungal and bacterial disease control can potentially address disease resistance to viruses. However, viral disease resistance has been widely demonstrated in many species against many viruses by using pathogen-derived resistance approaches. The greatest number of success stories involved the use of coat protein mediated resistance. Examples of using coat proteins are demonstrated resistance to papaya ring spot virus; PVX and PVY virus resistance in potato; and TMV resistance in tomato. Viral replicase genes introduced into transgenic plants have also proved successful in conferring resistance, as exemplified in bananas to banana bunchy top virus using BBTV replicase and resistance to potato leaf roll virus using the PLRV replicase gene.

There are also other virus resistance genes that can be considered to be pathogen derived resistance. These are ribozymes, ribosomal-inactivating proteins, ribonucleases, and RNA mediated gene silencing which target viral genes.

**Issues in applying disease resistance technology.** Issues in applying disease resistance technology to restore Heritage Trees® relate to the complexity of the defense response in plants. Although there appear to be many potential approaches, not all approaches will work for a specific disease problem in a particular species. Many of the disease resistance genes expressed in transgenic plants have had negative effects on vegetative growth and development. It will be important to be able to express these genes only where and when they are needed, so inducible and tissue specific promoters will be important for success. In addition, for durable resistance it is most likely important to have multiple resistance mechanisms working simultaneously.

Another issue is that patents may protect much of the technology available, and application to trees would require licensing. Examples of sources of technology are:

- Monsanto, Dow, Syngenta – Bt genes and associated intellectual property
- Monsanto, Syngenta, Mendel – fungal disease resistance genes and associated intellectual property
- Monsanto – virus resistance and associated intellectual property

In addition, patents may also protect the enabling technologies required to introduce and express the genes. Examples of some key technology owners are:

- Agrobacterium transformation – Monsanto, Syngenta
- Biolistics transformation – DuPont
- Selectable markers – Monsanto, Syngenta,
- Promoters – various sources from companies and academics

**APPENDIX 4**  
**MEMBERS OF THE HERITAGE TREES® COMMITTEE AS OF JANUARY 2004**

|                |                                 |
|----------------|---------------------------------|
| Kevan Gartland | University of Abertay           |
| Maud Hinchee   | ArborGen                        |
| Alan Lucier    | NCASI (chair)                   |
| John Pait      | CellFor                         |
| Ron Sederoff   | North Carolina State University |

**APPENDIX 5**  
**OVERVIEW OF INITIAL FUNDRAISING EFFORTS**

*National Science Foundation*

Dr. Mary Clutter is assistant director of the National Science Foundation (NSF). She is responsible for the Biological Sciences Directorate that supports all major areas of fundamental research in biology. Bob Kellison and Ben Box met with her at the NSF offices in Arlington, Virginia on December 3, 2003. She indicated an interest in a genomics project on American chestnut and suggested a proposal be submitted to Jane Silverthorne, Program Manager, at NSF. A well coordinated and directed effort needs to be outlined for this proposal. Interested participants will be solicited from those who have been working tirelessly in this area such as The American Chestnut Foundation, University of Georgia, State University of New York, American Chestnut Cooperators Foundation and ArborGen. Others involved in mapping, sequencing, tissue culture and transformation will be invited to participate.

*The Center for the Advancement of Genomics*

Initial discussions have included Dr. Robert Friedman, IFB Board Member and Vice President at TCAG. There is interest in a possible collaboration with IFB to include TCAG and The Institute for Genomic Research (TIGR) in a sequencing project for American chestnut. This could be part of the proposal to be submitted to NSF.

*Park Foundation*

The Park Foundation is involved in conservation. Richard Robb, a Park Foundation board member, has been working with Bob Kellison in finding undisturbed land in the southeast to set aside for conservation studies. Mr. Robb has indicated an interest in the Heritage Trees® program and has encouraged submission of a proposal.

*Biotechnology Companies*

Several agriculture biotechnology companies have been identified to solicit donations of funds and intellectual property for the purposes of the Heritage Trees® program. These companies are:

- Syngenta
- Dupont
- Monsanto
- Dow
- Mendel

*USDA Forest Service*

Preliminary discussions indicate that leaders of the Forest Service R&D organization may be receptive to collaboration with IFB on Heritage Trees® research. Specific options for collaboration will be developed and presented later this year.

*Heritage Trees® Symposium*

Identified prospective donors of funds and intellectual property will be invited to a small symposium to be held at the North Carolina Biotechnology Center in the Summer of 2004. The purpose is to communicate the vision, scope and plans of the Heritage Trees® Program to prospective donors. Ideally, several donors will come to the Symposium prepared to make commitments and help catalyze support from others for the concept of the Heritage Trees® Program.

**APPENDIX 6  
DRAFT STRATEGY FOR  
PROTECTING INTELLECTUAL PROPERTY RIGHTS OF TECHNOLOGY DONORS  
IN THE HERITAGE TREES® PROGRAM**

**Question:** How can the Heritage Trees® Program ensure that discoveries enabled by donated technologies do not compromise the intellectual property rights of the technology donors?

The IFB (or a separate Foundation within IFB) obtains funding for Heritage Trees® research and develops subcontracts with universities and other research organizations to achieve specific science objectives. Subcontracting allows IFB to hold and control new intellectual property derived from or enabled by donated technologies licensed to IFB and sublicensed to subcontractors individually or as part of a technology package. Subcontractors would not own any of the biological materials or intellectual property developed pursuant to subcontracts but would be allowed to publish their results (with IFB approval, and potential technology donor approval if required). Subcontractors would be selected by IFB's Heritage Trees® Committee or a scientific advisory panel, based on research capabilities and ability to meet specific objectives defined in a Heritage Trees® R&D plan.

**Question:** How can the Heritage Trees® Program ensure that efforts to restore Heritage Trees® do not infringe on the intellectual property rights of the technology donors and do not unduly compromise the ability of IFB to objectively monitor and evaluate the restoration efforts.

Heritage Trees® research supported through IFB may progress to a point where it becomes technologically feasible and ecologically desirable to attempt restoration of Heritage Trees® by producing substantial numbers of disease resistant seedlings for deployment in arboreta, research forests, parks, or other landscapes. In this circumstance, IFB (or its Foundation) would be responsible for negotiating, writing and executing licenses with third parties interested in undertaking a restoration effort. Such restoration partners would have to meet criteria established in licenses granted to IFB by the technology donors. For example, third parties undertaking restoration could be required to develop business plans that address topics such as royalty rates (if seedlings are to be sold), ecological monitoring and stewardship, regulatory measures and compliance, oversight rights of technology donors, and safeguards against piracy. Final agreements between IFB and third parties undertaking restoration would need to be approved by the donors of technologies that have a material role in the proposed restoration activities. Any royalties paid to IFB by a restoration partner would be earmarked for the continuing R&D activities of the Heritage Trees® Program.

Restoration partners would be responsible for developing and submitting regulatory packages, gaining regulatory approval, and all regulatory compliance associated with seedling deployment. Restoration partners would also be responsible for post-restoration stewardship and compliance with terms of licenses granted by IFB and its technology donors. The IFB may provide monetary support, within the resources available to do so, and guidance to the regulatory efforts of the restoration partners. IFB will monitor this process through annual reporting requirements.

**APPENDIX 7**  
**DRAFT R&D STRATEGY FOR TRANSFERRING SPECIFIC RESISTANCE GENES**  
**FROM CHINESE CHESTNUT TO AMERICAN CHESTNUT**

**A Path to a Blight Resistant American Chestnut.**

It is now possible to establish a comprehensive plan to select and create a blight resistant American chestnut. None of the current approaches alone is likely to be able to do this, however, a combination of existing methods combined with genomic information and gene transfer technology is now an apparent approach. To do this, a new organization of scientists is required which includes expertise in tree breeding, vegetative propagation, genome mapping, genome sequencing, physical mapping, microarray technology, gene transfer and improved disease screening. Existing pedigrees and each of the above components are essential to this strategy. The strategy has several parallel tracks.

Part of the strategy is to identify the genes underlying the existing resistance in the Chinese chestnut. Several have been identified as QTL loci in an F2. The genes underlying those QTLs may now be identified using genomic methods.

1. First, it is necessary to sequence the regions of the QTLs. This requires construction of a high resolution genetic map with good definition of the disease tolerant/resistant loci QTLs. Then a physical map is needed to defining the DNA segments spanning the QTLs. is needed.
2. Then the regions spanning the QTLs will be sequenced to define all of the genes in the intervals that contribute to resistance.
3. The genes in the intervals will be on the order of a thousand to two thousand genes. A microarray approach can define the ones that contribute to resistance.
4. Microarray methods have been developed to identify the genes underlying QTLs because *bona fide* candidate genes contribute more to phenotypic variance than do genes associated with a QTL by linkage. If all of the genes in the interval can be put on arrays, then there is a good chance of identifying the ones that are the basis of the QTL. Comparative genomics of resistance genes from other species may help in the identification.
5. Gene transfer technology is needed to verify if a *bona fide* candidate gene has been identified. Robust vegetative propagation methods, DNA transformation methods, and plant regeneration methods are needed.
6. Improved methods for screening resistance in young plants are an important technology that needs to be developed.
7. In addition to identification of genes that contribute to resistance from the Chinese chestnut, it will be necessary to increase and buffer the level of resistance by adding other genes providing defense against the pathogen from other sources, such as resistance genes used in other crops that are not based on gene for gene resistance. Mechanisms for hypovirulence may also be incorporated to increase or buffer resistance. It may be possible to improve resistance by increasing the timing or level of expression of genes that are to be identified as QTLs.
8. Finally a transgenic resistant chestnut with multiple inserted gene modifications would be created and tested using all of the information and technology assembled following this strategy.